

Role of Thyroid Hormone Deiodination in the Hypothalamus

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Iodothyronine deiodinases (D1, D2, and D3) comprise a family of selenoproteins that are involved in the conversion of thyroxine (T_4) to active triiodothyronine (T_3), and also the inactivation of both thyroid hormones. The deiodinase enzymes are of critical importance for the normal development and function of the central nervous system. D1 is absent from the human brain, suggesting that D2 and D3 are the two main enzymes involved in the maintenance of thyroid hormone homeostasis in the central nervous system, D2 as the primary T_3 -producing enzyme, and D3 as the primary inactivating enzyme. While the coordinated action of D2 and D3 maintain constant T_3 levels in the cortex independently from the circulating thyroid hormone levels, the role of deiodinases in the hypothalamus may be more complex, as suggested by the regulation of D2 activity in the hypothalamus by infection, fasting and changes in photoperiod. Tanycytes, the primary source of D2 activity in the hypothalamus, integrate hormonal and probably neuronal signals, and under specific conditions, may influence neuroendocrine functions by altering local T_3 tissue concentrations. This function may be of particular importance in the regulation of the hypothalamic-pituitary-thyroid axis during fasting and infection, and in the regulation of appetite and reproductive function. Transient expression of D3 in the preoptic region during a critical time of development suggests a special role for this deiodinase in sexual differentiation of the brain.

Introduction

IODOETHYRONINE DEIODINASES (D1, D2, and D3) comprise a family of membrane associated enzymes that are involved in the conversion of thyroxine (T_4) to triiodothyronine (T_3), and also the inactivation of both thyroid hormones (1). D1 catalyzes the deiodination of T_4 at both the outer and inner tyrosyl rings with equal efficiency, resulting in either the generation of T_3 or inactive reverse triiodothyronine (rT_3) (2). Because the preferred substrate of D1 is rT_3 (1), however, D1 may also be involved in the degradation of iodothyronines. D2 is an obligate, outer ring deiodinase enzyme that catalyzes the conversion of T_4 to T_3 , and rT_3 to $3,3'$ - T_2 (1) with 10^3 times higher affinity for T_4 than D1, and is the primary deiodinase responsible for T_3 generation in the central nervous system, pituitary, brown fat, placenta, thyroid, skeletal muscle and heart (1,3). D3 has almost exclusively inner ring deiodinase activity, catalyzing the conversion of T_4 to rT_3 and the conversion of T_3 to $3,3'$ - T_2 (1), and therefore the major T_4 and T_3 inactivating enzyme.

Drawing from the experience of numerous studies (reviewed in Bianco et al. [1] and Kohrle [4]), it is presumed that the major function of the deiodinases in the brain is to regulate local bioavailability of T_3 . This function is impor-

tant for neuronal proliferation, organization, arborization, synapse formation, migration, myelination and performance (5,6), but also to protect the brain from the adverse effects of excess amounts of thyroid hormone (7–9). Thus, in the face of reduced circulating levels of T_4 , a coordinated mechanism in which specific deiodinases are increased or decreased, assures that intracellular levels of T_3 within the brain remain constant and normal. Accordingly, D2 activity in the cortex is markedly increased by hypothyroidism and progressively reduced by varying degrees of hyperthyroidism, and cerebral cortical levels of T_3 are maintained at normal or near-normal levels even over a wide range of circulating T_4 levels that expand the gambit of hypothyroidism to hyperthyroidism (10–18). Thus, when the thyroid gland is absent (and thereby incapable of secreting T_3), constant subcutaneous infusion of various doses of T_4 that do not fully replace or exceed normal circulating levels, still result in normal levels of T_3 in the cerebral cortex (12,14). This regulation of D2 appears to be exerted both at the pretranslational and posttranslational levels, as hypothyroidism also increases D2 mRNA in the cerebral cortex (19).

Conversely, D3 activity is up regulated by hyperthyroidism and reduced by hypothyroidism (20–22) or iodine deficiency (23), thereby contributing to the maintenance of normal T_3 levels in the brain by degrading T_4 and T_3 (1,3).

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This view, however, is not universally accepted (12–14). Nevertheless, D3 activity and D3 mRNA increase in the cortex in response to the systemic administration of T_3 in rats (12,21). Furthermore, an inverse association between D2 activity and T_3 content in the cerebral cortex has been observed in man (24), implying that D3 contributes to local regulation of T_3 levels. As D3 increases rT_3 as a result of reductive deiodination of T_4 (1), it is conceivable that any increase of D3 activity may also contribute to inactivation of D2 as a result of the effects of rT_3 to promote the ubiquitination of D2 conjugates. The separate compartmentalization of D2 in glial cells and D3 primarily in neurons (22,25–27), however, would necessitate reciprocal neuronal–glial interactions in which rT_3 is released from neurons to affect adjacent glia expressing D2.

The role of D1 is of uncertain significance because of its apparent absence in the human brain (28). However, evidence for 5' deiodination of rT_3 and T_4 by extracts from rat cerebral cortex that can be inhibited by propylthiouracil is characteristic of D1 (15). In addition, the presence of D1 activity in several regions of the rat brain (14,29), and immunocytochemical evidence for D1 in the chicken brain (30), suggests a physiologic role for this deiodinase in the central nervous system. Because D1 has a greater substrate preference for rT_3 resulting in the degradation of rT_3 (1), it is conceivable that D1 may help maintain D2 activity by preventing tissue accumulation of rT_3 . On the other hand, D1 activity is increased by hyperthyroidism and reduced by hypothyroidism (3) and catalyzes the deiodination of sulfated forms of T_3 (1), supporting a primary role for this deiodinase in thyroid hormone degradation.

Importance of Deiodinase in the Regulation of Tissue Levels of T_3 in the Hypothalamus

While the regulation of deiodinases in the hypothalamus by circulating levels of thyroid hormone would seem to parallel that observed in the cortex (14,25,31) including diminution of D2 immunostaining in the hyperthyroid human hypothalamus (32), differences have been described. For example, in some studies, hypothyroidism induces only a moderate increase in D2 mRNA in the hypothalamus of approximately 1.3- to 1.6-fold over euthyroid tissue (25,33) and no increase in D2 activity (33). Similarly, no increase in D2 activity in the hypothalamus has been observed in association with iodine deficiency, contrary to other regions in the brain (34). In addition, only modest increases in D3 mRNA have been observed in the hypothalamus following the systemic administration of thyrotoxic doses of T_3 (21). Furthermore, contrary to the cortex, the systemic infusion of high doses of T_4 into hypothyroid rats increases tissue and nuclear T_3 content in the hypothalamus rather than maintaining normal levels (14).

Part of the explanation for the above differences may be that hypothalamic deiodinases have a broader function that extends beyond maintaining normal tissue levels of T_3 , and also allows T_3 to serve as a regulatory signal for specific hypothalamic functions by increasing or decreasing T_3 levels in discrete neuronal populations. In support of this concept are the studies by Diano et al. (33) showing that only D2 mRNA in the arcuate nucleus/median eminence area is inhibited by the systemic administration of high doses of T_4 , whereas D2 mRNA in the periventricular area is unaffected. Given that hypothalamus contains important neuroregula-

tory centers responsible for modulation of the hypothalamic-pituitary-thyroid (HPT) axis under conditions of hypothyroidism and hyperthyroidism, cold exposure, starvation, and illness, there may be an advantage for certain subregions of the hypothalamus to increase or decrease T_3 content rather than simply maintain normal levels (see below). In addition, increasing or decreasing levels of thyroid hormone in discrete regions of the hypothalamus may provide important regulatory signals for the control of appetite and satiety, and reproductive function (see below). The hypothalamus also contains a number of gene products that are not necessarily involved in neuronal growth and/or differentiation, for which changes in thyroid hormone exert important regulatory effects (Table 1).

Anatomic Distribution of Deiodinase in the Hypothalamus

The presence of D1 in the brain is species-specific because it is completely absent from the human brain (28). While evidence for D1 activity has been identified in extracts of whole rat hypothalamus (35,36), the distribution of its mRNA and/or immunoreactivity is unknown.

D2 activity is relatively low in whole hypothalamic blocks (37), but micropunches of the arcuate nucleus/median eminence regions show substantial activity, three to four times higher than D2 activity in any other brain region (38). D2 activity has also been detected in the ventromedial nucleus, dorsomedial nucleus, and lateral hypothalamus, although less (38). In contrast to the cerebral cortex, therefore, D2 is highly concentrated within discrete regions of the hypothalamus rather than diffusely dispersed.

Unique to the hypothalamus is the synthesis of D2 in a specialized type of glial cell, the tanycyte (25,27,35). By *in situ* hybridization histochemistry, the most intense D2 hybridization signal in the rat hypothalamus is located in the mediobasal hypothalamus between the rostral and caudal poles of the median eminence and the infundibular recess (Fig. 1). In this region, D2 mRNA is localized primarily in the floor and infralateral walls of the third ventricle, abruptly ceasing one half to two thirds up the third ventricular wall and extending into the adjacent arcuate nucleus in long cytoplasmic processes that envelop blood vessels (Fig. 2). No hybridization is present in the roof of the infundibular recess or in ependymal cells in other regions of the third ventricle rostral to the anterior pole of the median eminence. Hybridization is also present overlying a cell-clear zone above the tuberoinfundibular sulci, and in the substance of the median eminence and pituitary stalk where it extends into the external zone adjacent to the portal capillaries. A similar distribution of D2 or D2 mRNA has been identified in the mouse (personal observations), chicken (39) and human hypothalamus (32) (Fig. 3). No hybridization is seen in other circumventricular organs or other nuclear regions in the hypothalamus, including the paraventricular nucleus (25).

That tanycytes are one of the major sources for hypothalamic expression of D2 has been established by double-labeling techniques using specific markers for tanycytes including dopamine and cyclic adenosine monophosphate (cAMP)-regulated phosphoprotein (DARPP-32) (Fig. 4) (26,40,41) and vimentin (27). The significance of DARPP-32 in D2 tanycytes is uncertain, but by inhibiting phosphatase

TABLE 1. HYPOTHALAMIC GENES AFFECTED BY HYPOTHYROIDISM

Gene	Location	Effect of hypothyroidism	References
Peptides			
CART	PVN	Increase	(96, 152)
CRH	PVN	Decrease	(153–155)
Galanin	PVN	Increase	(155)
	DMN	Decrease	(155)
GALP	ARC	Decrease	(156)
TRH	PVN	Increase	(61)
VIP	PVN	Increase	(155,157,158)
Nuclear receptors/ transcription factors			
TR α_2	PVN	Increase	(159)
TR β_1	PVN	Increase	(159)
TR β_2	PVN	Increase	(159)
CBP	PVN	Increase	(159)
SRC-1	PVN	Increase	(159)
SMRT	PVN	Increase	(159)
NCoR	PVN	Increase	(159)
Enzymes			
Tyrosine hydroxylase	ARC	Decrease	(160)
Prohormone convertase 1	PVN, PON	Increase	(161,162)
G-Proteins			
G α	ARC, VMN	Increase	(163)

ARC, arcuate nucleus; PON, preoptic nucleus; PVN, paraventricular nucleus; VMN, ventromedial nucleus.

activity, DARPP-32, like other phosphatase inhibitors, may have an important role in the amplification of physiologic responses that are mediated by the phosphorylation of specific proteins during kinase activation (42). Because the promoter of the D2 gene contains a cAMP response element or CRE, which allows binding by the phosphorylate form of the cAMP response element binding protein (CREB) (43), DARPP-32 may have a unique role that contributes to tissue-specific regulation of D2 gene activity in tanycytes.

While excellent morphologic descriptions of tanycytes are present in the literature (44–47), their physiologic function remain poorly understood. Originally believed to serve as part of the blood–brain barrier, preventing material in the perivascular spaces in the median eminence from leaking into the adjacent arcuate nucleus or the cerebrospinal fluid

(CSF) and to restrict movement of blood-borne molecules traversing the arcuate from entering the median eminence (48,49), it is now believed that tanycytes have much more complicated functions and likely have an active role in endocrine regulation (50). The fact that tanycytes are in direct contact with the CSF and have large, 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, ferritin, and [3 H]dopamine injected into the cerebrospinal fluid are avidly concentrated by tanycytes (51,52). In addition, protrusions at the apical surface of tanycytes connected by a narrow pedicle have suggested that tanycytes can extrude substances into the cerebrospinal fluid (53). Furthermore, horseradish peroxidase in-

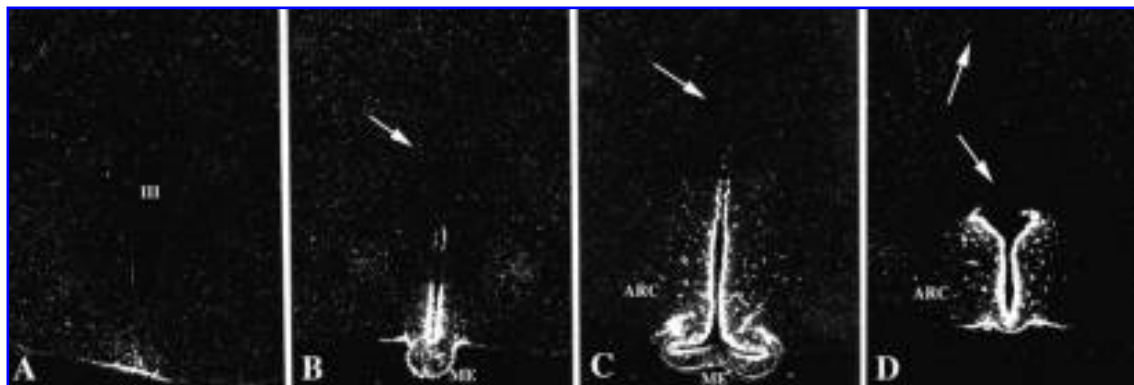


FIG. 1. Rostral–caudal distribution of D2 mRNA in the rat mediobasal hypothalamus by *in situ* hybridization histochemistry. Note hybridization in the floor and infralateral walls of the third ventricle (III), in the arcuate nucleus (ARC) surrounding blood vessels and in the median eminence (ME). The absence of hybridization in dorsal portions of the third ventricle is denoted by the arrow. (Reprinted from Tu et al. [25], with permission from The Endocrine Society.)

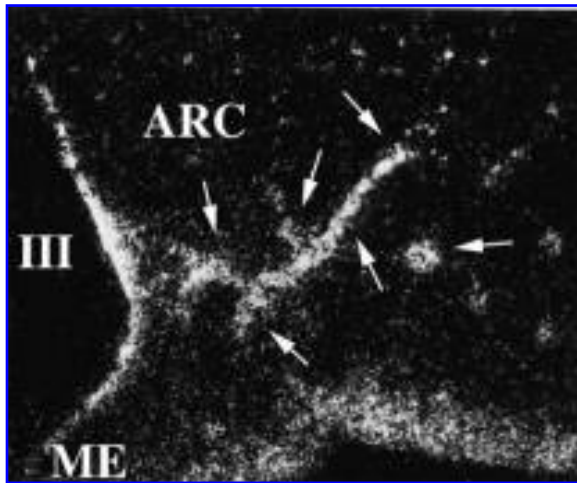


FIG. 2. High-power darkfield photomicrograph showing D2 mRNA in tanycyte processes surrounding blood vessels (arrows) in the arcuate nucleus. ARC, arcuate nucleus; ME, median eminence; III, third ventricle.

jected into the bloodstream and apolipoprotein A-IV, a circulating lipoprotein, accumulate in tanycytes (54,55), suggesting that tanycytes are also capable of extracting and concentrating specific substances directly from the bloodstream. Thus, tanycytes may provide a bidirectional, cytoplasmic conduit between the CSF and the vascular elements in the arcuate nucleus and/or the median eminence, allowing the movement of substances from one compartment to the other.

In addition to tanycytes, D2 is also expressed in astrocytes within the hypothalamus (35,56), more characteristic of the expression of D2 in other regions of the brain such as the cerebral cortex (27,35). Using immunocytochemical techniques to identify D2-producing cells in the hypothalamus, Diano et al. (35) have shown D2 immunostaining in astrocytes in the arcuate nucleus. Similar to observations made by *in situ* hybridization histochemistry, no D2 immunostaining has been identified in neurons.

A moderate amount of D3 mRNA can be detected throughout the hypothalamus in the adult animal using *in situ* hybridization, with the exception of the supraoptic nucleus, where in some hyperthyroid animals, the level of D3 mRNA just reaches detection limit (21).

Developmental Changes of Deiodinase Activity in the Hypothalamus

Very little information is known about the developmental changes in deiodinase expression or activity in the hypothalamus. D2 mRNA is present in cell clusters throughout the hypothalamus of E8-D15 chicken embryos, but the cell type is unknown (39). No specific hybridization is seen in the wall of the third ventricle at this age, whereas in the adult, D2 mRNA is decreased throughout the hypothalamus but a strong hybridization signal is present in the wall of the third ventricle between the rostral pole of the median eminence and the mammillary recess (39).

D3 mRNA is differentially expressed in the neonate and adult rat hypothalamus (22). At birth, D3 mRNA is present in the bed nucleus of the stria terminalis and hypothalamic medial and median preoptic nuclei. High D3 levels in these regions have been proposed to protect the normal process of sexual differentiation of the brain from high T_3 levels (see below). By postnatal day 10, however, the distribution of D3 mRNA becomes diffuse and more characteristic of the adult (22).

Role of Hypothalamic Deiodinase in Feedback Regulation of Hypophysiotropic TRH Neurons

The origin of thyrotropin-releasing hormone (TRH)-containing neurons that regulate anterior pituitary TSH secretion is the hypothalamic paraventricular nucleus (PVN). TRH-synthesizing neurons are found primarily in the anterior, medial, and periventricular parvocellular subdivisions, but only those in medial and periventricular parvocellular subdivisions project to the median eminence (often referred to as hypophysiotropic neurons as they are involved in regulation of the anterior pituitary) (57,58). These neurons are under inverse feedback regulation by circulating levels of thyroid hormone (59–62). 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 (63–65). Conversely, increased circulating levels of T_4 cause marked suppression of pro-TRH mRNA in the PVN and a reduction in the secretion of TRH into the portal plexus (63,66), establishing an inverse relationship between thyroid hormone and the biosynthesis and secretion of hypophys-

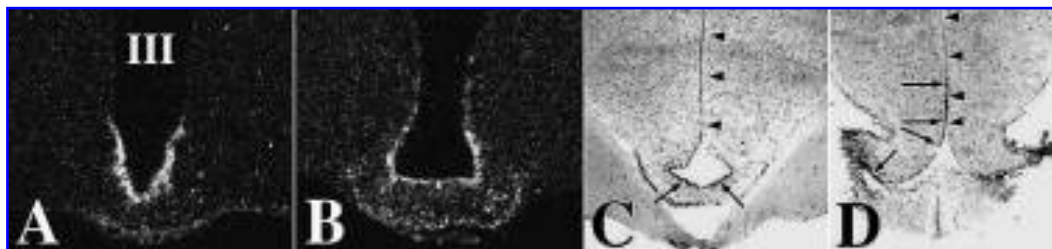


FIG. 3. Rostral-caudal distribution of D2 mRNA in the mediobasal hypothalamus of a normal mouse (A, B) and chicken (C, D) by *in situ* hybridization histochemistry. Note hybridization in the floor and infralateral walls of the third ventricle (III), and in the median eminence (ME). At the rostrocaudal 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 reprinted from Gereben et al. [39], with permission from The Endocrine Society.)

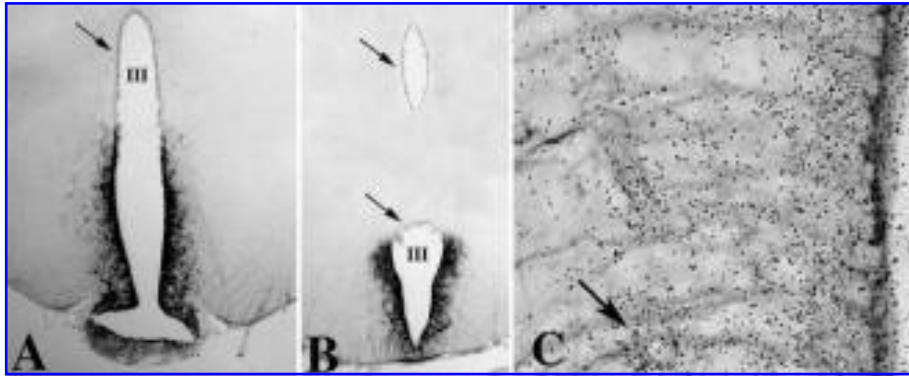


FIG. 4. (A and B): DARPP-32 immunostaining in caudal sections of the arcuate-median eminence area of a rat. Cell bodies of tanyocytes are located in the ependymal lining of the third ventricle but extend cytoplasmic processes into the substance of the arcuate nucleus (ARC) and median eminence (ME). Arrows denote absence of DARPP-32 immunostaining in the dorsal portions of the third ventricle (III). C: Colocalization of D2 mRNA (silver grains) and DARPP-32 (brown immunolabeling) in tanyocyte cell bodies lining the infralateral wall of the third ventricle and tanyocyte cytoplasmic processes. Arrow denotes colocalization of signals in a cytoplasmic process surrounding a blood vessel. (C reprinted from Fekete et al. [26], with permission from Elsevier Science.)

iotropic TRH. The increase or decrease in the amount of hypophysiotropic TRH secreted into the portal system for conveyance to the anterior pituitary as dictated by variations in circulating levels of thyroid hormones, is important to establish the setpoint for feedback regulation by thyroid hormone on anterior pituitary TSH secretion (67–69).

Because the PVN contains little, if any, D2 activity or D2 mRNA (25,27,38), and thereby is incapable of intracellular conversion of T_4 to biologically active T_3 , hypophysiotropic TRH neurons in the PVN must be dependent upon exogenous sources of T_3 . The possibility that feedback regulation by thyroid hormone on TRH neurons in the PVN is mediated exclusively by circulating levels of T_3 alone is unlikely because the systemic infusion of T_3 to hypothyroid animals that restore plasma levels of T_3 to normal, does not suppress pro-TRH mRNA levels in the PVN to euthyroid levels (60) (Fig. 5). Only after constant infusion with higher concentrations of T_3 that raise plasma T_3 into the supranormal range is there an apparent reduction in hybridization signal for pro-TRH mRNA to euthyroid levels. Thus, the calculated plasma level of T_3 required to suppress pro-TRH mRNA to normal in the absence of T_4 is approximately 1.6 times euthyroid levels (60), suggesting that like other regions of the brain, feedback regulation of thyroid hormone on TRH neurons in the PVN is partially dependent on the uptake of circulating T_4 into the brain and its conversion to T_3 .

One potential source of T_4 to T_3 conversion are the tanyocytes, lining the infralateral walls and base of the third ventricle (25,27). The location and high concentration of D2 mRNA and D2 enzymatic activity in these cells, places them in a strategic position to extract T_4 from the bloodstream by tanyocyte end processes terminating on portal capillaries or from blood vessels in the arcuate nucleus, or from the CSF *via* apical specializations after T_4 has traversed the choroid plexus (70). T_4 can then be converted to T_3 in the cytoplasm of tanyocytes, and released back into the CSF or bloodstream, thereby providing a source of T_3 to the central nervous system (CNS). The above mechanisms may explain the levels of free T_3 in the CSF that are equal to or exceed that in the systemic circulation in human subjects (71–74). T_3 released into

the CSF could diffuse into the substance of the brain by volume transmission (75), moving between ependymal cells lining the dorsolateral wall of the third ventricle that have poorly developed tight junctions (76), and provide a source of T_3 to hypophysiotropic TRH neurons in the hypothalamic PVN (25). Alternatively, tanyocytes may release T_3 into the median eminence for uptake and retrograde transport by axon terminals of hypophysiotropic TRH neurons, or into the arcuate nucleus through tanyocyte-neuronal interactions, where it could influence the activity of arcuate neurons that have known projections to TRH neurons in the PVN (77,78). A similar paracrine mechanism has been hypothesized by Guadano-Ferraz et al (27) to take place in the cerebral cortex, whereby after conversion of T_4 to T_3 in astrocytes, locally released T_3 can regulate neurons involved in specific cortical functions. 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. (56).

Hypothalamic Deiodinase and the Nonthyroidal Illness Syndrome

Fasting and infection alter the normal mechanisms of feedback regulation by circulating levels of thyroid hormone on hypophysiotropic TRH. Under these circumstances, there is a decrease in thyroid hormone levels in the peripheral blood, but a seemingly paradoxical reduction of pro-TRH 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 (79–89). Thus, the normal feedback mechanism described previously would appear to be overridden, and a state of central hypothyroidism is induced, commonly referred to as nonthyroidal illness or the sick euthyroid syndrome in humans (90). By reducing thyroid thermogenesis and preserving nitrogen stores (84,91,92), this mechanism is presumed an important adaptive response to reduce energy expenditure until the adverse stimulus has been removed.

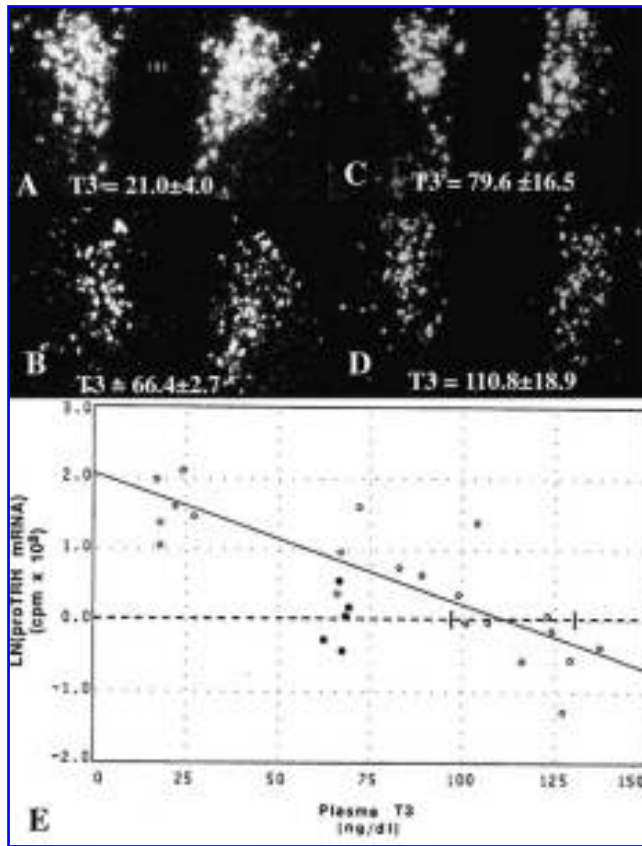


FIG. 5. *In situ* hybridization autoradiographs of pro-thyrotropin-releasing hormone (pro-TRH) TRH mRNA in the periventricular nucleus (PVN) in (A) hypothyroid, (B) euthyroid, and hypothyroid animals receiving a constant infusion of (C) 0.5 μ g or (D) 0.75 μ g triiodothyronine (T_3)/100 gm BW/d. Mean plasma T_3 levels (\pm standard error of the mean [SEM]) are shown for each group at the bottom of the photomicrographs. Note that only the higher dose of T_3 that raise plasma T_3 levels into the supranormal range is capable of suppressing TRH mRNA to euthyroid levels. E: Regression analysis of above experiment. Interrupted line represents the mean $\ln(\text{TRHmRNA})$ for euthyroid animals and its intercept with the regression line estimates the plasma T_3 concentration required to suppress pro-TRH mRNA to euthyroid levels. Ninety-five percent confidence intervals for each intercept are bracketed. Open dots denote values for hypothyroid animals and hypothyroid animals infused with graded doses of T_3 . Closed dots denote values for euthyroid controls. (Reprinted from Kakucska et al. [60], with permission from The Endocrine Society.)

Fasting

On the basis of studies from several laboratories, it is clear that the state of central hypothyroidism induced by fasting is primarily orchestrated by a circulating protein, leptin, and if leptin is administered exogenously to fasting animals, the reduction in circulating levels of thyroid hormone, TSH, and hypophysiotropic pro-TRH mRNA in the PVN can be prevented (87,93). The actions of leptin are mediated both directly on the PVN (94,95), and via the hypothalamic arcuate nucleus through an arcuate-PVN monosynaptic pathway. The latter mechanism utilizes two anatomically distinct pop-

ulations of neurons with opposing functions, α -MSH-producing neurons that coexpress CART, and the NPY-producing neurons that coexpress AGRP (96–99), ultimately lowering the set point for feedback sensitivity of pro-TRH-producing neurons in the PVN to thyroid hormone when leptin levels are suppressed during fasting.

Food restriction also has an effect to increase hypothalamic D2 activity and D2 mRNA (33,100). In a recent study by Diano et al (33), an approximately 2-fold increase in D2 mRNA in the mediobasal hypothalamus and 1.6-fold increase in D2 activity was observed in fasted animals compared to fed controls. These increases were unaffected by the administration of levo-thyroxine, despite substantial elevations in circulating levels of T_4 . On the basis of these observations, Diano et al. (33) have proposed that the increase in D2 in the hypothalamus in association with fasting may increase local tissue T_3 , ultimately contributing to the suppression of TRH production and release. Pascual-Leone et al. (101) have also observed a modest rise in D2 activity in the hypothalamus of neonatal rats subjected to a chronic, 35% food restricted diet, but found the content of T_3 and T_4 in extracts of whole hypothalamus to be reduced.

Cachexia and infection

While the reduction in circulating leptin levels and alteration in arcuate nucleus peptides contribute to fasting-induced suppression of the HPT axis, this mechanism does not appear to explain suppression of the HPT axis in the nonthyroidal illness syndrome associated with cachexia and infection. As opposed to reduced melanocortin signaling associated with fasting, substantial evidence would suggest increased melanocortin signaling in animal models of cachexia and following the administration of bacterial lipopolysaccharide (LPS) (102,103). Thus, antagonists of the MC4 receptor including SHU-9119 and AGRP, partially reverses the anorexia caused by LPS and spares lean body mass in animals bearing tumors (103,104). In addition, endotoxin increases circulating levels of leptin (105) and CART mRNA in the arcuate nucleus (106), does not increase NPY or AGRP expression (106), and increases norepinephrine release (107), despite suppression of TRH mRNA in the PVN (82). Paradoxically, each of these alterations would be expected to activate TRH gene expression in hypophysiotropic neurons rather than result in suppression as observed (78). Presumably, therefore, more potent inhibitory regulatory controls over the HPT axis that supercede the stimulatory action of each of these substances on hypophysiotropic TRH must come into play under these conditions. This overriding effect does not appear to be due to increased level of glucocorticoids, because adrenalectomized animals replaced with corticosterone to simulate normal basal levels, still show suppression of TRH and TSH mRNA levels following LPS (108).

Recent studies by Fekete et al. (109) have demonstrated that D2 mRNA and D2 activity in the rat mediobasal hypothalamus is increased approximately fourfold by immune activation. Following an intraperitoneal injection of bacterial LPS, D2 activity in the mediobasal hypothalamus shows an early maximal response at 12h followed by a decline at 24 hours (Fig. 6). Similar observations have been made by Boelen et

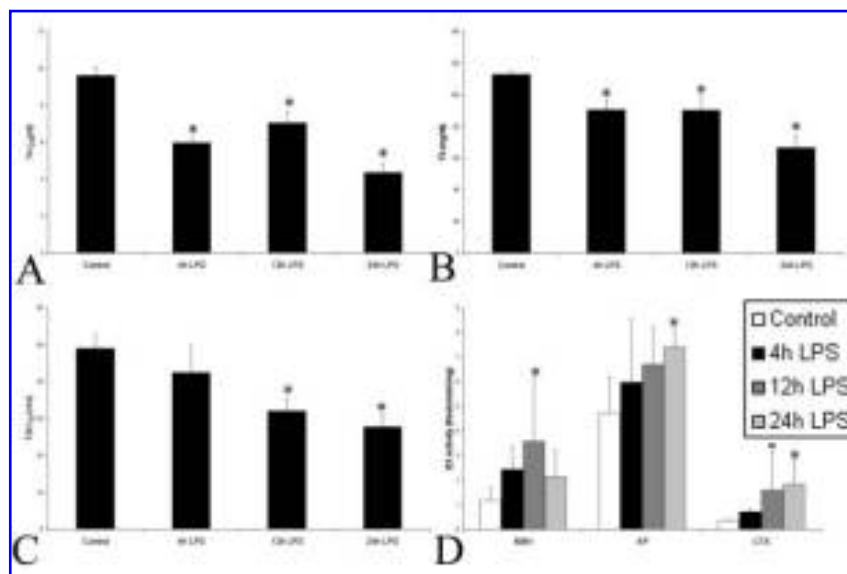


FIG. 6. Effects of intraperitoneal bacterial lipopolysaccharide (LPS) administration (250 $\mu\text{g}/100\text{g BW}$) on (A) thyroxine [T_4], (B) triiodothyronine (T_3) and (C) thyrotropin (TSH) levels and (D) type 2 iodothyronine deiodinase (D2) activity in the mediobasal hypothalamus (MBH), anterior pituitary (AP), and cerebral cortex (CTX) of Sprague-Dawley rats. Plasma T_3 , T_4 , and TSH levels are lowest at 24 hours after LPS treatment. While D2 activity in the cortex and anterior pituitary shows a steady linear increase over time, peak activity in the MBH occurs 12 hours after LPS administration, prior to the maximal decline in thyroid hormone levels. * $p < 0.05$ compared to baseline. (Reprinted from Fekete et al. [109], with permission from The Endocrine Society.)

al (110) in the mouse hypothalamus. The increase in D2 activity in the mediobasal hypothalamus occurs primarily in tanycytes (Fig. 7), which by semiquantitative *in situ* hybridization histochemistry and real-time polymerase chain reaction (PCR), increases up to 400% compared to control animals (109). Because the D2 response substantially exceeds that induced by even severe hypothyroidism (25), it is most likely not mediated by the decrease in circulating thyroid hormone levels. In addition, replacing circulating levels of thyroid hormone to normal in LPS-treated animals with exogenously administered T_4 has no effect on LPS-induced increase in D2 or D2 mRNA in the mediobasal hypothalamus, although T_4 prevents the endotoxin-induced rise in D2 in the cerebral cortex (personal observations). D3 expression in the mediobasal hypothalamus is low after LPS administration, and not significantly different than control animals (110).

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 (111) and express macrophage migration inhibitory factor (MIF) (112), 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- κB signaling, as the promoter of the D2 gene (*dio2*) contains multiple putative NF- κB binding sites (109). In addition, coexpression of p65 (RelA), a required component of the activated NF- κB heterodimer, together with a 6.5-kb human *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 *hdio2* promoter, that can be abolished by truncation of the promoter (Fig. 8). This would be in keeping with evidence that NF- κB is activated in the me-

dian eminence and wall of the third ventricle after LPS administration (111), although the precise cell types have not yet been identified.

It is possible, therefore, that after LPS administration, TNF- α derived from local macrophages in the external zone of the median eminence, perhaps recruited to this region by the release of chemotactic factors from tanycytes or local endothelial cells, or derived from the peripheral blood, may bind to TNF (p55) receptors on tanycytes, ultimately leading to the activation of D2 through activation of NF- κB . Alternatively or in addition, LPS may induce CD14 receptors in tanycytes (113) and either activate NF- κB directly and/or increase the expression of p55 receptors, potentiating the effect of locally released TNF- α . 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- κB activation (114), 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 (115,116).

The potential role of interleukin (IL)-1 β , IL-6, leptin, and other proinflammatory cytokines induced by LPS or TNF- α , either locally or present in the circulation that signal through gp130 receptor complexes (such as ciliary neurotrophic factor [CNTF], LIF (leukemia inhibitory factor), IL-11, oncostatin M, others) is not known, but may also contribute to transduction signals in tanycytes by the activation of NF- κB , STATs or other second messenger systems (117). This is based on the observation that in addition to NF- κB binding sites, we have also identified putative STAT binding sites in the *dio2* gene (personal observations). Thus, tanycytes 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 tis-

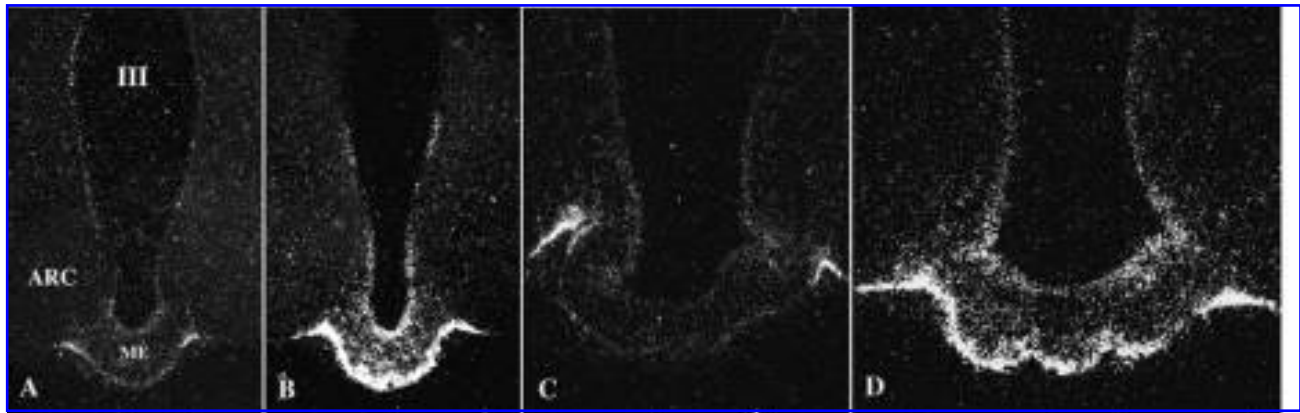


FIG. 7. Low-power darkfield micrographs from two different rostro-caudal levels of the median eminence (ME) showing the effect of lipopolysaccharide (LPS) treatment on type 2 iodothyronine deiodinase (D2) gene expression the mediobasal hypothalamus. (A and C) Controls. (B and D) LPS-treated animals. The density of silver grains denoting D2 mRNA is markedly increased after LPS, particularly in the external zone of the ME.

sue-specific D2-mediated thyrotoxicosis in the mediobasal hypothalamus caused by increased T_4 to T_3 conversion by tanycytes. Similar to that described previously, the increase in T_3 may suppress the synthesis of TRH in hypophysiotropic neurons either by local feedback inhibition through the release of T_3 from tanycyte apical processes into the CSF, or uptake from hypophysiotropic TRH axonal processes in the median eminence and retrograde transport to the hypothalamic PVN. T_3 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 Figure 9.

Hypothalamic Deiodinase and Appetite Regulation

It is well recognized that thyrotoxicosis is associated with

increased appetite (100,118–120), although the mechanism(s) by which this occurs have been uncertain. Reports on the effects of thyroid hormone on leptin have been inconsistent (121–127), but most suggest a small reduction, possibly secondary to the effects of hyperthyroidism to reduce fat accumulation in white adipose tissue (127). Along these lines, Ishii et al. (128) have shown an increase in hypothalamic NPY mRNA after the administration of thyrotoxic doses of T_3 , as might be expected if circulating leptin levels were suppressed. Nevertheless, T_3 -associated hyperphagia is only partially attenuated by a NPY receptor antagonist, indicating that other mechanisms must also be operable.

Recent studies by Kong et al. (100) have demonstrated that the systemic administration of superphysiologic doses of T_3 that still maintain free T_3 levels within the normal range and have no effects on plasma leptin levels, cause weight loss or increased locomotor activity, increase food intake and in-

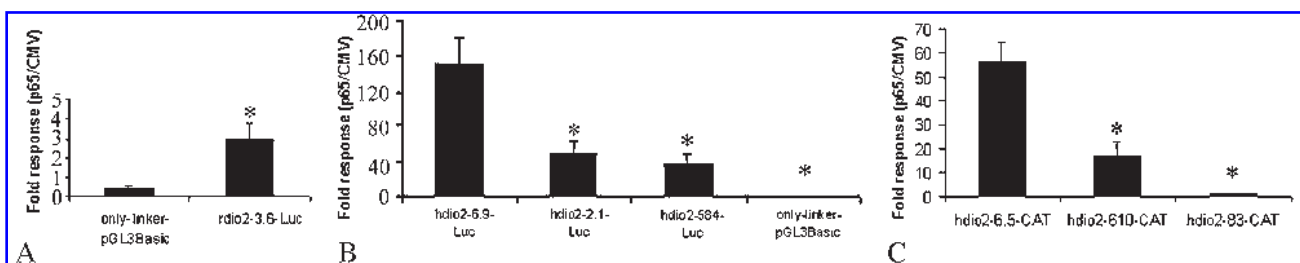
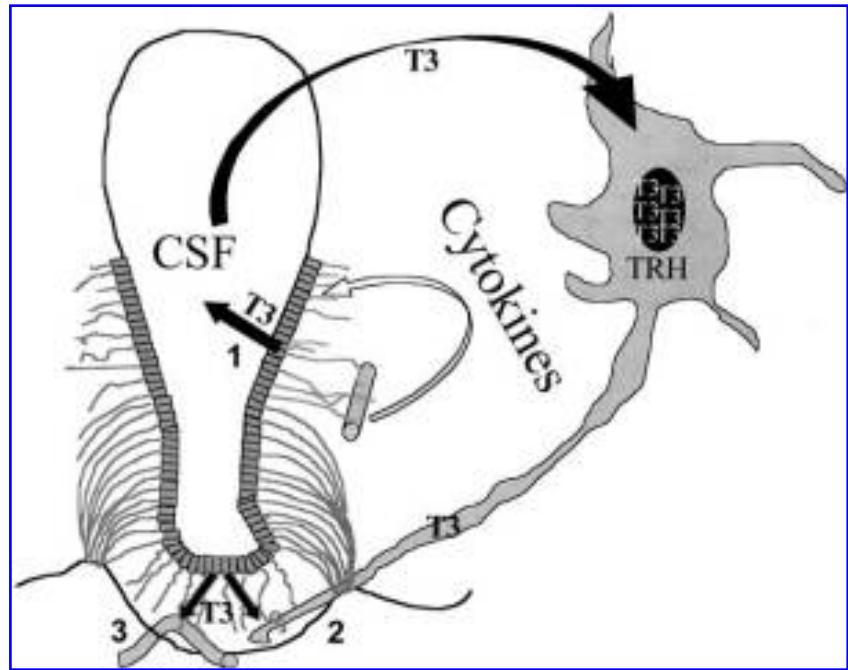


FIG. 8. Effect of the p65 NF- κ B subunit on the activity of the rat and human *dio2* promoter in HC11 and HEK cells. Results are shown as the ratio of reporter expression (corrected for transfection efficiency) in the presence and absence of p65 (p65/CMV). The ratios in experiments using the *Renilla* construct were divided by 3 to correct for the suppression of SV40-*Renilla* by p65. **A:** The *rdio2*-3.6-Luc construct containing approximately 3.6 kb rat *dio2* 5'-flanking region (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 approximately 6.9 kb of the *hdio2* 5'-FR), *hdio2*-2.1-Luc (containing the 3' approximately 2.1 kb of the *hdio2* 5'-FR), and *hdio2*-584-Luc (containing the 3' approximately 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 approximately 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 standard error of the mean (SEM) of at least 4 separate experiments based on Luc/*Renilla* or CAT/hGH ratios. (Reprinted from Fekete et al. [109], with permission from The Endocrine Society.)

FIG. 9. Proposed mechanism for type 2 iodothyronine deiodinase (D2) regulation of the hypothalamic-pituitary-thyroid axis after the administration of lipopolysaccharide (LPS). LPS increases D2 activity in tanyocytes resulting in increased thyroxine (T_4) to triiodothyronine (T_3) conversion. [1] T_3 is released from tanyocyte apical processes into the cerebrospinal fluid (CSF) for conveyance to the paraventricular nucleus, or [2] taken up from hypophysiotropic thyrotropin-releasing hormone (TRH) axonal processes in the median eminence and transported retrogradely back to its cell body in the paraventricular nucleus. [3] T_3 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.



crease immediate early gene expression in the hypothalamic ventromedial nucleus. In addition, microinjection of T_3 into the hypothalamic ventromedial nucleus but not the arcuate nucleus induces a fourfold increase in food intake during the first hour after injection. These data argue against changes in leptin or arcuate nucleus NPY as mediators of T_3 -induced hyperphagia, but rather suggest a direct effect of T_3 on the hypothalamus to induce feeding. Evidence that the nuclear T_3 concentrations in the hypothalamus are elevated after the systemic administration of thyrotoxic doses of T_3 or T_4 (14) further substantiates this hypothesis. The coincidence that rats display a nocturnal pattern of feeding and that hypothalamic D2 activity follows a circadian pattern that increases during the night that may have the effect of increasing local tissue levels of T_3 (100,129), provides circumstantial evidence for the potential importance of hypothalamic D2 to regulate feeding.

Hypothalamic Deiodinase and Reproductive Function

Photoperiod regulation

Photoperiodic control of reproduction is an important adaptive mechanism observed in many temperate zone animal species as a way to avoid giving birth during the harshest portions of the seasons. Thus, in some species, exposure to short photoperiods results in gonadal involution, reduced spermatogenesis and ovulation, and reduced mating behavior, whereas exposure to long photoperiods has the opposite response (130). While melatonin is the principal endogenous mediator of day length on reproduction in mammals that are seasonal breeders (131), there is considerable evidence that thyroid hormone is also of importance in mammals and of critical importance in birds. In the Japanese quail, for example, the peripheral administration of T_4 (132) or intracerebroventricular infusion of T_3 (133) can induce testicular growth and gonadotropin secretion, even though the animals are exposed to short photoperiods. Of interest, a sig-

nificant increase in D2 mRNA is found in the mediobasal hypothalamus of the Japanese quail when exposed to long photoperiods compared to D2 mRNA associated with short photoperiods. In addition, exposure to long photoperiods is associated with a dramatic rise in the content of T_3 in the mediobasal hypothalamus but not other parts of the brain (133). Further support for the importance of D2 in transducing photoperiod regulation of reproductive function is that administration of the D2 inhibitor, iopanoic acid, can reduce testicular growth under long photoperiod conditions (133). Presumably, therefore, the ability of thyroid hormone to induce seasonal reproductive changes in the Japanese quail is dependent upon upregulation of D2 in the mediobasal hypothalamus, mediated by changes in the light/dark cycle. Similar photoperiodic regulation of D2 mRNA in the mediobasal hypothalamus has been observed in the Djungarian hamster (134).

Thyroid hormone is also important for reproductive function in sheep, although contrary to the effects observed in the Japanese quail, the action of thyroid hormone is primarily to terminate the breeding season by causing anestrus. The breeding season in sheep occurs in the fall when days are short, followed by a prolonged anestrus period that begins as day length increases in the winter until the following fall (135). Reduction in thyroid hormone levels either by thyroidectomy or the administration of propylthiouracil, allows the animals to remain cyclic by preventing the seasonal increase in responsiveness to the negative feedback action of estradiol on episodic gonadotropin-releasing hormone (GnRH) secretion (136-139). This action of thyroid hormone occurs directly in the brain, as the central infusion of low doses of T_4 that restore physiologic concentrations of the hormone in the cerebrospinal fluid of thyroidectomized sheep at the end of the breeding season, or microimplants of T_4 directly into the preamillary region, allow the animals to enter anestrus (140,141). While it is unknown whether this

regulatory mechanism is dependent upon seasonal variations in deiodinase activity in the mediobasal hypothalamus of the sheep, it is intriguing to note that in the hamster, D2 expression can be inhibited by melatonin (134). Because melatonin has an important role in mediating the effects of day length on reproductive function (131), it is possible that D2 may also be secondarily affected by photoperiod changes in the sheep.

Sexual differentiation and lordosis behavior

The possibility that hypothalamic deiodinase contributes to sexual differentiation of the brain is hypothetical, and based on observations by Escamez et al. (22) that D3 mRNA is transiently expressed in high concentrations in preoptic area and bed nucleus of the stria terminalis during a critical time of brain development in the early postnatal period. High levels of thyroid hormone have been shown to antagonize the effects of estradiol either as a result of competition of liganded thyroid hormone receptors and estrogen receptors for binding to the same DNA sequences in estrogen-regulated genes, or due to squelching by either receptor for coactivators, such as SRC-1, that are essential for the action of each hormone (142). Escamez et al. (22) propose, therefore, that regional expression of D3 mRNA in the preoptic region may prevent T₃ from antagonizing the action of estrogen on the hypothalamus during this critical time in postnatal development, ultimately permitting normal sexual differentiation of the brain.

It is of further interest that cold exposure, which is associated with both an increase in circulating thyroid hormone levels and an increase in D2 activity in the hypothalamus (31,143,144), also reduces lordosis behavior in Syrian hamsters and mice (145,146). Lordosis behavior is mediated primarily in the hypothalamic ventromedial nucleus, and dependent upon the action of estradiol on estrogen receptors that are highly expressed in this nucleus (147,148) together with thyroid hormone receptors (149). Because the lordosis response can be attenuated by thyroid hormone (150,151) and the ventromedial nucleus is in proximity to D2-producing astrocytes and tanycytes (25,27), it is possible that D2 may have a role in modulating sexual responsiveness under this particular aversive circumstance by increasing local tissue levels of T₃.

Conclusions

The deiodinating enzymes are of critical importance for the normal development and function of the central nervous system. Because D1 is absent from the human brain, D2 and D3 probably have the greatest functional importance by maintaining constant, local tissue levels of T₃. The role of deiodinases in the hypothalamus may be more complex, however, as suggested by the unique localization of D2 primarily to tanycytes in the floor and infralateral walls of the third ventricle. These special glial cells integrate hormonal and probably neuronal signals, and under specific conditions, may influence neuroendocrine functions by altering local T₃ tissue concentrations. This function may be of particular importance in the regulation of the HPT axis during fasting and infection, and in the regulation of appetite and reproductive function. Transient expression of D3 in the pre-

optic region during a critical time of development suggests a special role for this deiodinase in sexual differentiation of the brain.

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