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Short communication

Hypothalamic type II iodothyronine deiodinase: a light and electron microscopic study

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Abstract

In the central nervous system, the active form of thyroid hormone, T3, derives from the cellular uptake and intracellular 5′-monodeiodination of T4 by type II 5′-monodeiodinase (DII). Here, we report that using an antiserum raised against the C-terminus of the full-length SeDII, immunolabeled cells were found in the rat hypothalamus in agreement with the DII mRNA distribution. Light and electron microscopy shows that DII is localized in astrocytes and tanycytes, supporting the hypothesis that these cells play an important role in the mediation peripheral signals, such as thyroid hormones, on hypothalamic functions.

Theme: Endocrine and autonomic regulation

Topic: Neuroendocrine regulation: other

Keywords: Deiodinase; Hypothalamus; Immunocytochemistry

Thyroid hormones are important for embryogenesis and remain critical in the appropriate regulation of cellular metabolism in central and peripheral tissues throughout life. Of great importance is the action of triiodothyronine (T3) in the hypothalamic feedback effect of the thyroid gland on the hypothalamic thyrotropin-releasing hormone (TRH)-producing cells [7,10,16]. During failure of the thyroid gland, declines in circulating thyroxine (T4) and T3 levels lead to the elevation in the production and release of TRH in the hypothalamic paraventricular nucleus (PVN), which, in turn, acts on thyroid-stimulating hormone (TSH)-producing cells of the anterior pituitary to induce the thyroid gland to increase the production of T4 and T3.

Hypothyroidism leads to a change in the activity of iodothyronine deiodinases, enzymes that are necessary for activation and inactivation of circulating thyroid hormones. Deiodinase type II is present in the central nervous system [14,18], pituitary [19], brown adipose tissue [17], and placenta [11], and shows increased activity when plasma T4 declines. It has been suggested that the major role of type II deiodinase is to maintain T3 homeostasis producing adequate intracellular levels of T3 in order to ensure all T3-dependent cellular functions in the tissue [4,12]. In fact, Escobar-Morreale et al. [8] demonstrated that in hypothyroid adult rats, the increased activity of type II deiodinase is able to normalize T3 levels in the brain and other tissues after infusion of T4 at doses insufficient to adjust plasma T4 and T3 levels.

A previous report [14] indicated the lack or minimal activity of DII in the PVN where hypophysiotropic TRH neurons are located and a higher activity in the hypothalamic arcuate nucleus/median eminence region (ARC/ME) of adult rat brain. The appearance of DII mRNA in the ependymal zone and the ME [5,6,18], together with our previous observation of glial fibrillary acidic protein (GFAP) in cells expressing DII mRNA [6] and an earlier report [9] on the expression of DII in glial cells in neonatal rat brain strongly indicate that DII producing cells are astrocytes and tanycytes of which communication with

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local neurons is critical in the regulation of TRH. Thus, to determine the anatomical proximity between DII producing glial processes and neurons becomes quintessential. For that, it is necessary that DII protein can be visualized by the means of immunocytochemistry. In this study, in an attempt to determine the subcellular localization of DII in brain cells, we used polyclonal antisera against the C-terminus of the full-length DII for immunocytochemistry and compared the results with DII mRNA distribution.

Five male, Sprague–Dawley rats (200–250 g BW) from Taconic Farms (Germantow, NY, USA) were used in this study. Animals were kept under standard laboratory conditions, with tap water and regular rat chow ad libitum, 12-h light–12-h dark cycle, at a temperature of 21–23 °C. Animals were killed under ether anesthesia by trans-aortic perfusion with heparinized saline, followed by 250 ml fixative (4% paraformaldehyde, 0.08% glutaraldehyde and 15% saturated picric acid, in 0.1 M phosphate buffer (PB), pH 7.35). Brains were removed from the skull and a tissue block containing the entire hypothalamus was dissected out and postfixed for an additional 2 h in glutaraldehyde-free fixative at 4 °C. After fixation, the tissue blocks were rinsed several times in PB and, 60-μm thick coronal vibratome (Lancer, St. Louis, MO, USA) sections were cut

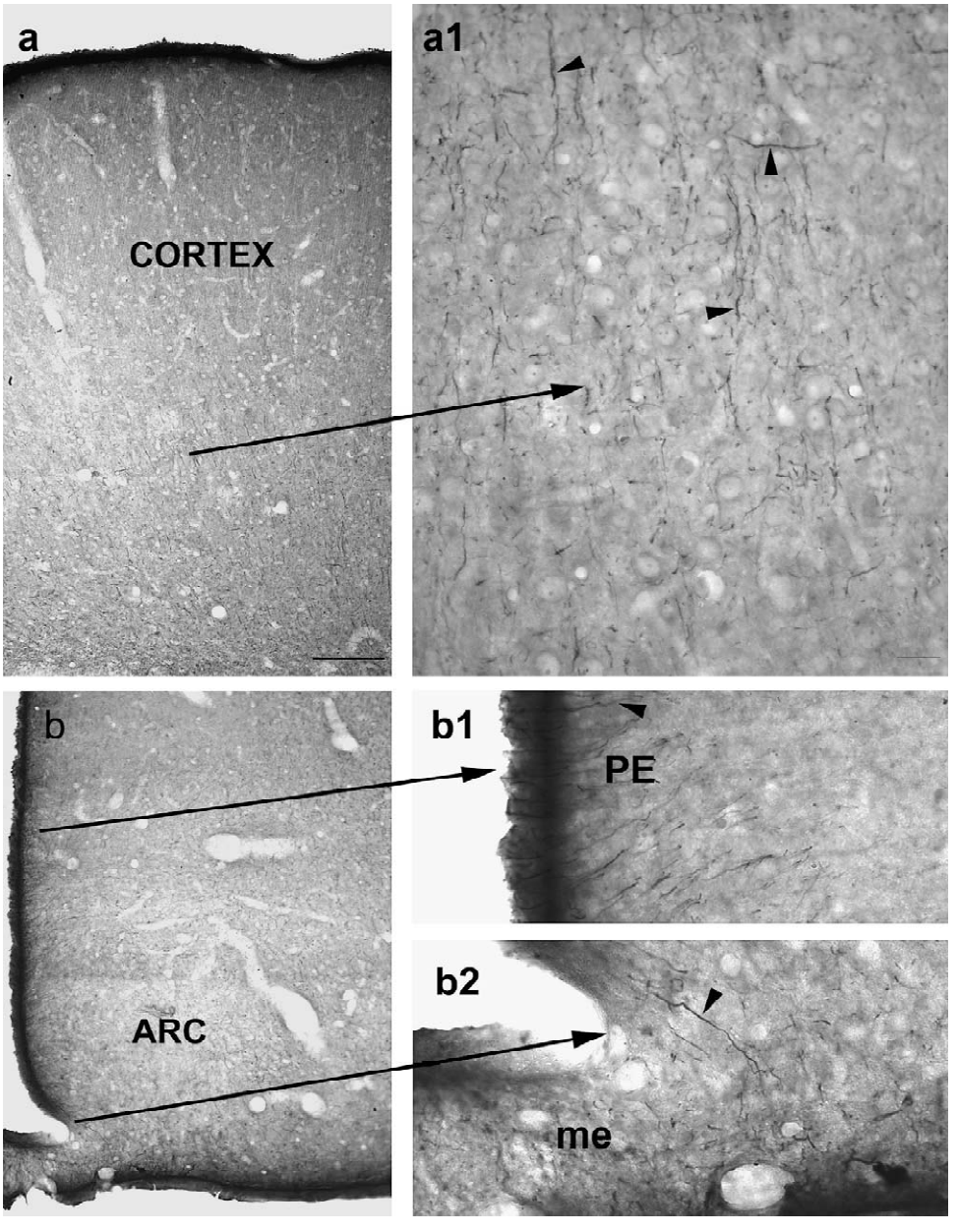

Fig. 1. (a) Immuolabeling for DII resulted in staining of astrocytic processes in all layers of the cerebral cortex; (a1) higher magnification of the area indicated by arrow in (a). (b) In the hypothalamus, DII immunopositivity was associated with astrocytes particularly tanycytes of the arcuate nucleus; (b1, b2) higher power magnification images of the periventricular area (PE; b1) and ARC (b2) indicated by arrows in (b). Arrowheads in (a1), (b1) and (b2) point to DII immunolabeled processes. Bar scale on (a) represents 100 μm for (a) and (b), on (a1) represents 10 μm for (a1), (b1) and (b2).

and rinsed 4×15 min in PB. Sections were washed for 30 min in 1% H_2O_2, rinsed several times in PB and incubated for 10 min in 1% sodium borohydride to eliminate unbound aldehydes.

The brain sections were incubated with rabbit anti-DII (1:500 in PB-Triton X-100) raised against the C-terminus of the full-length protein (anti-SeDII-long) overnight at room temperature. Detailed characterization of this antiserum can be found elsewhere [13]. The specificity of the primary antiserum was thoroughly tested previously [13]. After several washes with PB, sections were incubated in the secondary antibody (biotinylated goat antirabbit IgG; 1:250 in PB; Vector Labs., Burlingame, CA, USA) for 2 h at room temperature, then rinsed in PB three times 10 min each time, and incubated for 2 h at room temperature with avidin–biotin–peroxidase (ABC, 1:50 in PB; ABC Elite Kit, Vector Labs.). The immunolabeling was visualized with a modified version of the nickel-diaminobenzidine (Ni–DAB) reaction (15 mg DAB, 0.12 mg glucose oxidase, 12 mg ammonium chloride, 600 μl 0.05 M nickel ammonium sulfate, and 600 μl 10% β-D-glucose in 40 ml PB) for 10–30 min at room temperature resulting in a dark blue reaction product. When the primary antiserum was omitted, no immunoreactivity was found in the brain.

For electron microscopy, section were postosmocated (1% OsO_4 in PB) for 30 min, dehydrated trough increasing ethanol concentrations (using 1% uranyl acetate in the 70% ethanol, 30 min) and flat embedded in Araldite between liquid release (Electron Microscopy Science, Fort Washinghton, PA, USA) coated slides and coverslips, and placed in the oven to polymerize for 48 h at 60 °C. Flat embedded section were fixed with a drop of embedding medium on the top of cylindrical Araldite blocks and cured again for 48 h at 60 °C. Ribbons of ultrathin sections (Reichert-Jung Ultramicrotome) were collected on Formvar-coated single slot grids, and examined using a Philips CM-10 electron microscope.

Immunostaining using the antiserum raised against the C-terminus of SeDII and previously characterized [13], showed the same pattern of distribution of DII mRNA [5,6,18]. DII immunoreactivity was widely distributed in the rat brain including the cerebral cortex and hypothalamus (Fig. 1).

In the cerebral cortex (Fig. 1a), numerous glial pro-

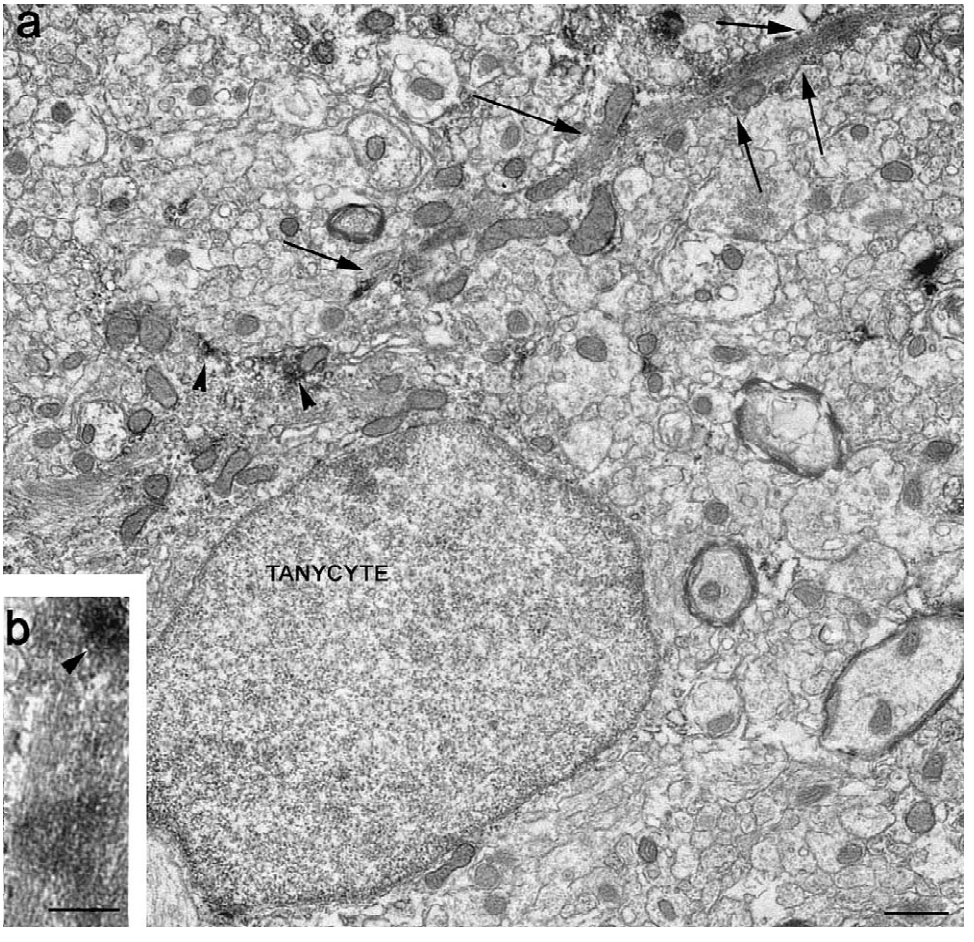

Fig. 2. (a) DII immunolabeled cell body and process (arrows) in the arcuate nucleus. Immunoperoxidase is associated with the cytosol (arrowheads). Bar scale represents 1 μm. (b) Higher power magnification of a tanycytic process in the arcuate nucleus. Note the association of immunolabeling with endoplasmic reticulum (arrowhead). Bar scale represents 0.5 μm.

cesses, present mostly on layers V and VI, were found immunolabeled for SeDII.

In the hypothalamus (Fig. 1b), the strongest immunolabeling was in the arcuate nucleus/median eminence and in the periventricular region of the third ventricle (Fig. 1b). Based on morphological characteristic, light microscopic examination showed that DII-immunolabeling was present in glial cells including astrocytes and tanycytes that provide an extensive network of cellular processes in the ARC [1–3,15]. In the median eminence, strongly immunolabeled glial processes were found around fenestrated vessels. In the arcuate nucleus, astroglia cells were found immunoreactive for SeDII, while in the periventricular area processes from tanycytes, arcing between the third ventricle and the basal surface of the brain and the portal vessels of the median eminence, were found immunopositive for this T3-activating enzyme. Electron microscopic survey confirmed that SeDII-immunoreactivity was associated with glial elements, including tanycytes, in the hypothalamus (Fig. 2). Electron microscopy showed that immunoperoxidase for SeDII was predominantly associated with the cytosol avoiding the nucleus. The patchy labeling most frequently was in the immediate vicinity of mitochondria or endoplasmic reticuli (Fig. 2).

Very few glial processes immunopositive for DII were found in the paraventricular nucleus of the hypothalamus, in agreement with the in situ hybridization as well as the activity studies.

The present results provide the first immunocytochemical evidence that SeDII is localized in brain cells. Light and electron microscopic analyses of DII immunolabeling showed staining in glial cells with no apparent association with neuronal profiles. Either astrocytic or tanycytic processes immunolabeled for SeDII were found in the hypothalamic arcuate nucleus and surrounding vessels in the median eminence while only few processes were present in the hypothalamic paraventricular nucleus (PVN), where thyrotropin-releasing hormone (TRH)-containing neurons are located. These results are in agreement with a previous study in which higher enzymatic activity for DII was found in the arcuate nucleus/median eminence fragment compared to the paraventricular nucleus of the hypothalamus [14] and, furthermore, completely overlap the distribution pattern of DII mRNA [5,6,18] by in situ hybridization.

Taken together, these observations establish that DII enzyme expression corresponds with DII mRNA expression thus revealing the specificity of our polyclonal antisera. Future immunocytochemical studies will be mandatory to determine the anatomical relationship between DII producing glial cells and other neurons to better understand the pleiotropic role of DII in brain functions.

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