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Dynamic Nongenomic Actions of Thyroid Hormone in the Developing Rat Brain

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Two well-characterized nongenomic actions of thyroid hormone in cultured brain tissues are: 1) regulation of type 2 iodothyronine 5′deiodinase (D2) activity and 2) regulation of actin polymerization. In particular, the latter is likely to have profound effects on neuronal migration in the developing brain. In this study, we determined whether these nongenomic actions also occurred *in vivo* during brain development. Neonatal hypothyroidism was induced by propylthiouracil given to pregnant dams beginning on d17 of gestation and continued throughout the neonatal period. On postnatal d 14, rats were injected with either cold or [^{125}I]-labeled iodothyronines and killed sequentially after injection. In contrast to reports in the adult rat, all three iodothyronines readily and equally entered developing brain tissues. As expected, cerebrocortical D2 activity was markedly elevated in the hypothyroid brain and both reverse T_3 (rT_3) and T_4 rapidly decreased D2 to euthyroid levels within 3 h. Furthermore, cerebellar G-actin content in the hypothyroid rat was approximately 5-fold higher than in the euthyroid rat. Again, both rT_3 and T_4 rapidly decreased the G-actin content by approximately 50%, with a reciprocal increase in F-actin content to euthyroid levels without altering total actin. Neither T_3 nor vehicle had any effect on D2 activity in the cortex or G- or F-actin content in the cerebellum. The thyroid hormone-dependent regulation of actin polymerization in the rat brain provides a mechanism by which this morphogenic hormone can influence neuronal migration independent of the need for altered gene transcription. Furthermore, these data suggest a prominent role for rT_3 during brain development. (*Endocrinology* 147: 2567–2574, 2006)

THYROID HORMONE has long been recognized as essential for regulation of the brain developmental program (1–7). The absence of thyroid hormone during the first 3 months of life in humans results in severe mental retardation and, in a rat model, irreversible morphological abnormalities in the brain (1–7). Adequate thyroid hormone replacement in the infant with congenital hypothyroidism instituted soon after birth before these abnormalities develop successfully prevents these morphological abnormalities and allows brain development program to progress normally (8, 9). Although the consequences of hypothyroidism on brain development are well recognized, the biochemical and molecular basis of the effects of this morphogenic hormone on neuronal integration remain elusive.

The major thyroid hormone produced by the thyroid gland is T_4, which is deiodinated in peripheral tissues to the transcriptionally active iodothyronine, T_3 (10, 11). T_4 is also deiodinated in peripheral tissues to the transcriptionally inert 3,3′,5′-triiodothyronine [reverse T_3 (rT_3)], which is the predominant iodothyronine produced during fetal life (12). The traditional model for thyroid hormone action has T_3 interacting with specific chromatin-bound ligand-activated transcription factors [thyroid hormone receptors (TRs)] (13, 14). However, the identification of developmentally important, T_3-responsive genes in the brain has been difficult at best and few, if any, obvious abnormalities in brain development have been observed in mutant mice lacking all known TRs (15, 16), suggesting that T_3-induced transcriptional regulation is not the sole contributor to the brain developmental program.

Nongenomic actions of the members of the steroid hormone super family are becoming increasingly important as mechanisms of hormone action (7, 17, 18). In contrast to T_3-dependent transcriptional actions that occur over hours to days, nongenomic actions of thyroid hormone typically occur in seconds to minutes and often involve T_4 as the ligand. Thus, for a nongenomic action to be relevant, the hormones must have rapid access to the tissue target of action. Nongenomic actions of thyroid hormone that have been described in brain cells include regulation of microfilament organization (19, 20) and regulation of type II iodothyronine 5′-deiodinase (D2) activity (21–26). In particular, thyroid hormone-dependent regulation of microfilament organization would likely have profound effects on the developing brain because the microfilaments are essential for the pathfinding and guidance of the migrating neurite (27–32).

We have described the nongenomic, thyroid hormone-dependent regulation of the organization of the actin cytoskeleton both in cultured rat astrocytes (19, 33, 34) and in cultured rat granular neurons (20). In astrocytes, approximately 90% of the cellular actin is polymerized and assembled into bundles of F-actin under euthyroid conditions. In the absence of thyroid hormone, the F-actin content falls by approximately 50% and the bundled microfilaments disappear; total cellular actin remains unchanged. T_4 and its metabolite, rT_3, are equipotent in rapidly (~10 min) promoting actin polymerization in astrocytes, returning the F-actin content to normal and restoring the stress fibers in the absence of transcription or translation. T_3 has no effect on actin po-

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Abbreviations: CNS, Central nervous system; D2, type II iodothyronine 5′-deiodinase; DTT, dithiothreitol; F-actin, filamentous actin; IOP, iopanoic acid; PTU, propylthiouracil; TR, thyroid hormone receptor.

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lymerization at equimolar concentrations. Similarly, the F-actin content in T_3-treated and hormone-free granular neurons is decreased by up to 35% compared with the T_4- or rT_3-treated cells, with no change in total cellular actin (20). Immunocytochemical analysis suggests that it is the F-actin that is found in the neuronal processes that is regulated by T_4 and rT_3 (20). These *in vitro* studies correlate with a report that the F-actin content in the cerebellum of 14-d-old hypothyroid rats is significantly decreased and that T_4 replacement for 2 d restores the F-actin content to normal (35).

In this study, we determined the time course of action of thyroid hormone on actin polymerization and D2 activity *in vivo* and correlated the alterations in actin polymerization and D2 activity with entry of iodothyronines into the developing rat brain. We show that T_4, T_3, and rT_3 all rapidly enter brain tissues after ip injection. Furthermore, we show that T_4 and rT_3, but not T_3, dynamically regulate actin polymerization and D2 activity in the developing brain.

Materials and Methods

Animals and reagents

Pregnant (16–17 d gestation) Sprague Dawley rats were obtained from Charles-River Labs (Kingston, NY). The study was approved by the Animal Research Committee and complies with the institutional assurance certificate of the University of Massachusetts Medical School. Neonatal hypothyroidism was induced by adding propylthiouracil (PTU) (2 mg/liter) to the drinking water of pregnant dams beginning at d 17 of gestation and continuing throughout the neonatal period.

Iodothyronines were obtained from Henning GmBH (Berlin, Germany), iopanoic acid (IOP) was obtained from Sterling Winthrop Research Institute and BSA was purchased from Sigma (St. Louis, MO). Antirabbit IgG-horseradish peroxidase conjugate was purchased from Promega (Madison, WI), rabbit polyclonal anti-actin IgG was purchased from Biomedical Technologies (Stoughton, MA), and Hybond ECL nitrocellulose was obtained from Amersham (Arlington Heights, IL). The Lumiglo chemiluminescent was obtained from Kirkegaard & Perry Laboratories (Gaithersburg, MD). All other reagents were of the highest grade available.

Tissue analysis of iodothyronines

[^{125}I]-labeled iodothyronines were synthesized by iodination of precursors with [^{125}I]Na obtained from NEN Life Science Products (Boston, MA) following established methods (36). The entry of iodothyronines into the brain was determined in euthyroid rat pups on postnatal d 14 by ip injection of [^{125}I]-labeled iodothyronine (20×10^6 cpm). Where indicated, animals were also injected ip with IOP (50 μg/10 g body weight), a potent inhibitor of 5′-deiodinase activity (11), to block T_4 to T_3 conversion. Animals were exsanguinated via the abdominal aorta and perfused with 10–20 ml iced 8 mM sodium phosphate (pH 7.4), 2.7 mM KCl, 137 mM NaCl, 1 mM IOP (PBS/IOP) at 0.5, 1, 3, and 6 h after injection. Blood samples obtained when the animals were killed were counted to determine the amount of [^{125}I]-labeled iodothyronine absorbed after injection. Cerebral cortex and cerebellum were harvested, washed in PBS/IOP, and frozen in liquid nitrogen and weighed. Iodothyronines were extracted and analyzed by HPLC according to established procedures (37, 38). In brief, tissues were homogenized in 4 vol methanol/1 mM IOP and then extracted with 8 vol chloroform. The upper phase was collected and the bottom phase was extracted again with chloroform:methanol (2:1). The two upper phases were combined and extracted with 0.05% $CaCl_2$. The aqueous phase was frozen, lyophilized, and resuspended in methanol. Samples were clarified by centrifugation, and the methanol was blown off under a nitrogen stream. Samples were then analyzed by HPLC and counted. The iodothyronine fractions were determined by parallel HPLC analysis of [^{125}I]-labeled iodothyronine standards subjected to the same extraction procedures.

Tissue harvest and hormone assays

On postnatal d 14 (P14), PTU-treated rat pups were injected ip with T_4, rT_3, T_3, or vehicle (1 M NaCl, 0.1% BSA, 100 μl volume) and animals were killed at 1, 3, and 6 h after injection. Where indicated, animals were also injected ip with IOP (50 μg/10 g body weight) to block T_4 to T_3 conversion. Control animals included postnatal d 14 euthyroid and PTU-treated animals and were killed at 0 and 6 h. In all experiments, animals were weighed, killed by decapitation and their blood collected for hormone assays. The cerebral cortex and cerebellum were rapidly isolated, washed in iced 8 mM sodium phosphate (pH 7.4), 2.7 mM KCl, 137 mM NaCl (PBS), frozen in liquid nitrogen, and kept at −70 C until use.

Serum TSH was measured in duplicate by RIA using materials obtained from the National Pituitary Agency, National Institutes of Health (Bethesda, MD). Serum T_4, T_3, and rT_3 were determined in duplicate by specific RIAs.

Actin analysis

Cerebellar samples were thawed and tissues were homogenized in 5 vol actin buffer [50 mM Tris (pH 6.8), 2 mM EDTA, 2 mM phenylmethylsulfonyl fluoride] and diluted to 1 mg protein/ml. Samples were solubilized by the addition of an equal volume of actin buffer containing 1% Triton X-100 (final Triton concentration 0.5%). The insoluble cytoskeletal pellet, containing F-actin, was separated from the soluble fraction, containing G-actin, by centrifugation in a microfuge at room temperature (39–41). The cytoskeletal pellet was solubilized in depolymerization buffer [1.5 M guanidine HCl, 1 M sodium acetate, 1 mM $CaCl_2$, 1 mM ATP, 20 mM Tris HCl (pH 7.4)]. Both fractions were analyzed by immunoblot using a polyclonal rabbit antiactin IgG followed by an antirabbit IgG-horseradish peroxidase conjugate. Slot blots were developed with the Lumiglo chemiluminescent system and analyzed by scanning densitometry.

D2 analysis

Cerebral cortex samples were thawed and homogenized in 40 vol of 250 mM sucrose, 10 mM dithiothreitol (DTT), 1 mM EDTA, 20 mM HEPES (pH 7.0). D2 activity was determined in homogenates by the iodide release method at 2 nM rT_3 and 20 mM DTT in the presence of 1 mM PTU (22). Units are expressed as fmol I^- released/h.

Statistical methods

Results were analyzed by single-factor ANOVA.

Results

Analysis of the entry of iodothyronines into brain tissues

Before iodothyronines are able to enter the central nervous system (CNS), they must pass across the blood-brain barrier or through the choroid plexus-cerebrospinal fluid barrier (42–44). Although many studies have shown that iodothyronines readily enter the brain (24, 38, 45–49), there is still some debate as to possible differential entry of iodothyronines into the CNS. To study rapid actions of iodothyronines in the brain, we examined the time course of entry of iodothyronines into the cerebral cortex and the cerebellum after ip injection of [^{125}I]-labeled iodothyronines into postnatal d 14 euthyroid rat pups.

All three iodothyronines were readily absorbed in to the bloodstream after ip injection (5–10% injected dose/ml at 1 h) and remained detectable throughout the experimental period (2–5% injected dose/ml at 6 h). In the cerebral cortex (Fig. 1, *top*), total [^{125}I] reached a peak at 1 h after the injection of T_4 and rT_3, whereas a peak was not observed until 3 h after the injection of T_3. At all time points, T_3 was 2- to 3-fold more abundant in the cortex than either T_4 or rT_3 (Fig. 1, *bottom*).

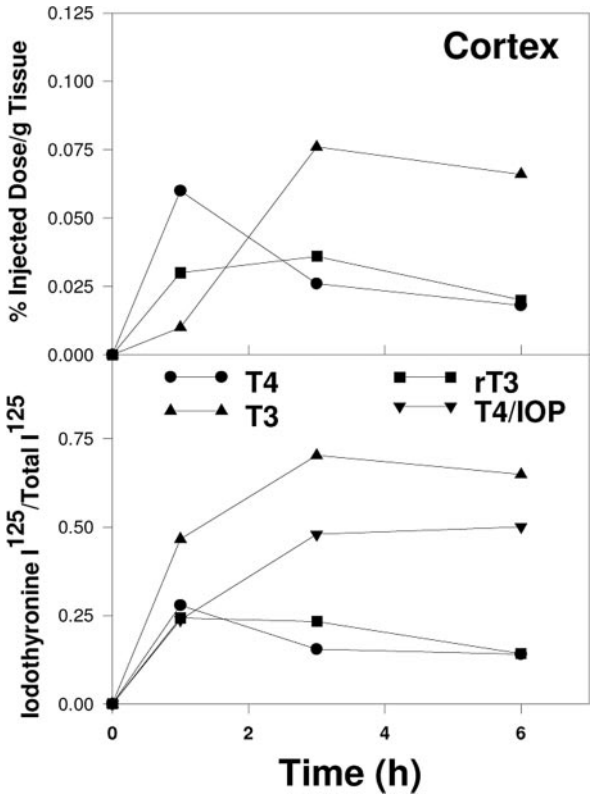

FIG. 1. Entry of iodothyronines into the developing rat cerebral cortex. Postnatal d 14 euthyroid rat pups were injected ip with [^{125}I]-labeled iodothyronines (20×10^6 cpm) and tissues were harvested at the indicated times. Tissues were extracted with chloroform:MeOH and analyzed by HPLC as discussed in *Materials and Methods*. *Top*, Total tissue [^{125}I]. Results are presented as the percent injected dose of each individual iodothyronine normalized to the amount of tissue and represent the mean of results obtained in two separate experiments with six animals/experiment (n = 12 total). *Bottom*, Tissue iodothyronine content. Results are presented as the ratio of [^{125}I]-labeled iodothyronine to the total tissue [^{125}I] normalized to the amount of tissue and represent the mean of results obtained in two separate experiments with six animals/experiment (n = 12 total). T4/IOP, [^{125}I]-labeled T_4 (20×10^6 cpm) and IOP (50 μg/10 g body weight) were injected ip at the same time.

The tissue:plasma ratio at 3 h was similar with all three iodothyronines, indicating equal access to the cerebral cortex (Table 1). The steady decline of T_4 from 1–6 h in the cortex was blocked by the concurrent administration of IOP, a potent inhibitor of both type I and type II 5′-deiodinases (50). Levels of rT_3 remained relatively stable over the 6-h period. In the cerebellum, peak tissue levels of total [^{125}I] were achieved within 1 h after injection of rT_3 and within 3 h after the injection of T_4 and T_3 (Fig. 2, *top*). Peak tissue levels of rT_3 were achieved in the cerebellum by 1 h and then fell to a plateau level from 3–6 h (Fig. 2, *bottom*). T_3 steadily increased to peak tissue levels in the cerebellum by 3 h then plateaued, whereas T_4 reached peak tissue levels by 3 h, followed by a slow decrease by 6 h. As observed in the cortex, the concurrent administration of IOP blocked the decrease in T_4 tissue levels from 3–6 h; instead T_4 levels continued to steadily rise the cerebellum for up to 6 h after IOP administration. Similarly, the tissue:plama ratio at 3 h in the cerebellum was the same for all three iodothyronines and approximately 2-fold higher than in the cortex (Table 1). These studies show that all iodothyronines rapidly and equally enter developing brain tissues and remain in the CNS for at least 6 h after a bolus injection.

TABLE 1. Plasma and tissue concentrations of iodothyronines 3 h after ip injection

Iodothyronine	Plasma (% dose/ml)	Tissue (% dose/g tissue)	Tissue/plasma ratio
	Cortex		
[^{125}I]T_4	1.77	0.026	0.015
[^{125}I]rT_3	1.81	0.036	0.020
[^{125}I]T_3	2.69	0.076	0.032
	Cerebellum		
[^{125}I]T_4	1.77	0.103	0.058
[^{125}I]rT_3	1.81	0.098	0.054
[^{125}I]T_3	2.69	0.091	0.039

The data represent the mean of results obtained in two separate experiments with six animals per experiment (n = 12 total).

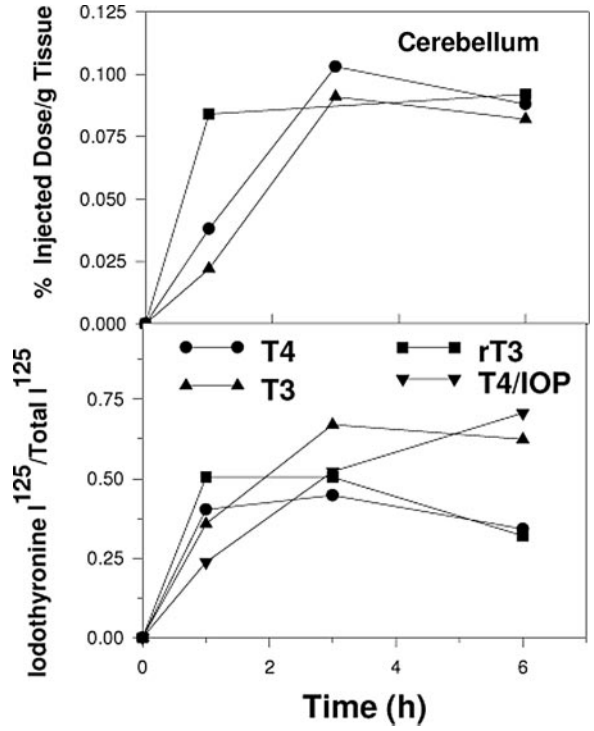

FIG. 2. Entry of iodothyronines into the developing rat cerebellum. Postnatal d 14 euthyroid rat pups were injected ip with [^{125}I]-labeled iodothyronines (20×10^6 cpm) and tissues were harvested at the indicated times. Tissues were extracted with chloroform:MeOH and analyzed by HPLC as discussed in *Materials and Methods*. *Top*, Total tissue [^{125}I]. Results are presented as the percent injected dose of each individual iodothyronine normalized to the amount of tissue and represent the mean of results obtained in two separate experiments with six animals/experiment (n = 12 total). *Bottom*, Tissue iodothyronine content. Results are presented as the ratio of [^{125}I]-labeled iodothyronine to the total tissue [^{125}I] normalized to the amount of tissue and represent the mean of results obtained in two separate experiments with six animals/experiment (n = 12 total). T4/IOP, [^{125}I]-labeled T_4 (20×10^6 cpm) and IOP (50 μg/10 g body weight) were injected ip at the same time.

Analysis of the rapid actions of thyroid hormone in the developing cerebral cortex and cerebellum

The above data clearly show that all iodothyronines rapidly enter the developing brain; thus, we were able to proceed and examine and compare the dynamic effects of iodothyronines on brain processes. Neonatal hypothyroidism was induced by adding PTU (2 mg/liter) to the drinking water of pregnant dams beginning at d 17 and continuing throughout the neonatal period. On postnatal d 14, rats were injected ip with T_4 (200 ng/10 g body weight), T_3 (40 ng/10 g body weight), rT_3 (200 ng/10 g body weight). Animals were killed and blood and tissues harvested 1–6 h after injection. Shown in Fig. 3 are the serum hormone concentrations in the experimental animals. PTU treatment resulted in undetectable T_4 and rT_3 concentrations and TSH concentrations 5- to 6-fold greater than that observed in the euthyroid animals. As observed previously (24, 51, 52), serum T_3 levels remained detectable in the PTU-treated animals at approximately 25% the level seen in the euthyroid animals. After the injection of T_4, serum T_4 concentrations rose steadily to a maximum level at 3 h that was approximately 2- to 3-fold higher than in the euthyroid animals. Importantly, the only animals with detectable circulating T_4 were the euthyroid and T_4-treated animals. Also after T_4 injection, serum T_3 concentrations steadily increased to euthyroid levels and rT_3 concentrations increased to approximately 8-fold greater than that observed in euthyroid animals over the 3- to 6-h time period, whereas TSH concentrations steadily fell to euthyroid levels by 6 h. After T_3 injection, serum T_3 concentrations increased to slightly above euthyroid levels (and higher than observed after T_4 injection) by 3 h then declined to euthyroid levels over the following 3 h. TSH concentrations also decreased by approximately 50% by 6 h after injection of T_3, with no change in serum T_4 or rT_3 concentrations in the T_3-treated animals. Similarly, rT_3 injection altered only serum rT_3 concentrations, increasing the levels to approximately 8-fold above that observed in euthyroid animals over the 3- to 6-h time period. As expected, there were no changes in serum hormone concentrations from the PTU-treated baseline in the animals injected with vehicle.

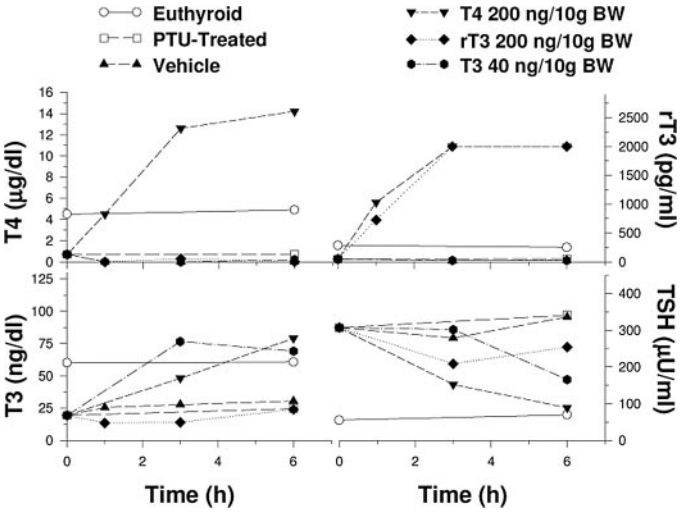

FIG. 3. Serum levels of iodothyronines after ip injection. On postnatal d 14, PTU-treated rat pups were injected ip with T_4 [200 ng/10 g body weight (BW)], T_3 (40 ng/10 g BW), rT_3 (200 ng/10 g BW), or vehicle and animals were killed at 1, 3, and 6 h after injection. Control postnatal d 14 euthyroid and PTU-treated animals were killed at 0 and 6 h. Blood was collected for hormone assays as discussed in *Materials and Methods*. Results are presented as the mean of values obtained in triplicate animals in three experiments (n = 9).

Dynamic regulation of D2 activity in the cerebral cortex

Regulation of D2 activity in the brain is a well-characterized nongenomic action of thyroid hormone and is a sensitive indicator of the cellular levels of thyroid hormone in the cerebral cortex (7, 22, 24, 26, 34, 40, 53). D2 catalyzes T_4 to T_3 conversion within the brain and enzyme activity is inversely proportional to serum T_4 levels. In rat astrocytes, T_4 dynamically regulates D2 activity by activating actin-based endocytosis leading to the internalization of the enzyme and directing it to recycling pathways within the cell (26, 34, 40, 54, 55). Using D2 as a marker of nongenomic thyroid hormone action, we examined the effects of individual iodothyronines on D2 activity in the developing rat cerebral cortex.

As shown previously (21, 22, 56), D2 activity in the hypothyroid cerebral cortex is approximately 10-fold greater than that found in the euthyroid rat brain (Fig. 4). The injection of vehicle alone or of T_3 at sufficient doses to produce euthyroid serum levels (Fig. 3) had no effect on D2 activity in the cerebral cortex over the 6 h period. In contrast, injection of both T_4 and rT_3 decreased D2 activity in the cerebral cortex by approximately 50% and 75%, respectively, within 30 min of injection (Fig. 4). D2 activity decreased to levels observed in the euthyroid cortex within 3 h after either T_4 or rT_3 injection and remained stable from 3–6 h after injection. At

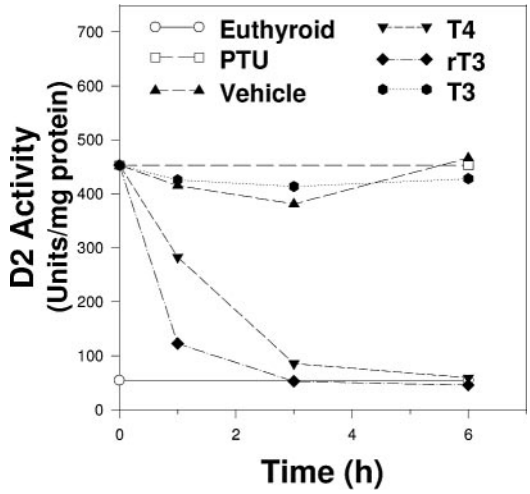

FIG. 4. Effect of iodothyronines on D2 activity in the developing rat cerebral cortex. On postnatal d 14, PTU-treated rat pups were injected ip with T_4 [200 ng/10 g body weight (BW)], T_3 (40 ng/10 g BW), rT_3 (200 ng/10 g BW), or vehicle, and animals were killed at 1, 3, and 6 h after injection. Where indicated, animals were also injected ip with IOP (50 µg/10 g BW). Control postnatal d 14 euthyroid and PTU-treated animals were killed at 0 and 6 h. Tissues were harvested and frozen in liquid nitrogen before use as discussed in *Materials and Methods*. D2 activity was determined in tissue homogenates at 2 nM rT_3 and 20 mM DTT in the presence of 1 mM PTU. Results are presented as the mean of values obtained in triplicate animals in three experiments (n = 9). Units = fmol I^- released/h.

all time points, D2 activity was significantly lower in either the T_4- or rT_3-treated rats compared with either the T_3 or vehicle-treated rats ($P < 0.05$). The effects of T_4 and rT_3 on D2 activity in the cerebral cortex are dose dependent, whereas T_3 continued to have no effect on D2 activity at equimolar concentrations (Table 1).

Dynamic regulation of actin polymerization in the cerebellum

We next examined the effect of iodothyronines on the polymerization state of the microfilaments in the cerebellum. Previous studies showed that the cerebellum of hypothyroid postnatal d 14 rats had a lower amount of F-actin than the euthyroid rat cerebellum, with no change in total actin content (35). We confirmed those findings and show that the F-actin content in the hypothyroid rat cerebellum is 30–40% lower than in the euthyroid rat cerebellum at postnatal d 14 (Fig. 5; Table 2, $P < 0.05$ *vs.* euthyroid animal). In addition, the G-actin content in the cerebellum in the hypothyroid rat pups is greater than 2-fold higher than that found in the euthyroid rats ($P < 0.001$ *vs.* euthyroid animal). As shown previously (35), total cerebellar actin content was unchanged in all conditions examined in this study (data not shown). Acute administration of either T_3 or vehicle did not affect either the G-actin or F-actin content in the hypothyroid rat cerebellum of the 6-h period. In contrast, T_4 and rT_3 both rapidly decreased G-actin content ($P < 0.001$ *vs.* PTU-treated animal) and increased the F-actin content in the PTU-treated rat cerebellum to that observed in the euthyroid cerebellum ($P < 0.05$ *vs.* PTU-treated animal) within 3 h (Fig. 5). These effects persisted for at least 6 h. The T_4- and rT_3-dependent changes in G- and F-actin content are concentration-dependent, whereas T_3 has no effect even at high levels (Table 2). These data indicate that T_4 and rT_3, but not T_3, dynamically regulate actin polymerization in the developing rat cerebellum.

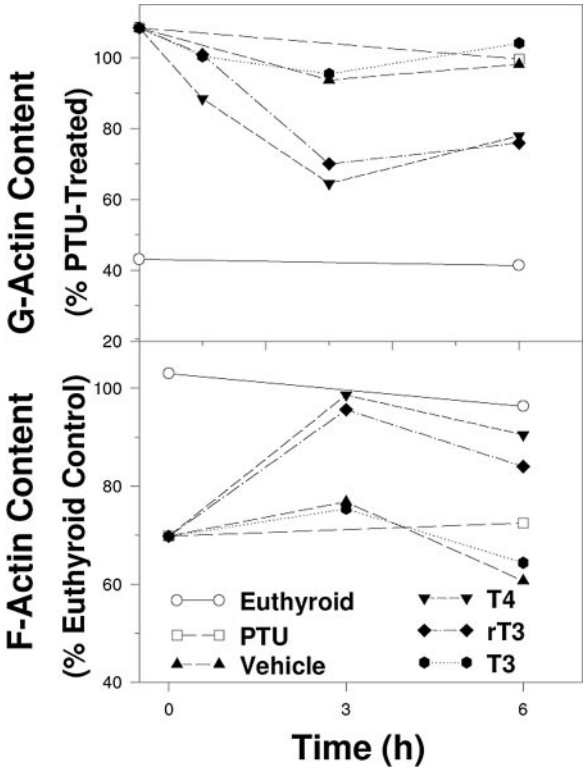

FIG. 5. Effect of iodothyronines on the G- and F-actin content in the developing rat cerebellum. On postnatal d 14, PTU-treated rat pups were injected ip with T_4 [200 ng/10 g body weight (BW)], T_3 (40 ng/10 g BW), rT_3 (200 ng/10 g BW), or vehicle, and animals were killed at 1, 3, and 6 h after injection. Where indicated, animals were also injected ip with IOP (50 μg/10 g body weight). Control postnatal d 14 euthyroid and PTU-treated animals were killed at 0 and 6 h. Tissues were harvested and frozen in liquid nitrogen before use as discussed in *Materials and Methods*. Tissues were homogenized then solubilized with 0.5% Triton X-100. The soluble G-actin was separated from the insoluble F-actin fibers by centrifugation and the actin content in the soluble and insoluble fraction was analyzed by Western blot with an antiactin IgG as discussed in *Materials and Methods*. The G-actin content is expressed as a percent of the PTU-treated control animals, whereas the F-actin content is expressed as a percent of the euthyroid control animals. Results are presented as the mean of values obtained in triplicate animals in at least two experiments (n = at least 6).

Discussion

In the adult rat, local deiodination of T_4 serves as the major source of brain tissue T_3 and rT_3 (24, 50, 57), potentially confounding the interpretation of T_4- and rT_3-specific actions in the brain. In particular, previous studies performed in adult rats suggested that access of rT_3 to brain tissues was markedly less than that of T_4 and T_3 (24, 45), with local deiodination of T_4 serving as the primary, if not sole, source of rT_3 in the cerebral cortex (24). In this paper, we show that serum T_4, rT_3, and T_3 all have equal and rapid access to cerebral cortical and cerebellar tissues in the developing rat. A likely explanation for this apparent discrepancy is the increased permeability to thyroid hormones of the blood: brain barrier in the developing brain (58–60). Because circulating T_4, rT_3, and T_3 absorbed after ip injection readily enter developing brain tissues, this study allows the direct evaluation of individual iodothyronines on the nongenomic regulation of D2 activity in the cerebral cortex and actin polymerization in the cerebellum of the developing rat.

As noted previously, regulation of D2 activity in the adult cerebral cortex is a well-characterized nongenomic action of thyroid hormone (21, 22, 53, 56). This study shows that regulation of D2 by T_4 and rT_3 also occurs in the developing brain. As reported in the adult brain (21, 22, 53, 56), D2 activity in the developing cerebral cortex is markedly increased in the absence of thyroid hormone. Acute administration of either T_4 or rT_3 caused cerebrocortical D2 levels in the hypothyroid rat pups to fall to euthyroid levels within 3 h, whereas injection of T_3 at concentrations sufficient to raise serum T_3 levels above the normal range failed to have any effect over that seen with vehicle alone (Fig. 4). Importantly, injection of the hypothyroid rat pups with rT_3 increased serum levels of only rT_3 (Fig. 3). Thus, because serum T_4 levels remained undetectable and serum T_3 levels remained low and unchanged, the fall in D2 activity in the hypothyroid rat cortex after injection of rT_3 is a direct effect of this inactive iodothyronine.

Underlying the regulation of D2 activity is the nongenomic regulation of actin polymerization by thyroid hormone in brain cells (19). The expression of actin is developmentally

TABLE 2. Dose-dependent effects of iodothyronines on D2 activity and G- and F-actin content in the P14 brain

Hormone dose (ng/10 g BW)	D2 activity (% vehicle injected)	G-actin content (% vehicle injected)	F-actin content (% vehicle injected)
Euthyroid	11.7[a]	54[a]	132.0[a]
PTU treated	110.1	90.4	100
Vehicle injected	100	100.1	100
T_4 100 ng	109.0	82.4	100.4
T_4 200 ng	21.9[a]	65.5[a]	130.7[a]
T_3 10 ng	90.8	101.1	105.6
T_3 20 ng	92.5	105.3	90.0
T_3 40 ng	112.9	100.7	112.1
rT_3 20 ng	102.8	104.2	103.9
rT_3 100 ng	82.5[a]	87.3	123.3[a]
rT_3 200 ng	42.7[a]	69.8[a]	126.8[a]

Data are presented at 3 h after ip injection and represent the mean of values obtained in triplicate animals in three experiments (n = 9). BW, Body weight.

[a] $P < 0.05$ as compared with vehicle injected.

regulated in the rat brain (35, 61, 62). At birth, the F-actin content in the rat brain is low and, beginning at approximately postnatal d 5, steadily increases to adult levels over the next 2 wk (35, 61, 62). Peak actin content is observed around postnatal d 8, after which levels fall to adult levels by the third week of life. Thyroid hormone has been reported to have no effect on the total actin content in the developing brain (35), despite the observation that the peak actin mRNA content in the cerebellum is delayed by a few days in the hypothyroid rat (63). However, the F-actin content in the developing hypothyroid rat cerebellum was reported to remain low until postnatal d 15–16, before steadily increasing to adult levels (35). Treatment of the hypothyroid rat with T_4 normalized the F-actin content in the cerebellum within 4 d (35).

In this study, we confirm the observation (35) that the F-actin content in the hypothyroid rat cerebellum at postnatal d 14 is significantly lower than in the euthyroid animal (Fig. 5). Importantly, we show that the F-actin content in the hypothyroid rat cerebellum is rapidly (within 3 h) restored to normal by the administration of either T_4 or rT_3. The increase in the F-actin content is associated with a reciprocal fall in the G-actin content, indicating changes in the polymerization state of the cellular actin as opposed to a change in the total cellular actin content. In contrast, T_3 had no effect on the F-actin content in the hypothyroid rat cerebellum (Fig. 5). Because T_3 readily enters cerebellar tissues (Fig. 2), the inability for T_3 to modulate F-actin content in the cerebellum is not due to restricted access to the brain intracellular compartment. Similar to the actions of rT_3 on D2 activity, the increase in cerebellar F-actin content after injection of rT_3 is clearly a direct action of this iodothyronine. These *in vivo* data correlate well with previous *in vitro* observations on the regulation of actin polymerization by T_4 and rT_3 in cultured rat astrocytes (19, 40, 41) and rat neurons (20). Similar to our observation in the cerebellum (Table 2), T_4 and rT_3 are greater than 100-fold more potent than T_3 in promoting actin polymerization in cultured rat astrocytes and neurons.

Thyroid hormone-dependent regulation of actin polymerization is likely to play a major role in the brain developmental program. The microfilaments in both the neurons and the astrocytes play a key role during neuronal migration in the developing brain. The ability to regulate actin polymerization in the migrating neurite is essential to interpret extracellular guidance cues used by the migrating cell to follow specific pathways to their destination (27–30, 32, 64). Chemical disruption of actin polymerization in cultured neurons markedly impairs neuronal growth cone motility and pathfinding ability *in vitro* (32, 65–67). Furthermore, the T_4- and rT_3-dependent regulation of actin polymerization in neurons markedly blunts neuronal migration and neuronal process outgrowth *in vitro* (20).

Regulation of actin polymerization in the supporting astrocytes is also essential for the organization of neuronal guidance molecules such as laminin on the astrocyte surface during brain development (68–74). Disruption of the microfilaments in cultured astrocytes also disrupts the formation of laminin arrays on the cell surface (75). We have previously shown that the nongenomic thyroid hormone-dependent regulation of F-actin content in astrocytes modulates integrin:laminin interactions in astrocytes that, in turn, modulates the ability of astrocytes to fix and position laminin on the cell surface after secretion (34, 75). This *in vitro* modulation of laminin patterning on the astrocyte surface also occurs *in vivo* because the appearance of laminin in the molecular layer in the developing hypothyroid rat cerebellum is both delayed and diminished compared with the euthyroid cerebellum (76), without any change in laminin mRNA expression. Because the present study shows that thyroid hormone dynamically regulates actin polymerization in the developing brain, these data suggest that the thyroid hormone-dependent modulation of F-actin:integrin:laminin interactions occurs *in vivo* and is the likely etiology of the altered laminin appearance in the hypothyroid cerebellum.

This study also shows that rT_3, an iodothyronine that has previously been considered an inactive metabolite of T_4, indeed has direct regulatory actions on *in vivo* processes in the developing brain. Previous studies reported that a single injection of rT_3 decreased the pyknotic index in the hypothyroid neonatal cerebellum to euthyroid levels (77). The ability of rT_3 to increase the F-actin content in the developing cerebellum provides a mechanism for this observation because pathfinding and migration would be restored to the migrating neurites in the presence of rT_3. This would lead to more cells reaching their target destination and surviving rather than dying and becoming pyknotic. The regulation of

F-actin content and D2 activity by rT_3 in the developing brain is the first demonstration of *in vivo* processes directly modulated by this iodothyronine.

Our data suggest that the nongenomic regulation of actin polymerization plays a major role in neuronal migration during brain development. Indeed, although transcriptional gene regulation is the major mechanism of thyroid hormone action, alternative mechanism(s) of action need to be considered since the demonstration that mutant mice lacking all known TRs exhibit apparently normal brain development (15, 16). Others have suggested a role for the unliganded receptor in thyroid hormone-mediated brain development because TRs bind to DNA in the unliganded state and *in vitro* studies have shown that the unliganded receptor can exert a negative effect on transcription (78). Furthermore, inactivation of TRα prevented a hypothyroid phenotype in congenitally hypothyroid Pax8$^{(-/-)}$ mice (79), although subsequent studies have suggested a more complex explanation for these observations (80). However, when considering unliganded TRs as mediators of the abnormities observed in the hypothyroid brain, it is important to remember that the obligatorily unliganded TRα2 is the most abundant TR isoform in the brain throughout development (81). Furthermore, like the unliganded T_3-binding TRs, TRα2 has been reported to function as a transcriptional inhibitor *in vitro* (82, 83). Importantly, there has been no direct *in vivo* demonstration of regulated gene repression by either the unliganded T_3-binding TRs or by TRα2; instead, all *in vivo* data have been inferential (*i.e.* hypothyroid mice exhibiting a more benign phenotype in the absence of expression, as noted above). Thus, it seems likely that non-TR-mediated processes are likely to contribute to the action of thyroid hormone on the developmental program of the brain.

In summary, we have shown that thyroid hormone dynamically regulates D2 activity and actin polymerization in the developing rat brain and that it likely does so via a nongenomic mechanism of action. The thyroid hormone-dependent regulation of actin polymerization in the rat brain provides mechanism by which this morphogenic hormone can influence neuronal migration and development independent of the need for altered gene transcription. Furthermore, because rT_3 is equipotent to T_4 in the nongenomic regulation D2 activity and microfilament organization in both astrocytes and neurons (7, 19, 20, 33, 34), and rT_3 is the most abundant iodothyronine during fetal life (12), these data suggest a prominent role for this iodothyronine during brain development.

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References

1. **Kilby MD** 2003 Thyroid hormones and fetal brain development. Clin Endocrinol (Oxf) 59:280–281
2. **Koibuchi N, Jingu H, Iwasaki T, Chin WW** 2003 Current perspectives on the role of thyroid hormone in growth and development of cerebellum. Cerebellum 2:279–289
3. **Anderson GW, Schoonover CM, Jones SA** 2003 Control of thyroid hormone action in the developing rat brain. Thyroid 13:1039–1056
4. **Bernal J, Guadano-Ferraz A, Morte B** 2003 Perspectives in the study of thyroid hormone action on brain development and function. Thyroid 13:1005–1012
5. **Zoeller RT** 2004 Editorial: local control of the timing of thyroid hormone action in the developing human brain. J Clin Endocrinol Metab 89:3114–3116
6. **Zoeller RT, Crofton KM** 2000 Thyroid hormone action in fetal brain development and potential for disruption by environmental chemicals. Neurotoxicology 21:935–945
7. **Farwell AP, Leonard JL** 2005 Nongenomic actions of thyroid hormone during fetal brain development. Curr Opin Endocrinol Metab 12:17–22
8. **Dubuis J-M, Glorieux J, Richer F, Deal CL, Dussault JH, van Vliet G** 1996 Outcome of severe congenital hypothyroidism: closing the developmental gap with early high dose levothyroxine treatment. J Clin Endocrinol Metab 81:222–227
9. **Bongers-Schokking JJ, Koot HM, Wiersma D, Verkerk PH, de Muinck Keizer Schrama SM** 2000 Influence of timing and dose of thyroid hormone replacement on development in infants with congenital hypothyroidism. J Pediatr 136:292–297
10. **Hernandez A, St Germain DL** 2003 Thyroid hormone deiodinases: physiology and clinical disorders. Curr Opin Pediatr 15:416–420
11. **Leonard JL, Koehrle J** 2000 Intracellular pathways of iodothyronine metabolism. In: Braverman LE, Utiger RD, eds. The thyroid. Philadelphia: Lippincott Williams & Wilkins; 136–173
12. **Roti E, Braverman LE, Fang SL, Alex S, Emerson CH** 1982 Ontogenesis of placental inner ring thyroxine deiodinase and amniotic fluid 3,3′,5′-triiodothyronine concentration in the rat. Endocrinology 111:959–963
13. **Viguerie N, Langin D** 2003 Effect of thyroid hormone on gene expression. Curr Opin Clin Nutr Metab Care 6:377–381
14. **Yen PM** 2001 Physiological and molecular basis of thyroid hormone action. Physiol Rev 81:1097–1142
15. **Gothe S, Wang Z, Ng L, Kindblom JM, Barros AC, Ohlsson C, Vennstrom B, Forrest D** 1999 Mice devoid of all known thyroid hormone receptors are viable but exhibit disorders of the pituitary-thyroid axis, growth, and bone maturation. Genes Dev 13:1329–1341
16. **Gauthier K, Plateroti M, Harvey CB, Williams GR, Weiss RE, Refetoff S, Willott JF, Sundin V, Roux JP, Malaval L, Hara M, Samarut J, Chassande O** 2001 Genetic analysis reveals different functions for the products of the thyroid hormone receptor α locus. Mol Cell Biol 21:4748–4760
17. **Bassett JH, Harvey CB, Williams GR** 2003 Mechanisms of thyroid hormone receptor-specific nuclear and extra nuclear actions. Mol Cell Endocrinol 213:1–11
18. **Davis PJ, Davis FB** 2003 Nongenomic actions of thyroid hormone. In: Braverman LE, ed. Diseases of the thyroid. Totowa, NJ: Humana Press; 19–38
19. **Siegrist-Kaiser CA, Juge-Aubry C, Tranter MP, Ekenbarger DM, Leonard JL** 1990 Thyroxine-dependent modulation of actin polymerization in cultured astrocytes. A novel, extranuclear action of thyroid hormone. J Biol Chem 265:5296–5302
20. **Farwell AP, Dubord-Tomasetti SA, Pietrzykowski AZ, Stachelek SJ, Leonard JL** 2005 Regulation of cerebellar neuronal migration and neurite outgrowth by thyroxine and 3,3′,5′-triiodothyronine. Brain Res Dev Brain Res 154:121–135
21. **Leonard JL, Kaplan MM, Visser TJ, Silva JE, Larsen PR** 1981 Cerebral cortex responds rapidly to thyroid hormones. Science 214:571–573
22. **Visser TJ, Leonard JL, Kaplan MM, Larsen PR** 1982 Kinetic evidence suggesting two mechanisms for iodothyronine 5′-deiodination in rat cerebral cortex. Proc Natl Acad Sci USA 79:5080–5084
23. **Leonard JL, Gogolin PJ** 1984 Thyroid hormone dependent regulation of thyroxine-5′-deiodinase in glial cell cultures. Ann Endocrinol (Paris) 45:61
24. **Silva JE, Leonard JL** 1985 Regulation of rat cerebrocortical and adenohypophyseal type II 5′-deiodinase by thyroxine, triiodothyronine, and reverse triiodothyronine. Endocrinology 116:1627–1635
25. **Leonard JL, Larsen PR** 1985 Thyroid hormone metabolism in primary cultures of fetal rat brain cells. Brain Res 327:1–14
26. **Leonard JL, Siegrist-Kaiser CA, Zuckerman CJ** 1990 Regulation of type II iodothyronine 5′-deiodinase by thyroid hormone. Inhibition of actin polymerization blocks enzyme inactivation in cAMP-stimulated glial cells. J Biol Chem 265:940–946
27. **Dodd J, Jessell TM** 1988 Axon guidance and the patterning of neuronal projections in vertebrates. Science 242:692–699
28. **Tessier-Lavigne M, Goodman CS** 1996 The molecular biology of axon guidance. Science 274:1123–1133
29. **Lockerbie RO** 1987 The neuronal growth cone: a review of its locomotory, navigational and target recognition capabilities. Neuroscience 20:719–729

30. **Smith SJ** 1988 Neuronal cytomechanics: the actin-based motility of growth cones. Science 242:708–715
31. **DeVoto SH** 1990 Neuronal growth cone migration. Experientia 46:916–922
32. **Rivas RJ, Hatten ME** 1995 Motility and cytoskeletal organization of migrating cerebellar granule neurons. J Neurosci 15:981–989
33. **Leonard JL, Farwell AP** 1997 Thyroid hormone-regulated actin polymerization in brain. Thyroid 7:147–151
34. **Farwell AP, Leonard JL** 1997 Extranuclear actions of thyroid hormone in the brain. In: Porterfield SP, Hendrich CE, eds. Recent research developments in neuroendocrinology—thyroid hormone and brain maturation. Trivandrum, India: Research Signpost; 113–130
35. **Faivre-Sarrailh C, Rabie A** 1988 A lower proportion of filamentous to monomeric actin in the developing cerebellum of thyroid-deficient rats. Dev Brain Res 41:293–297
36. **Weeke J, Orskov H** 1973 Synthesis of 125I monolabeled 3,5,3′-triiodothyronine and thyroxine of maximum specific activity for radioimmunoassay. Scand J Clin Lab Invest 321:357–360
37. **Morreale de Escobar G, Pastor R, Obregon MJ, Escobar del Ray F** 1985 Effects of maternal hypothyroidism on the weight and thyroid hormone content of rat embryonic tissues, before and after the onset of fetal thyroid function. Endocrinology 117:1890–1900
38. **Gordon JT, Crutchfield FL, Jennings AS, Dratman MB** 1982 Preparation of lipid-free tissue extracts for chromatographic determination of thyroid hormones and metabolites. Arch Biochem Biophys 216:407–415
39. **Goldman JE, Abramson B** 1990 Cyclic AMP-induced shape changes of astrocytes are accompanied by rapid depolymerization of actin. Brain Res 528: 189–196
40. **Farwell AP, Lynch RM, Okulicz WC, Comi AM, Leonard JL** 1990 The actin cytoskeleton mediates the hormonally regulated translocation of type II iodothyronine 5′-deiodinase in astrocytes. J Biol Chem 265:18546–18553
41. **Farwell A, Leonard J** 1992 Dissociation of actin polymerization and enzyme inactivation in the hormonal regulation of type II iodothyronine 5′-deiodinase activity in astrocytes. Endocrinology 131:721–728
42. **Chanoine JP, Braverman LE** 1992 The role of tranthyretin in the transport of thyroid hormone to cerebrospinal fluid. Acta Med Austriaca 19:25–28
43. **Robbins J, Lakshmanan M** 1994 The movement of thyroid hormones in the central nervous system. Acta Med Austriaca 19:21–25
44. **Pardridge WM** 1990 Transport of thyroid hormones into tissues in vivo. In: Wu S-Y, Hershman JH, eds. Thyroid hormone metabolism. Oxford: Blackwell; 123–143
45. **Cheng LY, Outterbridge LV, Covatta ND, Martens DA, Gordon JT, Dratman MB** 1994 Film autoradiography identifies unique features of [^{125}I]3,3′,5′-(reverse) triiodothyronine transport from blood to brain. J Neurophys 72:380–391
46. **Obregon M-J, Larsen PR, Silva JE** 1985 Plasma kinetics, tissue distribution, and cerebrocortical sources of reverse triiodothyronine in the rat. Endocrinology 116:2192–2200
47. **Dratman MB, Crutchfield FL** 1989 Thyroxine, triiodothyronine, and reverse triiodothyronine processing in the cerebellum: autoradiographic studies in adult rats. Endocrinology 125:1723–1733
48. **Dratman MB, Crutchfield FL, Schoenhoff MB** 1991 Transport of iodothyronines from bloodstream to brain: contributions by blood:brain and choroid plexus:cerebrospinal fluid barriers. Brain Res 554:229–236
49. **Dratman MB, Crutchfield FI, Axelrod J, Colburn RW, Thoa N** 1976 Localization of triiodothyronine in nerve ending fractions of rat brain. Proc Natl Acad Sci USA 73:941–944
50. **Leonard JL, Visser TJ** 1986 Biochemical basis of deiodination. In: Hennemann G, ed. Thyroid hormone metabolism. New York: Marcel Dekker, Inc.; 189–229
51. **Chanoine JP, Safran M, Farwell AP, Dubord S, Alex S, Stone S, Arthur JR, Braverman LE, Leonard JL** 1992 Effects of selenium deficiency on thyroid hormone economy in rats. Endocrinology 131:1787–1792
52. **Chanoine JP, Braverman LE, Farwell AP, Safran M, Alex S, Dubord S, Leonard JL** 1993 The thyroid gland is a major source of circulating T3 in the rat. J Clin Invest 91:2709–2713
53. **Visser TJ, Leonard JL, Kaplan MM, Larsen PR** 1981 Different pathways of iodothyronine 5′-deiodination in rat cerebral cortex. Biochem Biophys Res Commun 101:1297–1304
54. **Stachelek SJ, Kowalik TF, Farwell AP, Leonard JL** 2000 Myosin V plays an essential role in the thyroid hormone-dependent endocytosis of type II iodothyronine 5′-deiodinase. J Biol Chem 275:31701–31707
55. **Farwell AP, Dibenedetto DJ, Leonard JL** 1993 Thyroxine targets different pathways of internalization of type II iodothyronine 5′-deiodinase in astrocytes. J Biol Chem 268:5055–5062
56. **Leonard JL, Silva JE, Kaplan MM, Mellen SA, Visser TJ, Larsen PR** 1984 Acute posttranscriptional regulation of cerebrocortical and pituitary iodothyronine 5′-deiodinases by thyroid hormone. Endocrinology 114:998–1004
57. **Leonard JL** 1992 Regulation of T3 production in the brain. Acta Med Austriaca 1:5–8
58. **Saunders NR, Habgood MD, Dziegielewska KM** 1999 Barrier mechanisms in the brain, II. Immature brain. Clin Exp Pharmacol Physiol 26:85–91
59. **Mollgard K, Saunders NR** 1986 The development of the human blood-brain and blood-CSF barriers. Neuropathol Appl Neurobiol 12:337–358
60. **Robbins J, Lakshmanan M** 1992 The movement of thyroid hormones in the central nervous system. Acta Med Austriaca 19(Suppl 1):21–25
61. **Schmitt H, Gozes I, Littauer UZ** 1977 Decrease in levels and rates of synthesis of tubulin and actin in the developing rat brain. Brain Res 121:327–342
62. **Nona SN, Trowell SC, Cronly-Dillon JR** 1985 Postnatal developmental profiles of filamentous actin and of 200 kDa neurofilament polypeptide in the visual cortex of light- and dark-reared rats and their relationship to critical period plasticity. FEBS Lett 186:111–115
63. **Faivre-Sarrailh C, Ferraz C, Liautard JP, Rabie A** 1990 Effect of thyroid deficiency on actin mRNA content in the developing rat cerebellum. Int J Dev Neurosci 8:99–106
64. **Hatten ME, Heinz N** 1995 Mechanisms of neural patterning and specification in the developing cerebellum. Annu Rev Neuronsci 18:385–408
65. **Bentley D, Toroian-Raymond A** 1986 Disoriented pathfinding by pioneer neuron growth cones deprived of filopodia by cytochalasin treatment. Nature 323:712–715
66. **Forscher P, Smith SJ** 1988 Actions of cytochalasins on the organization of the actin filaments and microtubules in a neuronal growth cone. J Cell Biol 107: 1505–1516
67. **Marsh L, Letourneau PC** 1984 Growth of neurites withour filapodial or lamellipodial activity in the presence of cytochalasin B. J Cell Biol 99:2041–2047
68. **Venstrom KA, Reichardt LF** 1993 Extracellular matrix 2: Role of extracellular matrix molecules and their receptors in the nervous system. FASEB J 7:996–1003
69. **Liesi P** 1990 Extracellular matrix and neuronal movement. Experientia 46: 900–907 (Review)
70. **Liesi P, Silver J** 1988 Is astrocyte laminin involved in axon guidance in the mammalian CNS? Dev Biol 130:774–785
71. **Liesi P** 1985 Do neurons in the vertebrate CNS migrate on laminin? EMBO J 4:1163–1170
72. **Liesi P, Seppala I, Trenkner E** 1992 Neuronal migration in cerebellar microcultures is inhibited by antibodies against a neurite outgrowth domain of laminin. J Neurosci Res 33:170–176
73. **Liesi P, Narvanen A, Soos J, Sariola H, Snounou G** 1989 Identification of a neurite outgrowth-promoting domain of laminin using synthetic peptides. FEBS Lett [Erratum (1989) 251:283] 244:141–148
74. **Liesi P, Hager G, Dodt H-U, Seppala I, Zieglgansberger W** 1995 Domain-specific antibodies against the B2 chain of laminin inhibit neuronal migration in the neonatal rat cerebellum. J Neurosci Res 40:199–206
75. **Farwell AP, Dubord-Tomasetti SA** 1999 Thyroid hormone regulates the extracellular organization of laminin on astrocytes. Endocrinology 140:5014–5021
76. **Farwell AP, Dubord-Tomasetti SA** 1999 Thyroid hormone regulates the expression of laminin in the developing rat cerebellum. Endocrinology 140:4221–4227
77. **Dubuis JM, Sanchez-Menegay C, Burger AG** 1992 Effects of thyroxine, triiodothyronine and reverse triiodothyronine on the neonatal hypothyroid rat cerebellum. Acta Med Austriaca 19(Suppl 1):106–109
78. **Glass CK, Rosenfeld MG** 2000 The coregulator exchange in transcriptional functions of nuclear receptors. Genes Dev 14:121–141
79. **Flamant F, Poguet AL, Plateroti M, Chassande O, Gauthier K, Streichenberger N, Mansouri A, Samarut J** 2002 Congenital hypothyroid Pax8(−/−) mutant mice can be rescued by inactivating the TRα gene. Mol Endocrinol 16:24–32
80. **Mittag J, Friedrichsen S, Heuer H, Polsfuss S, Visser TJ, Bauer K** 2005 Athyroid Pax8−/− mice cannot be rescued by the inactivation of thyroid hormone receptor α1. Endocrinology 146:3179–3184
81. **Strait KA, Schwartz HL, Perez-Castillo A, Oppenheimer JH** 1990 Relationship of c-erbA mRNA content to tissue triiodothyronine nuclear binding capacity and function in developing and adult rats. J Biol Chem 265:10514–10521
82. **Katz D, Lazar MA** 1993 Dominant negative activity of an endogenous thyroid hormone receptor variant (α2) is due to competition for binding sites on target genes. J Biol Chem 268:20904–20910
83. **Hermann T, Zhang XK, Tzukerman M, Wills KN, Graupner G, Pfahl M** 1991 Regulatory functions of a non-ligand-binding thyroid hormone receptor isoform. Cell Regul 2:565–574

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