

Ontogenetic Changes in the Expression of Cytochrome c Oxidase Subunit I Gene in the Cerebellar Cortex of the Perinatal Hypothyroid Rat

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ABSTRACT

The thyroid hormone plays a critical role in normal development of the mammalian central nervous system. This study was designed to examine the effect of perinatal hypothyroidism on ontogenetic change in cytochrome *c* oxidase subunit I (COX I) gene expression in the rat cerebellum by using quantitative *in situ* hybridization histochemistry (ISH). Newborn rats were rendered hypothyroid by continuous administration of methimazole in the mothers' drinking water. The pups were then killed by decapitation on 1, 5, 10, 15, 20, and 30 days after birth (P1, P5, P10, P15, P20, and P30). Their cerebella were removed, and frozen sections were cut and processed for ISH with ³⁵S-labeled RNA probe for COX I messenger RNA. After hybridization, emulsion autoradiography was performed. The numbers of grains within the external granule cell layer, molecular layer, and internal granule cell

layer were then counted. A significant decrease in grain density was detected in the hypothyroid animal in all these areas on P5, P10, and P15. On P15, in the molecular layer, a greater hybridization signal was detected in the inner portion than in the outer portion in the euthyroid animal. No such difference was seen in the hypothyroid animal. Daily T₄ treatment for 15 days restored the effect of methimazole treatment. The significant effect of perinatal hypothyroidism on COX I gene expression was not detected after P20. These results indicate that altered thyroid states affect the COX I gene expression in the cerebellar cortex during development, suggesting that the COX I gene is one of the key genes regulated by the thyroid hormone and plays an important role in the morphogenetic changes observed in the perinatal hypothyroid cerebellum. (*Endocrinology* 137: 5096–5108, 1996)

THE IMPORTANCE of the thyroid hormone (T₄ and T₃) on growth and differentiation of many organs including the central nervous system (CNS) has been well documented (1). T₃ binds to nuclear thyroid hormone receptor (TR) to form a ligand-receptor complex, which binds to a specific DNA sequence called the thyroid responsive element located within the promotor region of specific genes and influences the transcription of these genes (2). TRs are distributed throughout the CNS, including the cerebellar cortex (3, 4).

In the rat cerebellum, neuronal development is largely postnatal (5–9), and neonatal hypothyroidism dramatically affects the morphogenesis of neurons (10–15). The proliferation and differentiation of granule cells in the external granule cell layer (EGL) are greatly influenced (10, 12). The EGL persists for a longer period in the hypothyroid rat than in the euthyroid animal, and the cells keep on proliferating. Disturbance of nerve process outgrowth of the Purkinje cells and a marked deficiency of synaptic connection in the molecular layer (ML) and internal granule cell layer (IGL) are also reported (10, 11, 13, 15). Although the molecular mechanism of morphogenetic actions of thyroid hormone during development is not fully understood, it is known that the expressions of several genes are changed in the rat cerebellum in association with altered thyroid states, such as actin (16),

several tubulin isotypes (17, 18), calbindin (19), IP3 receptor (19), p75 nerve growth factor receptor (18), growth-associated protein-43 (18), myelin proteolipid protein (18), myelin basic protein (18–20), and cerebellar Purkinje cell-specific gene-2 (19). In addition to these genes, we have previously screened cytochrome *c* oxidase (COX) subunit I (COX I) (21), which is encoded by the mitochondrial DNA (mtDNA) (22), in 15-day-old euthyroid and hypothyroid rat cerebellum by differential plaque screening.

Because the change in COX I gene expression during rat cerebellar development has not been fully studied, we considered it important to study its change by means of *in situ* hybridization histochemistry (ISH). We also considered it of interest to examine the effect of perinatal hypothyroidism on ontogenetic change in COX I gene expression for two reasons. First, it is generally considered that the thyroid hormone little affects oxygen consumption in the CNS, but a marked increase is induced in many peripheral organs (23). Nevertheless, several metabolic processes related to oxidative phosphorylation seem to be changed in the developing rat brain in association with altered thyroid states, such as glucose transport (24), Na⁺, K⁺-ATPase activity (25), and ketone body utilization (26). One previous study in particular has reported that the thyroid hormone regulates, at least in part, oxidative phosphorylation in brain mitochondria (27). Another study has found that perinatal hypothyroidism causes a decrease in the brain COX activity (28). Recent studies have shown that the expression of genes in mtDNA is, at least in part, regulated by the thyroid hormone in the cerebrum (29, 30) as well as in the cerebellum (21). It is therefore important

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to specify the area and period in which the COX I gene expression is affected by altered thyroid states. Second, a previous study has reported that COX activity and synaptogenesis are closely related in the developing rat cerebellum (31). Because synaptogenesis is dramatically depressed by hypothyroidism (11, 13), the COX I gene expression could be depressed within the area where synaptogenesis is proceeding. If so, retardation of synaptogenesis could be induced as a consequence of a decrease in COX I gene expression. To examine this hypothesis, we have studied the ontogenetic changes in COX I gene expression in perinatal hypothyroid rats by quantitative ISH.

Materials and Methods

Animals and treatment

Pregnant Sprague-Dawley rats raised in our animal facilities were housed under controlled temperature (24 ± 0.5 °C) and illumination (light 0500–1900 h). Food and water were available *ad libitum*. From 15 days after conception, 0.05% methimazole (2-mercapto-1-methylimidazole, Sigma Chemical Co., St. Louis, MO) dissolved in distilled water was administered as drinking water until the end of the experiment. Newborn pups were rendered hypothyroid (hypothyroid rats) because methimazole crosses the placenta and is excreted into milk (32). Other pregnant rats received only distilled water. Their pups were used as the control. The pups were weighed every day until sacrifice. Some hypothyroid pups (T_4 -replaced rats) received a daily sc injection of T_4 (0.2 µg/10 g body weight) dissolved in saline (20 µg/ml). Other pups received a vehicle injection. Control and hypothyroid pups were killed on 1, 5, 10, 15, 20, and 30 days after birth (P1, P5, P10, P15, P20, and P30) by decapitation. T_4 -replaced rats were killed on P15 and P30. Blood samples were collected from all pups killed on P15 and P30 to measure the TSH concentration in plasma. The cerebella were dissected out, immediately frozen on dry ice, and stored in liquid nitrogen until use.

The plasma TSH concentration was measured according to the procedures described elsewhere (33). Purified rat TSH (NIDDK-rTSH-I-9) for radiolabeling with 125 I, rat TSH antiserum (rabbit, NIDDK-anti-rTSH-S-5) and TSH reference standard (NIDDK-rTSH-RP-2) were generously supplied by the U.S. National Hormone and Pituitary Program (USNHPP) and National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK).

Probe preparation

To make the probe for ISH, a 290-bp *Eco*RI-*Hind*III fragment of COX I gene [bases 5319–5608, according to Gadaleta *et al.* (34)] cloned in our laboratory (21) was used. This fragment was subcloned into pBluescript II SK + transcription vector (Stratagene Cloning Systems, La Jolla, CA). Sense and antisense probes were transcribed with T3 and T7 RNA polymerases, respectively. 35 S-UTP was used to label the probe. After transcription, the probes were hydrolyzed to an average length of 200 bases (35).

Tissue preparation and *in situ* hybridization

Protocols for tissue preparation and ISH have been described elsewhere (21). In short, 10-µm thick frozen sagittal sections of the cerebellum were cut on a cryostat, mounted on a organosilane-coated glass slides, fixed with 4% paraformaldehyde, and dehydrated through a graded series of ethanol. Sections were hybridized with a 35 S-labeled sense or antisense RNA probe (400,000 cpm/section) for 24 h at 50 °C. The sections were then rinsed, treated with 20 µg/ml ribonuclease A (RNase A, type X-A, Sigma), dehydrated through a graded series of ethanol, and dried. They were then dipped in Kodak NTB3 photographic emulsion, exposed for 1 week at 4 °C, developed with Kodak D-19 and fixed with Kodak Fix. The sections were then counterstained with Cresyl Violet, dehydrated through a graded series of ethanol, and coverslipped.

Quantification and statistical analysis

The number of animals used for quantitative analysis is indicated in Table 1. All sections used for quantification were hybridized and exposed simultaneously. The number of silver grains in the cerebellar cortex was manually counted under a light microscope. A CCD (charge-coupled device) video camera (Sony, Tokyo, Japan) was directly attached to the microscope and counting was done under the video monitor attached to the camera. After the counting, the exact size of the counted area was measured with a computerized image analyzer (Mitani Cooperation Inc., Fukui, Japan). To maintain consistency, one section, which showed a similar plane in each animal, was carefully selected, and grain counting was done within lobule IX of the vermis along with fissure 2. On P1, because foliation was not complete, the dorsal part of the cerebellar cortex was used. Counting was started at the beginning of fissure 2 on the surface side and a 500-µm length was counted. For counting in the IGL, 100 µm in depth from the surface of the IGL was counted. For animals killed on P1, because it is rather difficult to delineate the boundary between ML and IGL, 100 µm in depth from the bottom of the EGL was counted. In the IGL, because COX I is ubiquitously expressed among closely packed cells, it is rather difficult to distinguish the hybridization signals in each cell. For this reason, no attempt was made to eliminate the grains that are concentrated within Purkinje cells from those in granule cells. The result was expressed as grain density (number of grain/mm²).

The treatment effect was examined by ANOVA. Post hoc comparison was made by Duncan's new multiple range test. When probability was less than 0.05, the results were considered to be significant.

Results

The effects of methimazole treatment and T_4 replacement were first confirmed by finding the body weight and measuring the plasma TSH concentrations on P15 and P30. Methimazole treatment significantly retarded the increase in body weight, and the daily T_4 replacement restored the effect of methimazole treatment [data not shown; the change in body weight after the same treatment was shown in our preceding paper (21)]. Mean plasma concentrations of TSH and its SEM were 3.04 ± 0.48 ng/ml (P15 control, n = 4), 8.61 ± 0.58 ng/ml (P15 hypothyroid, n = 4), 2.11 ± 0.28 ng/ml (P15 T_4 -replaced, n = 4), 0.35 ± 0.06 ng/ml (P30 control, n = 3), 5.51 ± 0.83 ng/ml (P30 hypothyroid, n = 3), and 0.19 ± 0.07 ng/ml (P30 T_4 -replaced, n = 3). Methimazole treatment significantly increased the plasma TSH level, and

TABLE 1. Number of animals used for the quantification

Day killed	Treatment	Number of animals
P1	Control	4
	Hypothyroid	5
P5	Control	4
	Hypothyroid	4
P10	Control	4
	Hypothyroid	4
P15	Control	4
	Hypothyroid	4
P20	T_4 -replaced	4
	Control	4
P30	Hypothyroid	5
	Control	3
	Hypothyroid	3
	T_4 -replaced	3

Mothers of the hypothyroid and T_4 -replaced pups received 0.05% methimazole dissolved in drinking water from the 15th day of conception until the end of the experiments. Control rats received distilled water. T_4 -replaced rats received a daily sc injection of thyroxine (0.2 µg/10 g BW).

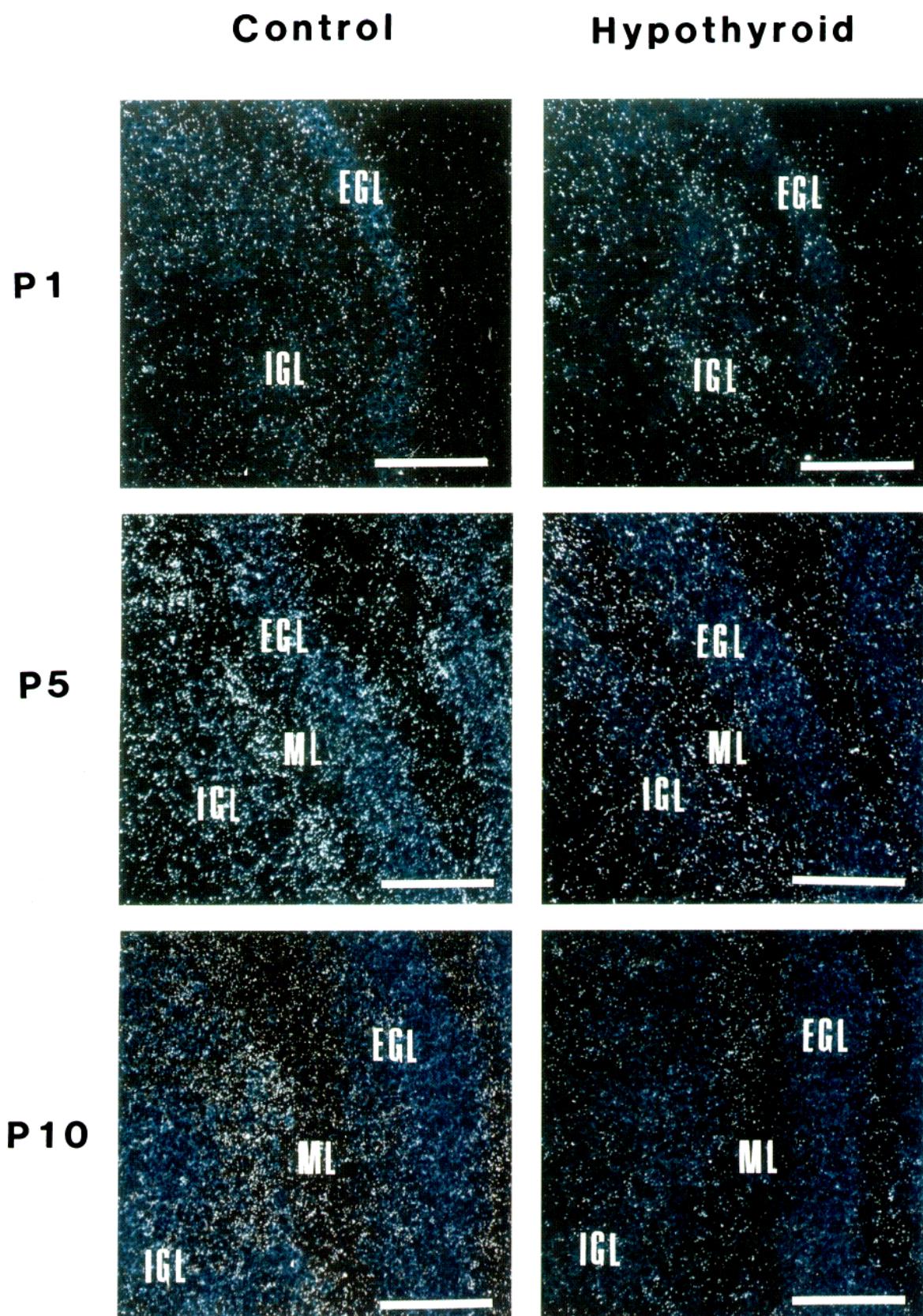


FIG. 1. Dark field photomicrographs showing the *in situ* hybridization detection of COX I mRNA within the cerebellar cortex (lobule IX of the vermis) of euthyroid (control) and methimazole treated (hypothyroid) rats. Rats were killed 1 (P1), 5 (P5), and 10 days (P10) after birth. Sagittal sections were hybridized with ^{35}S -labeled RNA probe for COX I mRNA. Hybridization was performed at 50°C for 24 h. Sections were exposed for 7 days for autoradiography. All sections were hybridized simultaneously and exposed together. Scale bar length, 100 μm .

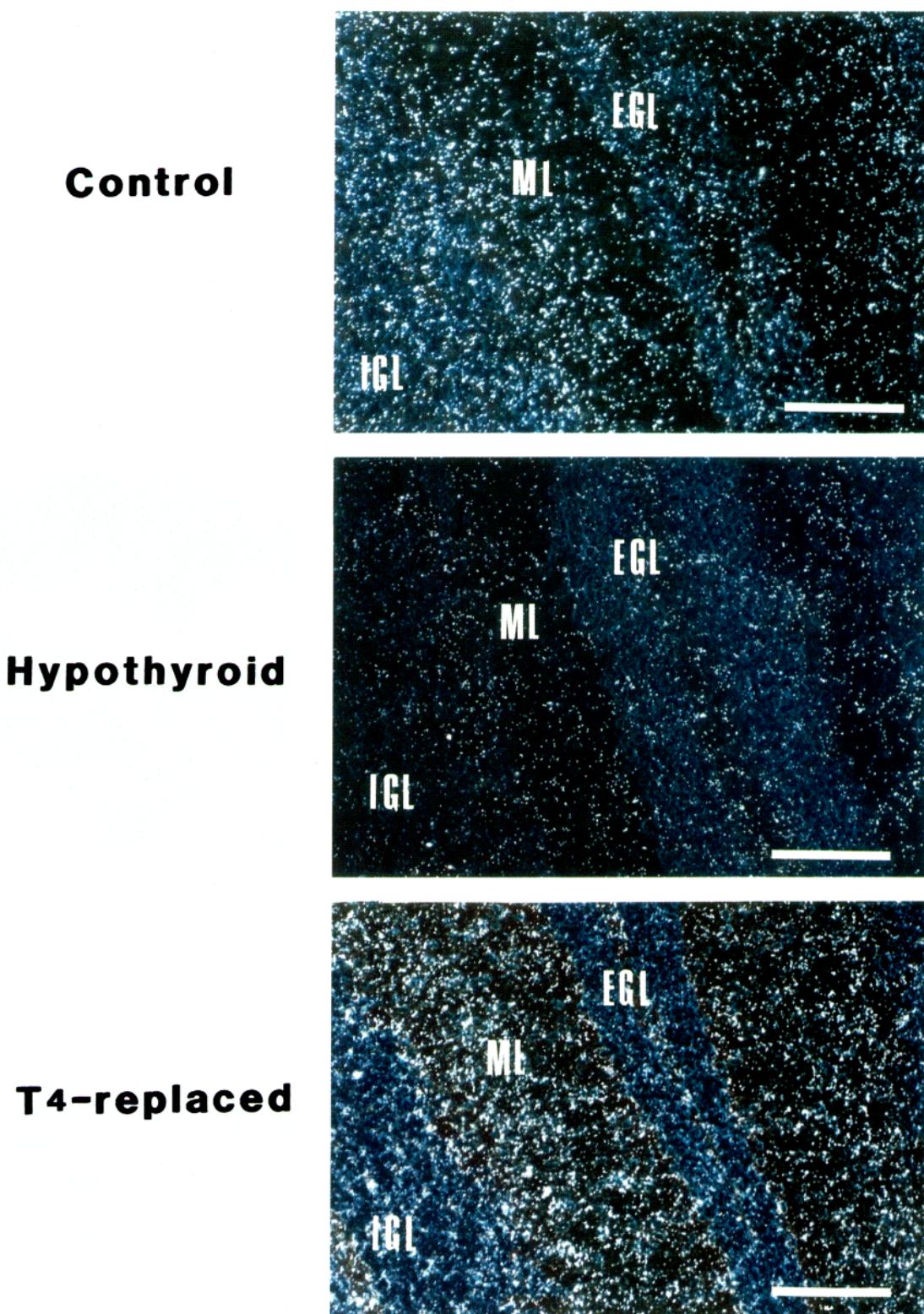


FIG. 2. Dark field photomicrographs showing the *in situ* hybridization detection of COX I mRNA within the cerebellar cortex (lobule IX of the vermis) of control, hypothyroid and T₄-replaced rats on P15. Hybridization and autoradiography were performed simultaneously with the sections shown in Fig. 1. Note that, in control and T₄-replaced animals, significant grain concentrations are seen not only in the EGL and IGL but also in the inner portion of the ML. Scale bar length, 100 μ m.

T_4 replacement abolished it on P15 and P30 ($P < 0.01$, by Duncan's new multiple range test).

Examples of the ISH for COX I messenger RNA (mRNA) in the cerebellar cortex are shown in Figs. 1–5. As many studies reported, perinatal hypothyroidism induced a retardation of disappearance of the EGL, and a decrease in the width in ML by P15 (Fig. 1 and 2). Daily T_4 replacement for 15 days restored the effect of methimazole treatment on cerebellar morphogenesis (Fig. 2). These results confirm the previous results indicating that the thyroid hormone regulates the morphogenesis of the rat cerebellum. When sections were hybridized with an antisense probe, a specific concentration of the silver grains over the section was observed. No such specific concentration was seen when sections were hybridized with a sense probe (data not shown), indicating that a specific hybridization signal for COX I mRNA was detected. The hybridization signal was distributed not only

over the EGL and IGL but also over the ML, in which fewer cells are located. On P1, no particular difference between the euthyroid (control) and hypothyroid animals was seen in the distribution of the hybridization signal (Fig. 1). On P5 and P10, Purkinje cells were clearly identified even under the dark field condenser because of the significant concentration of grains in the control animals (Figs. 1 and 5). No such significant concentration was clear in the hypothyroid animal. On P15, as reported previously (21), in the ML, grains were more densely concentrated in the inner portion than in the outer portion in the control animal, but were evenly distributed in the hypothyroid animal (Fig. 2). This change was restored by daily T_4 replacement. On P20, EGL was still clearly seen in the hypothyroid animal but was no longer clear in the control animal (Fig. 3). Distribution of the grain in the ML and IGL seemed to be essentially the same in the control and hypothyroid animals on P20. On P30, no differ-

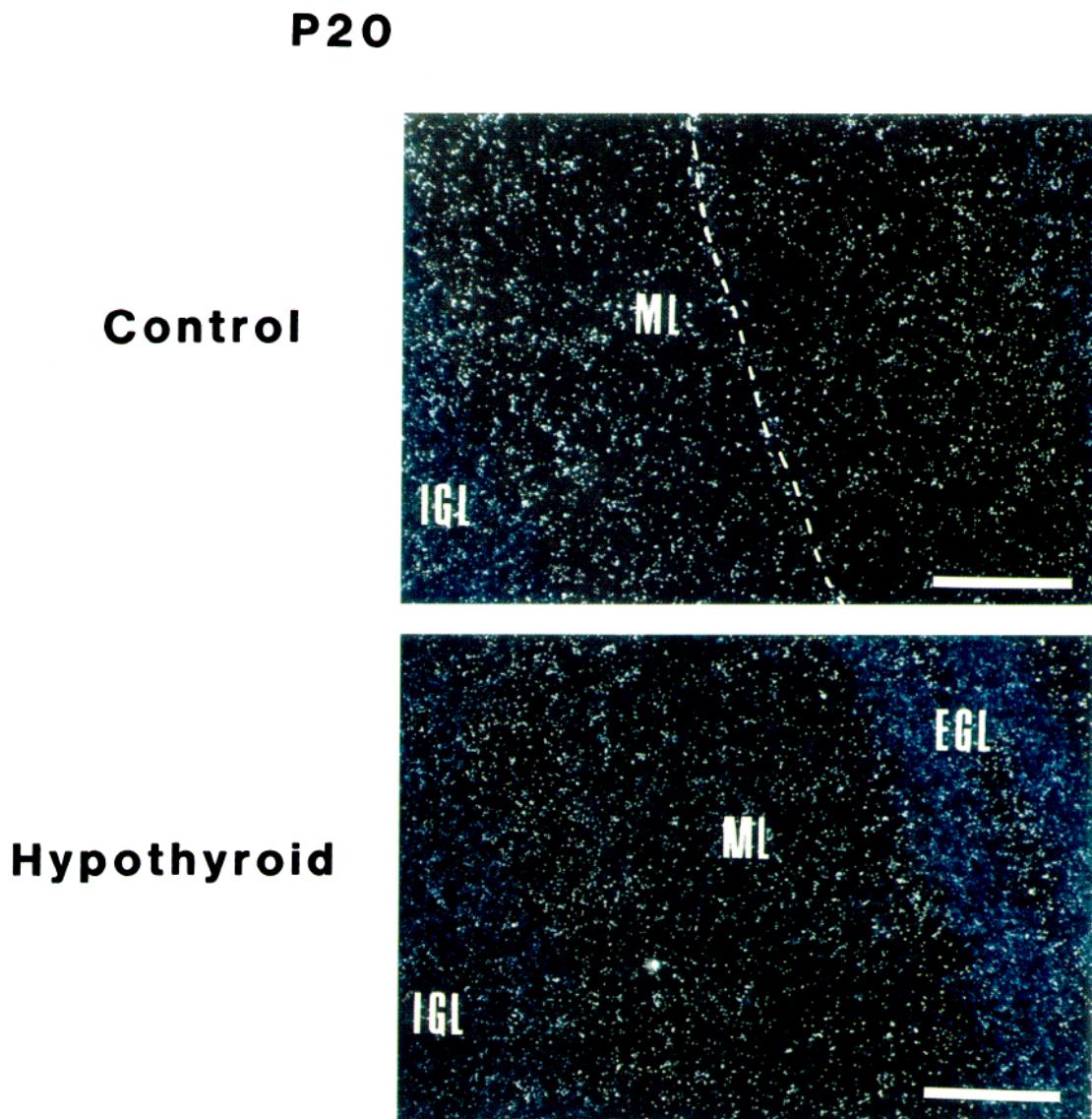


FIG. 3. Dark field photomicrographs showing the *in situ* hybridization detection of COX I mRNA within the cerebellar cortex (lobule IX of the vermis) of control and hypothyroid rats on P20. Hybridization and autoradiography were performed simultaneously with the sections shown in Figs. 1 and 2. The white line indicates the cerebellar fissure. Scale bar length, 100 μ m.

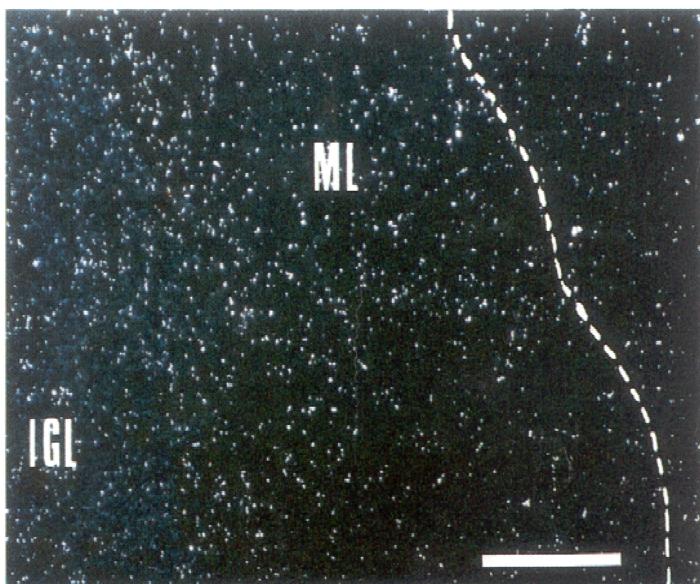
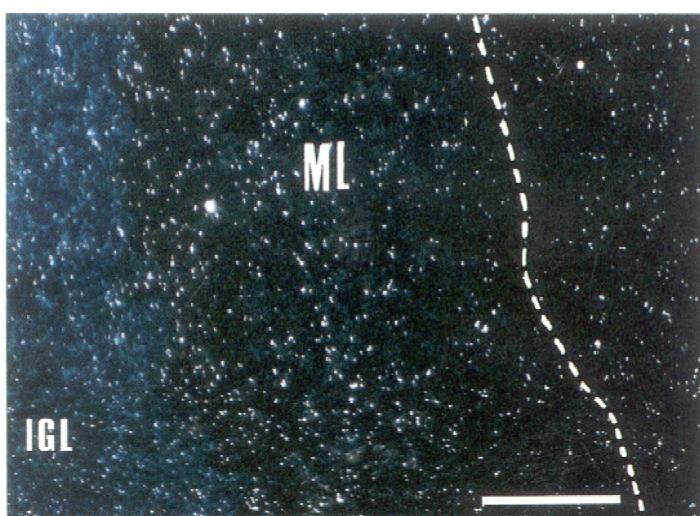
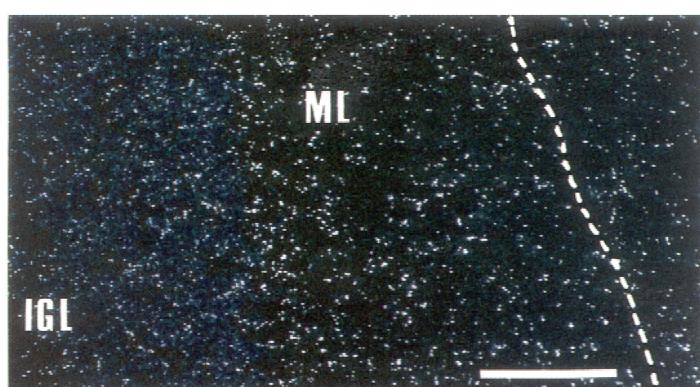
P30**Control****Hypothyroid****T₄-replaced**

FIG. 4. Dark field photomicrographs showing the *in situ* hybridization detection of COX I mRNA within the cerebellar cortex (lobule IX of the vermis) of control, hypothyroid, and T₄-replaced rats on P30. Hybridization and autoradiography were performed simultaneously with the sections shown in Figs. 1–3. White lines indicate the cerebellar fissure. Scale bar length, 100 μ m.

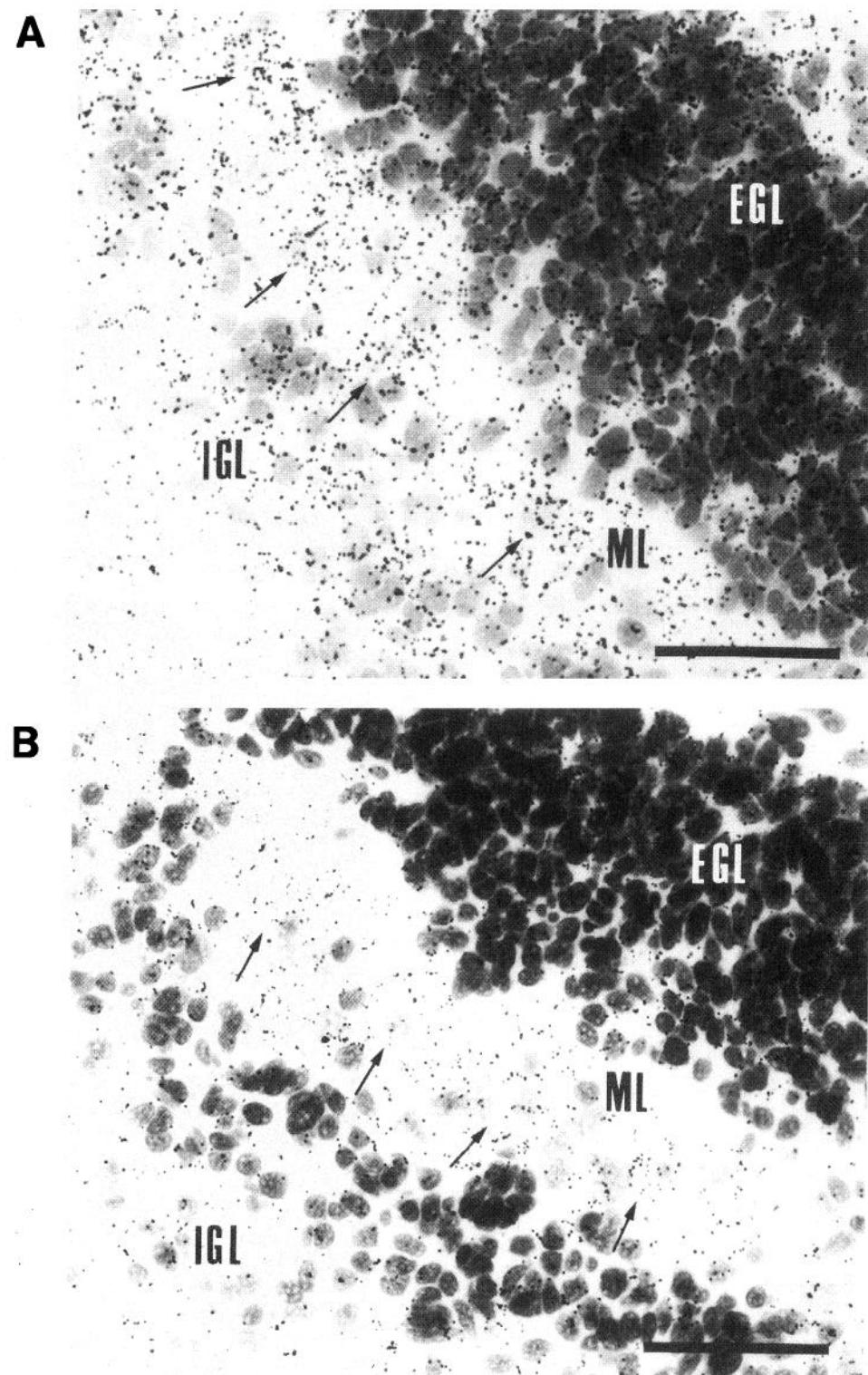


FIG. 5. Bright field photomicrographs showing the *in situ* hybridization detection of COX I mRNA in the euthyroid (A) and hypothyroid (B) rat cerebellar cortex on P5. Arrows indicate the localization of Purkinje cells. Scale bar length, 50 μ m.

ence in the grain distribution was seen among the control, hypothyroid and T_4 -replaced animals (Fig. 4).

The change in relative levels of COX I mRNA was expressed by the change in grain density, as shown in Fig. 6. On P1, no significant difference between the control and hypothyroid animals in the grain density was seen in any area. On P5, grain density was significantly lower in the

hypothyroid rats in all areas. The difference was greater in the EGL and IGL than in the ML. On P10 and P15, a marked difference in the grain density was seen in all areas. Daily T_4 replacement for 15 days abolished the effect of the methimazole treatment. No effect of methimazole treatment was seen in the ML and IGL on P20 and P30. On P20, however, the grain density was slightly less in the IGL and ML. North-

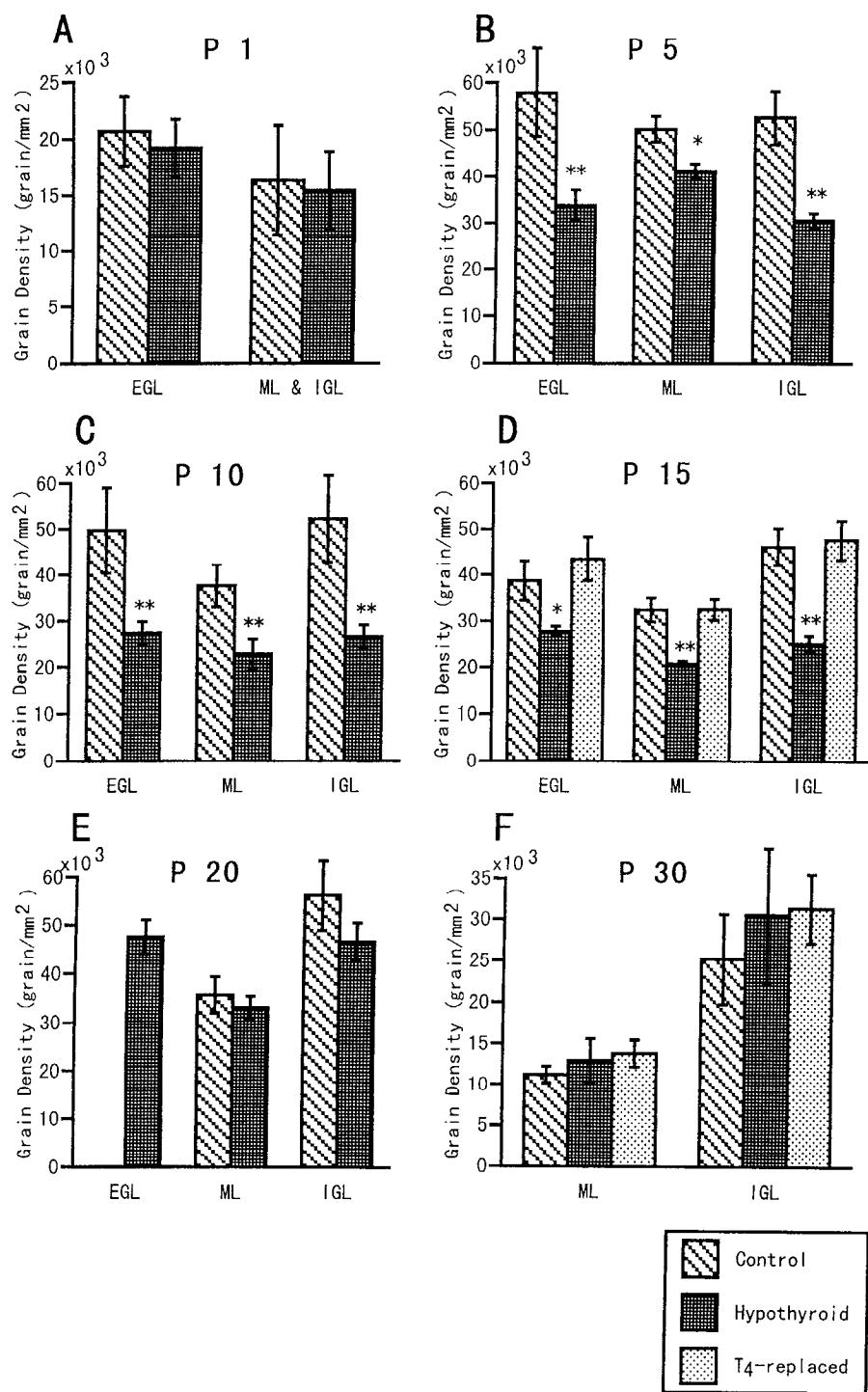


FIG. 6. Quantitative analysis of the change in relative levels of COX I mRNA within the EGL, ML, and IGL of the rat cerebellar cortex (lobule IX of the vermis) on P1 (A), P5 (B), P10 (C), P15 (D), P20 (E) and P30 (F). Data are expressed as grain density per unit area (grain/mm²). Shown are the mean \pm SEM. Note that, because the boundary between ML and IGL was not clear on P1, grain density was determined together. **, $P < 0.01$, *, $P < 0.05$ vs. same area in control animal

ern blot analysis showed a slight decrease in the hybridization signal for COX I mRNA in the rat cerebellum on P20 (data not shown). A slight difference could therefore still remain. On P30, no longer was any difference among the three groups seen in the grain density.

The ontogenetic changes in relative levels of COX I mRNA in each area are shown in Fig. 7. In the EGL, a marked increase in grain density on P5 and its gradual decrease thereafter were seen in the control animal. No such increase was seen in the hypothyroid animal by P15 but was signif-

icantly increased on P20. Because we quantified the grain density in the ML and IGL together on P1, whether the increase in grain density on P5 was induced in the ML and IGL was not clarified, but, by comparing the grain density of ML + IGL on P1 (Fig. 6A) to that of ML or IGL on P5 (Fig. 6B), it appears that such increase was also induced. In the ML, as width increased, grain density gradually decreased. Density was lower in the hypothyroid animal from P5 to P15. Furthermore, because we found that the grain density is greater in the inner portion of the ML, we measured the grain density

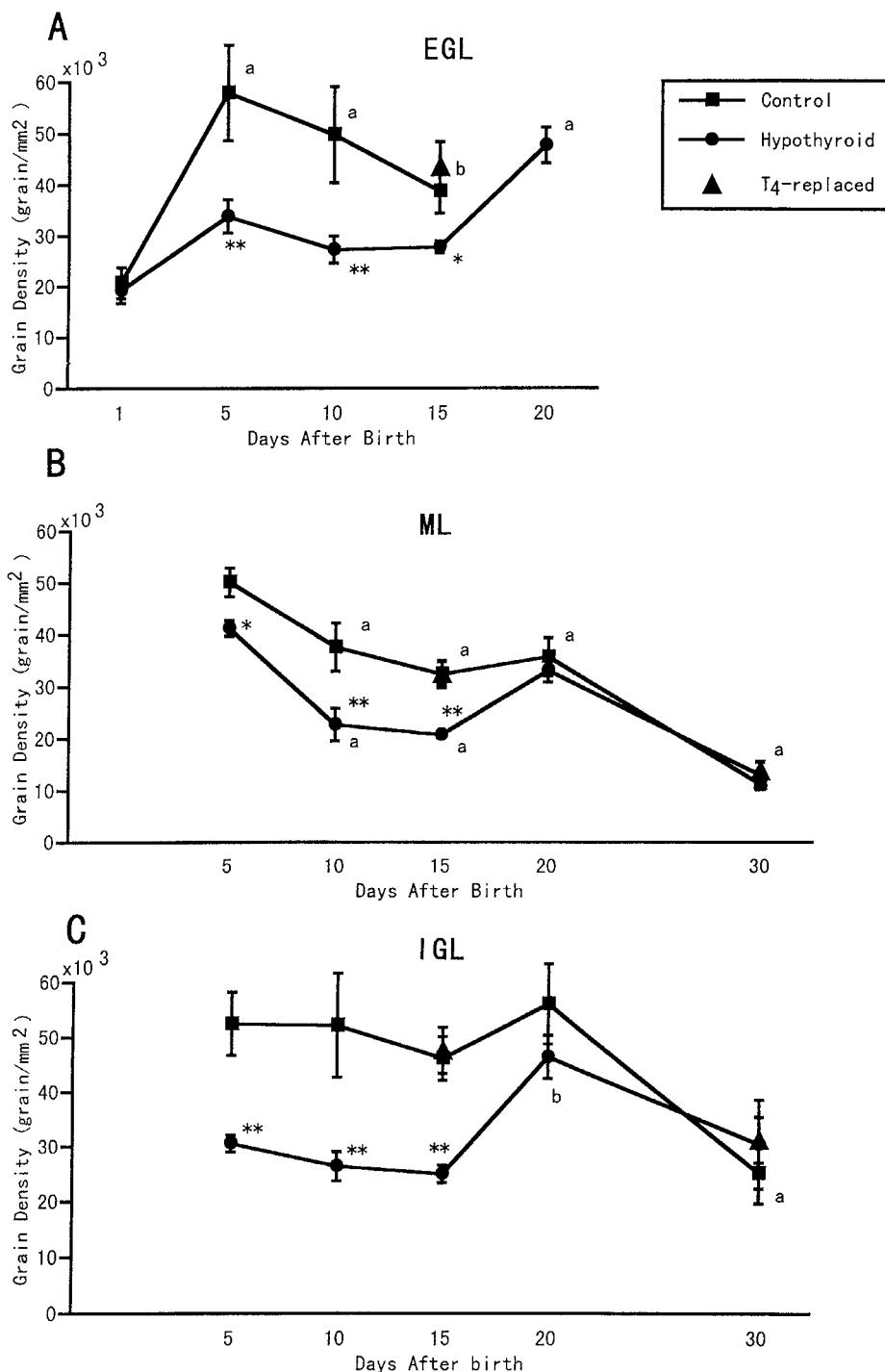


FIG. 7. Ontogenetic change in relative levels of COX I mRNA and the effect of perinatal hypothyroidism within EGL (A), ML (B), and IGL (C) of the cerebellar cortex. **, $P < 0.01$; *, $P < 0.05$ vs. control animal on the same day of sacrifice. a, $P < 0.01$; b, $P < 0.05$ vs. P1 (A) or P5 (B&C) with the same treatment.

of inner and outer portions of the ML separately on P15, P20, and P30 (Figs. 8 and 9). As shown in Fig. 8, grain density was greater in the inner portion in the control animal and T₄-replaced animal, but no such difference was seen in the hypothyroid animal. On P20, by ANOVA, grain density was found to be greater in the inner portion than in the outer portion ($P < 0.01$) (Fig. 8), but a statistical difference between the outer and inner portions was seen only in the control animal. This tendency was no longer seen on P30. As shown in Fig. 9, a marked increase in COX I gene expression is

induced in hypothyroid animal on P20 in both portions. In the IGL, the grain density was much lower in the hypothyroid animal by P15 (Fig. 7). This tendency was restored by daily T₄ replacement. Density was gradually increased in the hypothyroid animal from P15 to P20 (Fig. 7).

Discussion

In the present study, we examined the ontogenetic change in COX I gene expression and the effect of perinatal hypo-

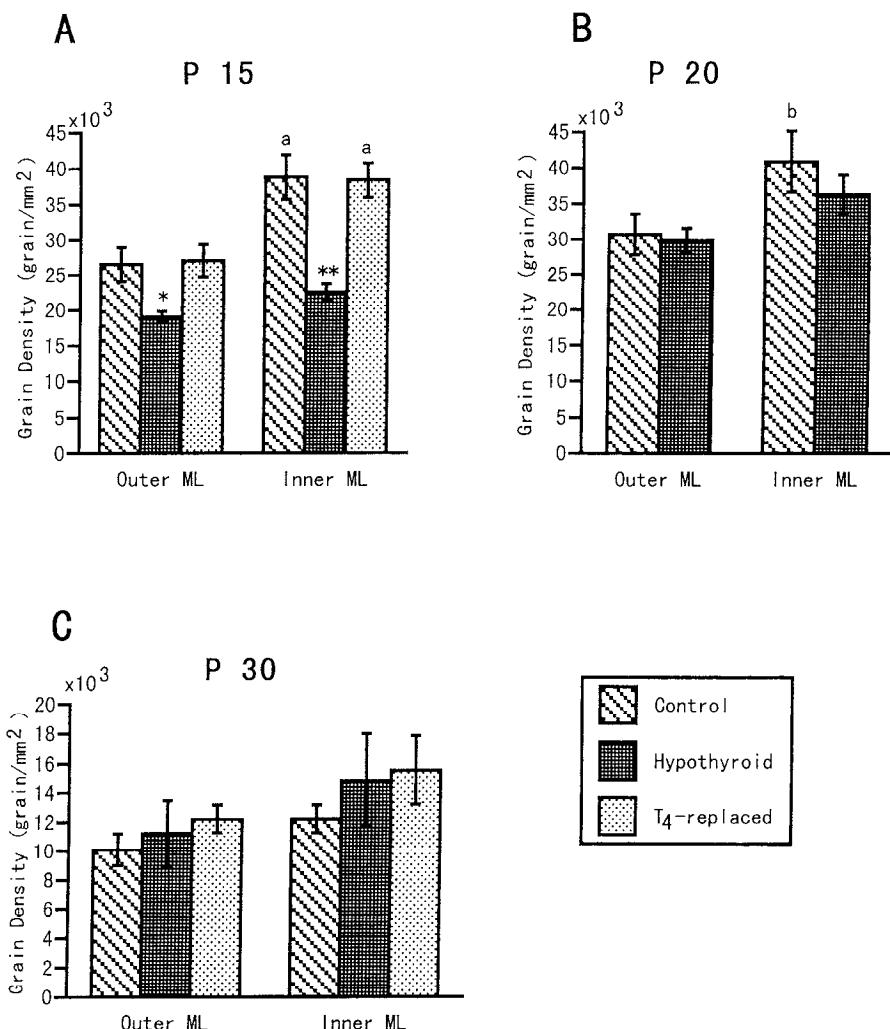


FIG. 8. Comparison of relative levels of COX I mRNA in outer and inner portions of the ML of the cerebellar cortex and the effect of perinatal hypothyroidism and T₄-replacement on P15 (A), P20 (B) and P30 (C). Note that, although no statistical significance was seen between the outer ML and the inner ML in the hypothyroid animal on P20, the grain density in the inner ML was greater than that in the outer ML by ANOVA ($P < 0.01$). **, $P < 0.01$; *, $P < 0.05$ vs. same area in control animal. a, $P < 0.01$; b, $P < 0.05$ vs. outer ML with the same treatment.

thyroidism on its expression in the cerebellar cortex by means of *in situ* hybridization technique. The results of the present study demonstrated the difference in timely expression of COX I gene among the EGL, ML, and IGL and a marked change in its expression in perinatal hypothyroid rats. We cannot determine whether the changes in COX I gene expression observed in the perinatal hypothyroid rat simply reflect the change in morphogenesis or are induced as a consequence of a specific effect of the thyroid hormone on COX I gene, but we consider that the effect is specific because the genes whose expression is not affected by the thyroid hormone during brain development are known. One study has shown that the expression of many genes in the developing rat brain is not affected by perinatal hypothyroidism (36). Furthermore, the content of c-erbA β 1 mRNA in the developing rat cerebellum is also not affected (37). As other genes whose expression is changed in association with altered thyroid states, COX I could therefore be one of the target genes of the thyroid hormone.

The thyroid hormone regulates the BMR through the change in the rate of oxygen consumption (32). It has been considered that this change is exerted by the effects of the thyroid hormone on mitochondrial metabolism (38). The COX is a complex of metalloproteins that plays a critical role

in cellular respiration in both prokaryotes and eukaryotes (25). Three subunits (I, II, and III) are encoded by genes on the mitochondrial chromosome (22, 34, 39), and other subunits are encoded on the nuclear chromosome (22). The thyroid hormone affects COX activity in the adult rat liver (40–43) and heart (44). These changes are induced in part by the change in transcription of COX subunit genes in the mitochondrial chromosome (45). On the other hand, the thyroid hormone little affects oxygen consumption in the CNS (23), but several metabolic processes related to oxidative phosphorylation (24–27) as well as COX activity (28) are changed in association with altered thyroid states in the developing rat brain. Furthermore, recent studies have indicated that the expression of several gene products that are encoded on the mitochondrial chromosome and related to oxidative phosphorylation is regulated by the thyroid hormone in the perinatal rat cerebrum (28, 30) and cerebellum (21). Taken together with the results of the present study, it is clear that the thyroid hormone affects the mitochondrial metabolism in the developing rat brain as well as in the peripheral organs.

COX I gene expression was affected by altered thyroid states in the rat cerebellum only during the critical period, during which the thyroid hormone plays a critical role in brain morphogenesis (46). As morphogenesis proceeds, en-

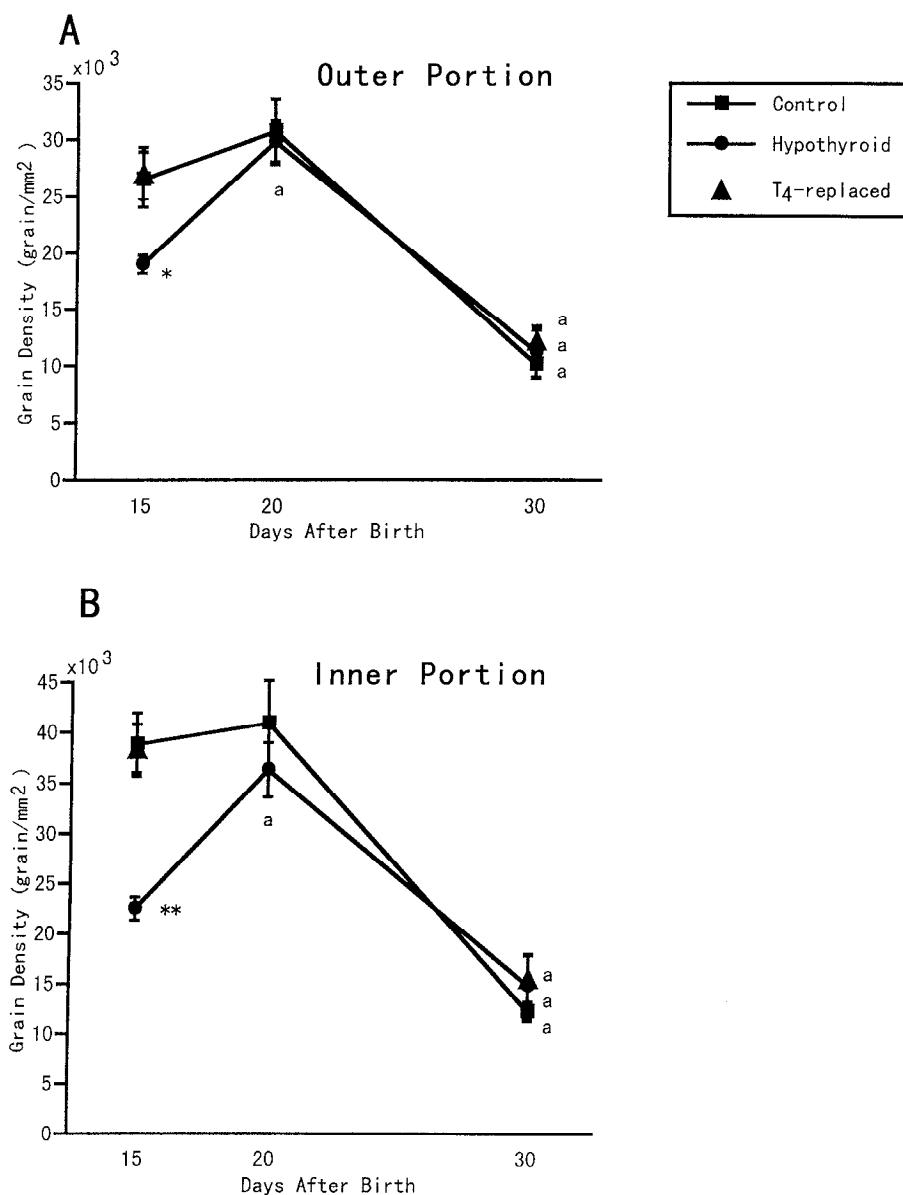


FIG. 9. Ontogenetic change in relative levels of COX I mRNA within outer portion (A) and inner portion (B) of the molecular layer and the effect of perinatal hypothyroidism and T₄-replacement. **, $P < 0.01$; *, $P < 0.05$ vs. control animal. a, $P < 0.01$; b, $P < 0.05$ vs. P15 (control and hypothyroid) or P20 (T₄-replaced) with the same treatment.

ergy demand must be increased. The COX I gene expression could be induced along with such morphogenetic processes within specific areas. After the critical period, when morphogenetic processes are terminated not only in the euthyroid but also in the hypothyroid rat and energy demand is decreased, the COX I gene expression could reach the same level regardless of the morphological differences.

In the EGL of the euthyroid animal, COX I gene expression was markedly increased on P5 and gradually decreased by P15. During this period, proliferation and differentiation of granule cells occur in this region (47). Because energy demand is increased with the increase in neurogenesis, COX I gene expression could be increased in parallel with the increase in neurogenesis. In the hypothyroid animal, the increase was seen on P20 (Fig. 7). These results suggest that the COX I gene expression to supply the energy for neurogenesis is retarded in the hypothyroid animal. Consequently, retardation of neurogenesis in the EGL of the hypothyroid animal, as reported previously (10, 11), could be induced.

Because COX I mRNA is transcribed from mtDNA, the hybridization signal could be located not only in the cell bodies but also in the dendrites, synaptic terminals, and neuronal fibers, so that a significant hybridization signal could be seen in the ML. As the ML developed, COX I mRNA was first exclusively located in the inner portion of the ML in the euthyroid animal and then it was diffusely expressed. This is consistent with the results of previous studies showing that intense staining of COX protein is first detected in the inner portion of the ML (6, 31). By electron-microscopy, synaptogenesis between Purkinje cells and parallel fibers also starts in the inner portion of the ML and gradually proceeds to the outer portion (6, 31), and a close correlation between synaptogenesis and COX activity has been reported (31), suggesting that energy demand is increased with synaptogenesis. On the other hand, no such significant concentration of COX I mRNA was seen in the hypothyroid animal. Previous studies have reported a decrease in dendritic arborization of the Purkinje cell and synaptogenesis between

the Purkinje cells and parallel fibers in the ML of the perinatal hypothyroid animal (11, 13, 15). In view of the fact that synaptogenesis and COX activity are closely correlated (31), we consider it likely that the decrease in synaptogenesis between Purkinje cells and parallel fibers is caused, at least in part, by the decrease in COX I gene expression.

Because the COX I gene is ubiquitously expressed in all closely packed cells, no attempt was made to differentiate the hybridization signals of individual cells in the IGL, but factors related to Purkinje cells and granule cells could be considered separately to explain the change in COX I gene expression in the IGL. The formation of somatic synapses between the Purkinje cells and climbing fibers starts around P3, reaches its peak at around P7, and gradually disappears by P15 (6, 31). The change in staining intensity of COX protein within the cell body of the Purkinje cells is closely correlated with the formation of synapses (31). In the present study, we saw a significant concentration of hybridization signals over the Purkinje cells on P5 and P10, but not on P15. These results indicate that synthesis of COX I in the Purkinje cells changes with synaptogenesis. In the hypothyroid animal, however, no such significant concentration was seen. It has been reported that synapses between Purkinje cell somata and climbing fibers in the hypothyroid rat cerebellum persist approximately 10 days longer than those in the euthyroid animal (48). The decrease in COX I gene expression observed in the present study could induce the retardation of the disappearance of axo-somatic synapses on the Purkinje cells. On the other hand, when granule cells migrate into the IGL, synaptogenesis between the granule cells and mossy fibers forms glomeruli (49). This synaptogenesis may also be involved in the significant concentration of hybridization signals in the IGL. The retardation of this synaptogenesis may also be caused by the decrease in COX I gene expression from P5 to P15 in the IGL. The increase in COX I gene expression in the ML from P15 to P20 in the hypothyroid rat could result in retarded synaptogenesis of the granule cells.

Whether the COX I gene expression in the cerebellum is directly regulated by the thyroid hormone is not yet known. Because replacement of thyroid hormone restored the decrease in COX I gene expression in all areas in the cerebellar cortex, COX I gene could be the gene that is regulated by the thyroid hormone and plays a critical role in thyroid hormone-dependent morphogenesis of the cerebellum. A recent study has shown the presence of thyroid hormone receptor within mitochondria (50). Furthermore, genomic analysis revealed that the NADH dehydrogenase subunit 3 gene, which is located in mtDNA, is capable of binding the thyroid hormone receptor (30). Although further study is required, COX I gene expression could also be directly regulated by the thyroid hormone at least during the critical period of brain development.

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