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Developmental Exposure to Polychlorinated Biphenyls Exerts Thyroid Hormone-Like Effects on the Expression of RC3/Neurogranin and Myelin Basic Protein Messenger Ribonucleic Acids in the Developing Rat Brain*

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ABSTRACT

Polychlorinated biphenyls (PCBs) are a class of industrial compounds consisting of paired phenyl rings with various degrees of chlorination. They are now ubiquitous, persistent environmental contaminants that are routinely found in samples of human and animal tissues and are known to affect brain development. The effects of PCBs on brain development may be attributable, at least in part, to their ability to reduce circulating levels of thyroid hormone. However, the developmental effects of PCB exposure are not fully consistent with hypothyroidism. Because some individual PCB congeners interact strongly with various thyroid hormone binding proteins, several investigators have speculated that these congeners may be producing thyroid hormone-like effects on brain development. Therefore, we tested whether a mixture of PCBs, Aroclor 1254 (A1254), would produce an antithyroid or thyromimetic effect on the expression of known thyroid hormone-responsive genes in the developing brain. Pregnant female rats were fed various doses of A1254 (0, 1, 4, and 8 mg/kg) from gestational day 6 to weaning on postnatal day (P) 21. Pups derived from these dams were sampled on P5, P15, and P30. Total T_4 was reduced by A1254 in a dose-dependent manner, but body weight of the pups or dams was not affected. The expression of RC3/Neurogranin and myelin basic protein was not affected by A1254 on P5 or P30. However, on P15, RC3/Neurogranin was elevated by A1254 in a dose-dependent manner, and myelin basic protein expression followed this general pattern. These data clearly demonstrate that the developmental effects of PCB exposure are not simply a function of PCB-induced hypothyroidism. (*Endocrinology* **141:** 181–189, 2000)

POLYCHLORINATED BIPHENYLS (PCBs) are a class of industrial compounds consisting of paired phenyl rings with various degrees of chlorination (1). Before their production was banned in the 1970s, over a billion kilograms of PCBs were produced (2); and they are now ubiquitous, persistent environmental contaminants that are routinely found in samples of human and animal tissues (1, 3). PCBs become concentrated especially in fatty tissues because they are highly lipophilic. The observation that PCBs become concentrated in human milk is particularly concerning because concentrations of individual congeners reported for milk samples taken from women exposed to local background PCB levels and actively breast-feeding their infants range from 38.3 ng/g lipid (4) to 395 ng/g lipid (5), corresponding to approximately 1.28 μg/ml milk (3.52 μM) to 13.2 μg/ml milk (36.3 μM) (6). Thus, the potential magnitude of PCB exposure to infants through breast milk and other sources justifies concern about potential effects on development.

PCBs are known to be developmental neurotoxicants at environmentally-relevant concentrations (7–11). The most commonly noted neurological abnormalities associated with low-levels of PCB contamination in humans are hypoactivity and impaired learning (3). Because the symptoms of PCB exposure can overlap with those of thyroid dysfunction, several investigators have speculated that the neurological consequences of incidental exposure to PCBs are caused by disruption of the thyroid axis (12, 13). Many reports document that PCBs reduce circulating levels of thyroid hormone (reviewed by Refs. 13, 14, and 15), an effect believed to be produced by the simultaneous activation of liver UDP-glucuronosyltransferase involved in thyroid hormone metabolism (16) and displacement of T_4 from serum proteins (14). These two effects seem to interact to produce a significant reduction in circulating levels of total and free T_4, and total and free T_3 (14, 17, 18, 19). These observations have lead to the prediction that PCBs effectively produce neurological deficits by producing hypothyroidism. Several lines of evidence support this prediction. For example, PCB exposure reduces circulating levels of thyroid hormone and produces hearing loss in rats (18) that can be partially ameliorated by T_4 administration (20). In addition, T_4 can normalize the PCB-induced suppression of choline acetyltransferase activity in the forebrain of neonatal rats (21). Finally, perinatal PCB exposure can increase testis size of the adult rat (22), an effect that is identical to that of perinatal treatment with goitrogens (23).

However, the structure of some PCB congeners may resemble that of thyroid hormone enough to interact with the thyroid hormone receptor (TR) (24), acting as agonists, antagonists, or mixed agonists (25). This hypothesis also is supported by several lines of evidence. For example, PCB exposure does not produce a compensatory increase in circulating TSH, despite profound hypothyroxinemia (re-

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viewed in Refs. 14 and 16), suggesting that unidentified individual PCB congeners are suppressing TSH (14, 16). In addition, developmental exposure to PCBs advances the onset of eye opening in rats (18), an event associated with hyperthyroidism. In humans, children exposed to high levels of PCBs can exhibit hyperactivity (26), a symptom that also may be associated with subclinical hyperthyroidism (27). Finally, Cheek *et al.* (28) have recently shown that some individual PCB congeners can bind to the human TRβ1. These studies support the speculation that individual PCB congeners can directly interact with TRs.

The hypothesis that individual PCB congeners are interacting with the TR suggests that the effects of PCBs on brain development may not be purely a function of PCB-induced hypothyroxinemia. To test this hypothesis, we examined whether PCBs produce antithyroid or thyromimetic effects on specific thyroid hormone-responsive gene expression in the developing brain. TRs are hormone-activated transcription factors (29, 30) that regulate the expression of specific genes in the brain during development (31–33). Therefore, we focused on two well-characterized thyroid hormone-responsive genes, RC3/Neurogranin (34) and myelin basic protein (MBP) (35–37).

Materials and Methods

Animal treatment

All procedures were performed in accordance with the NIH guidelines for the ethical treatment of animals and were approved by the University of Massachusetts-Amherst Institutional Animal Care and Use Committee before initiating these studies.

Timed-pregnant Sprague Dawley rats (n = 33) were purchased from Zivic-Miller Laboratories, Inc. (Pittsburgh, PA) and arrived in our animal facility 2 days after insemination [gestational day (GD)2]. They were individually housed in plastic cages, were provided with food and water continuously, and were maintained on a 12-h light, 12-h dark cycle (0600 h–1800 h). Beginning on the day of arrival, each dam was weighed in the morning and provided with a single wafer 1 h before lights off. This initial training period (GD2–GD6) was required for the animals to consume a wafer quickly during the experimental procedure. Beginning on GD6 and continuing until weaning on postpartum day (P) 21, the dams were weighed in the morning and provided daily with a wafer dosed with 50 μl/100 g BW of a solution calibrated to deliver specific doses of a commercial mixture of PCBs [Aroclor 1254 (A1254); AccuStandard, Inc., New Haven, CT]. Wafers were individually dosed each morning based on the dam's weight. PCBs were dissolved in contaminant-free methanol, pipetted onto the wafer, and allowed to dry in a fume hood throughout the day before feeding. Control wafers (0 mg/kg PCB) were dosed with methanol alone. Doses of A1254 included 0, 1.0, 4.0, and 8.0 mg/kg BW. Because earlier reports indicated that daily administration of 8 mg/kg A1254 may reduce the rate at which body weight increases in pregnant rats (18), we measured food consumption by dams in the 8-mg/kg group and pair-fed an additional control group (0-mg/kg) to this amount of food. However, we found no differences in food consumption or body weight in the 8-mg/kg group. Therefore, our control group (0-mg/kg) contains twice the number of animals as PCB-treated groups (n = 12 *vs.* n = 6, respectively).

On P5, P15, and P30, one pup from each litter was weighed and killed by decapitation. Trunk blood was collected for measurement of serum total T_4. The head (P5) or brain (P15 and P30) was frozen in pulverized dry ice, labeled, and stored at −80 C until it was sectioned for *in situ* hybridization. Additionally, one pup from each litter was also weighed and killed on P1, P10, and P20 for measurement of serum T_4. Only males were included on P30. A follow-up study in which male and female pups were compared for their responses to A1254 exposure on P5 and P15 revealed no treatment effects on body weight or thyroid hormone (data not shown).

In situ hybridization

Frozen brain tissues were sectioned in coronal plane at 12 μm in a cryostat (Reichert-Jung Frigocut 2800N, Leica Corp., Deerfield, IL). Coronal sections were made through the rostral hippocampus at approximately 2.8–3.8 mm caudal to bregma, corresponding to Figs. 29–33 of Paxinos and Watson (38), and through the cerebellum at approximately 12.72–13.68 mm caudal to bregma, corresponding to Figs. 68–72. Sections were thaw-mounted onto cold gelatin-coated microscope slides and stored at −80 C until hybridization. Prehybridization treatments, hybridization, and posthybridization washes were carried out for RC3/Neurogranin as described earlier (39), with a few exceptions as follows. First, the sections were immersed for 30 min in 4% formalin, and the hybridization was performed at 52 C. Second, the hybridization buffer did not contain single-stranded DNA. Finally, the ribonuclease treatment, after hybridization, was performed in a buffer containing 10 mM Tris/1 mM EDTA/2 × SSC, at pH 7.4. The *in situ* hybridization protocol for MBP messenger RNA (mRNA) was performed as described (40), except that the hybridization buffer contained 200 mM dithiothreitol.

Probe preparation

The RC3 RNA probes (complementary or sense-strand) were generated *in vitro* from an RC3 cDNA, kindly provided by Dr. Juan Bernal [pPRC/CMV-RC3 (34), Madrid, Spain]. The transcription reaction was performed in a final vol of 10 μl. RNA was synthesized in the presence of 1 μg DNA template (linearized plasmid); 500 μM each of GTP, ATP, and CTP; and 12 μM UTP (UTP + ^{33}P-UTP at a molar ratio of 1:1). pPRC/CMV-RC3 was linearized with *Hin*dIII and transcribed in the presence of SP6 RNA polymerase for complementary RNA production. After transcription, the DNA template was removed by deoxyribonuclease digestion, and the probe was purified by phenol(−)chloroform extraction followed by two ethanol precipitations. The size and integrity of the ^{33}P- RC3 probe (337 bp) was verified on a 6% sequencing gel. The probe used to quantify MBP mRNA was an oligonucleotide that we have previously characterized (40). This 48-base oligonucleotide was labeled with ^{33}P-deoxy-ATP using terminal deoxynucleotidyl transferase, as previously described (40).

Autoradiography and signal quantitation

Slides were arranged in x-ray cassettes and apposed to BioMax film (Eastman Kodak Co., Rochester, NY) for periods that depended on the specific activity of the probe and the abundance of the target message. For RC3/Neurogranin mRNA, these periods were 24 h, 15 h, and 17 h for P5, P15, and P30, respectively. For MBP mRNA, these periods were 10 days, 4 days, and 3 days for P5, P15, and P30, respectively. ^{14}C-standards (American Radiolabeled Chemicals, Inc., St. Louis, MO) were simultaneously apposed to the film to ensure that the film was not overexposed. For RC3/Neurogranin in P15 brains, the slides were dipped in Eastman Kodak Co. NTB-3 nuclear tract emulsion after film autoradiography. These emulsion autoradiograms were developed in Dektol, fixed in Eastman Kodak Co. Fixer, and counterstained with Methyl Green (Sigma, St. Louis, MO).

Regional analysis of gene expression was performed as follows. First, a 5× magnified image was captured using a Scion AG-5 capture board interfaced with the public domain NIH-Image 1.61/ppc (W. Rasband, National Institute of Mental Health, Bethesda, MD) being run on a Macintosh 7600. The optical system included a Dage-MTI 72 series video camera equipped with a Nikon macro lens mounted onto a bellows system over a light box. The relative level of expression of RC3/Neurogranin mRNA was measured by microdensitometry, in which the area of the specific brain region and the film density was measured. Brain areas included the occipital cortex (Oc2) (medial and lateral aspects of layer 2), retrosplenial granular cortex (RSG), piriform cortex (Pir), dentate gyrus (DG), CA1, CA2, and CA3 subfields of Ammon's horn (see Fig. 4C). The RSG, Pir, and DG have been shown previously to be areas in which RC3/Neurogranin expression is affected by thyroid hormone (34). For RC3/Neurogranin expression, the signal was evaluated using the greyscale setting by encircling the target brain region and acquiring the area of the identified region and the average density of that area of film. The density value was corrected by subtracting the density of an adjacent area of film. MBP expression was measured on film separately

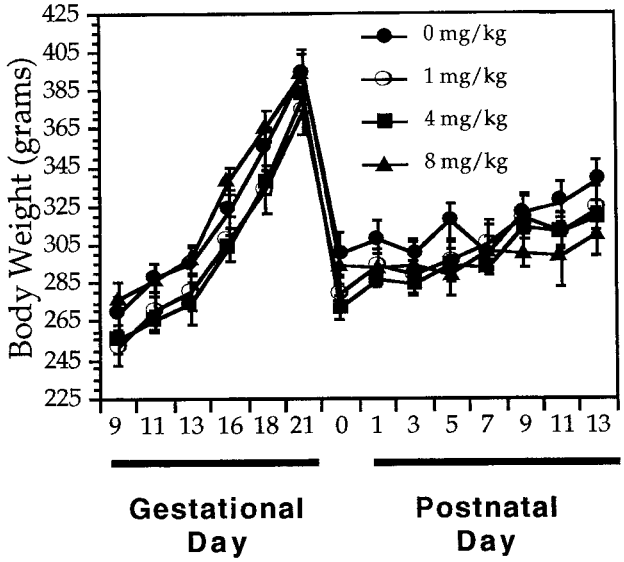

FIG. 1. Effect of A1254 on body weight of dams (grams) during the period of treatment. Dams were treated daily with different doses of A1254 as shown in the legend. No significant effect of treatment was observed ($F(\text{treatment})_{3,312} = 0.666$; $P = 0.5810$).

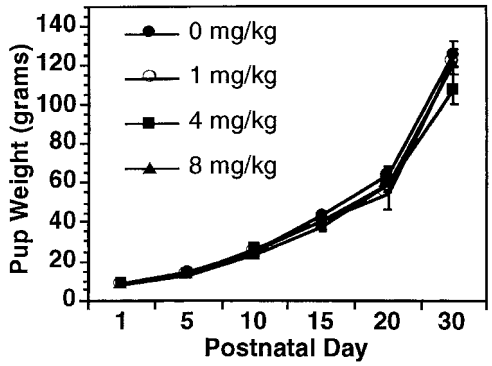

FIG. 2. Effect of A1254 on body weight of pups. Dams were treated daily with different doses of A1254 as shown in the legend. No effects were observed at any age.

over the entire cerebellum or the entire pons/medulla (Fig. 1B) using a threshold value that allowed us to electronically subtract background. This was required because of the striated nature of MBP expression in the cerebellum (see Fig. 4D). For both RC3/Neurogranin and MBP, the resulting values were averaged over four sections from each brain. Measurements were taken by two independent operators who were unaware of the identify of the signals. Concordance between operators was always observed.

Single-cell analysis of RC3/neurogranin mRNA

For single-cell analysis of RC3/Neurogranin expression in cells of the RSG, we used the technique described earlier (41), with some exceptions. First, we used a Nikon (Japan) ES-600 microscope at 400× magnification. Second, brain sections were counterstained with methyl green, which did not require the use of a blue filter. The study produced two microscope slides, each containing 2 sections through the RSG, for each of the 22 brains included in this part of the study. The slides were coded, randomized, and analyzed by an operator unaware of the identity of each sample. Ten individual cells within the RSG were chosen for analysis, based on the ability of the operator to identify the edges of the cell. We show (see Fig. 7) how cells appear in both clusters of cells and as individuals that can be reasonably measured as single cells. In this way, we limited the possibility of measuring grain density over multiple cells and ascribing the value to a single cell. The cluster of grains over individual cells were encircled, and grain number was evaluated. After all brain sections were analyzed, the data were pooled, the codes revealed, and statistical analysis performed.

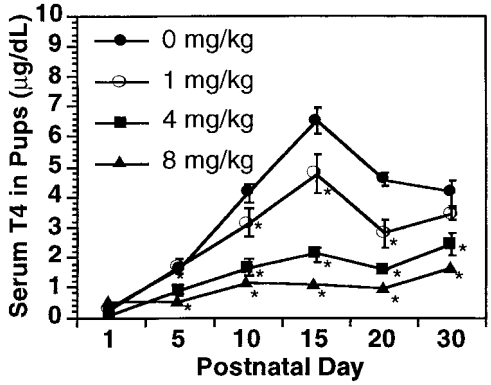

FIG. 3. Circulating concentrations of total T_4 in pups during A1254 exposure to the dams. Dams were treated daily with different doses of A1254 as shown in the legend. As expected, there was a postnatal rise in serum total T_4, peaking on P15. However, total T_4 was significantly reduced by A1254, in a dose-dependent manner, from P5 to P30. Pups derived from dams treated with 4 or 8 mg/kg/day did not exhibit a postnatal peak in total T_4. Note: lower limit of detection was 10 μg/dl.

RIA

Total T_4 was measured according to the manufacturer's instructions, using a total T_4 RIA kit (ICN Diagnostics, Costa Mesa, CA). This assay was performed at 40% binding with a standard range of 10–200 μg/dl and an intraassay variation of 3.5%. All samples were measured in duplicate in the same assay.

Statistical analysis

Effects of treatment on body weight of the dams was evaluated using a one-way ANOVA with repeated measures; hormone levels and body weight of the pups were evaluated using a two-way ANOVA with main factors of treatment and age. One-way ANOVA was performed on the average film density and area over various brain areas, and the average number of grains per cell was determined for emulsion autoradiograms. All analyses were performed using the SuperAnova software package (Abacus Concepts, Inc., Berkeley, CA) on all data. *Post hoc* tests, where appropriate, were performed by Bonferroni's t test, where the mean squared error term in the ANOVA table is used as the point-estimate of the pooled variance.

Results

Treatment with A1254 did not affect body weight (Fig. 1) or food consumption (data not shown) of the dams. In addition, treatment of the dams with A1254 did not alter the normal increase in body weight of the pups (Fig. 2; $F_{3,135} = 2.239$; not significant). In contrast, A1254 administration to the dams significantly reduced the concentration of circulating total T_4 in pups in a dose-dependent manner [Fig. 3; $F(\text{A1254})_{3,133} = 88.78$, $P \ll 0.001$; $F(\text{age})_{5,133} = 53.765$, $P \ll 0.001$]. The severity of hypothyroxinemia induced by A1254 was age-dependent, because the concentration of total T_4 in serum was age-dependent. On day 15, total T_4 levels were maximal in control animals and were progressively reduced in animals exposed to increasing doses of A1254; 8 mg/kg A1254 reduced total T_4 to below the detection limit for the assay.

Microdensitometry of the film autoradiograms after *in situ* hybridization for RC3/Neurogranin mRNA (Fig. 4) revealed that RC3 mRNA levels were not different among treatment groups in any brain region on P5 or P30 (Fig. 5). This could not be evaluated in the 4-mg/kg group on P5 because these samples were lost during processing. However, on P15, RC3 mRNA levels were significantly elevated in RSG, Pir, and DG of animals treated with 4 and 8 mg/kg A1254. In all cases, only signal density was different among treatment groups, not signal area. In contrast, RC3 mRNA levels were not different among treatment groups in the Oc2, or in CA1, CA2, or CA3 of the hippocampus.

To determine whether the results obtained for RC3/Neurogranin, using film autoradiograms, were attributable to a PCB-induced increase in cellular levels of mRNA, we evaluated the emulsion autoradiograms in the RSG, where single cells could be delineated. The average grain density over individual cells of the RSG was nearly 50% greater in animals exposed to 8 mg/kg A1254, compared with controls (Fig. 6; $F_{3,21} = 6.067$, $P = 0.0039$).

MBP mRNA levels in cerebellum and medulla were measured by microdensitometry of film autoradiograms after *in situ* hybridization (Fig. 7). MBP mRNA was not detected in the cerebellum of pups on P5, because MBP expression was below detection at this time (42). The relative levels of MBP mRNA were significantly reduced in the cerebellum and medulla of P15 animals treated with 1 mg/kg A1254 [$F(P15)_{3,23} = 4.926$; $P = 0.0087$].

Discussion

The purpose of this experiment was to test whether developmental exposure to PCBs produces antithyroid or thyromimetic effects on the developing brain. This question

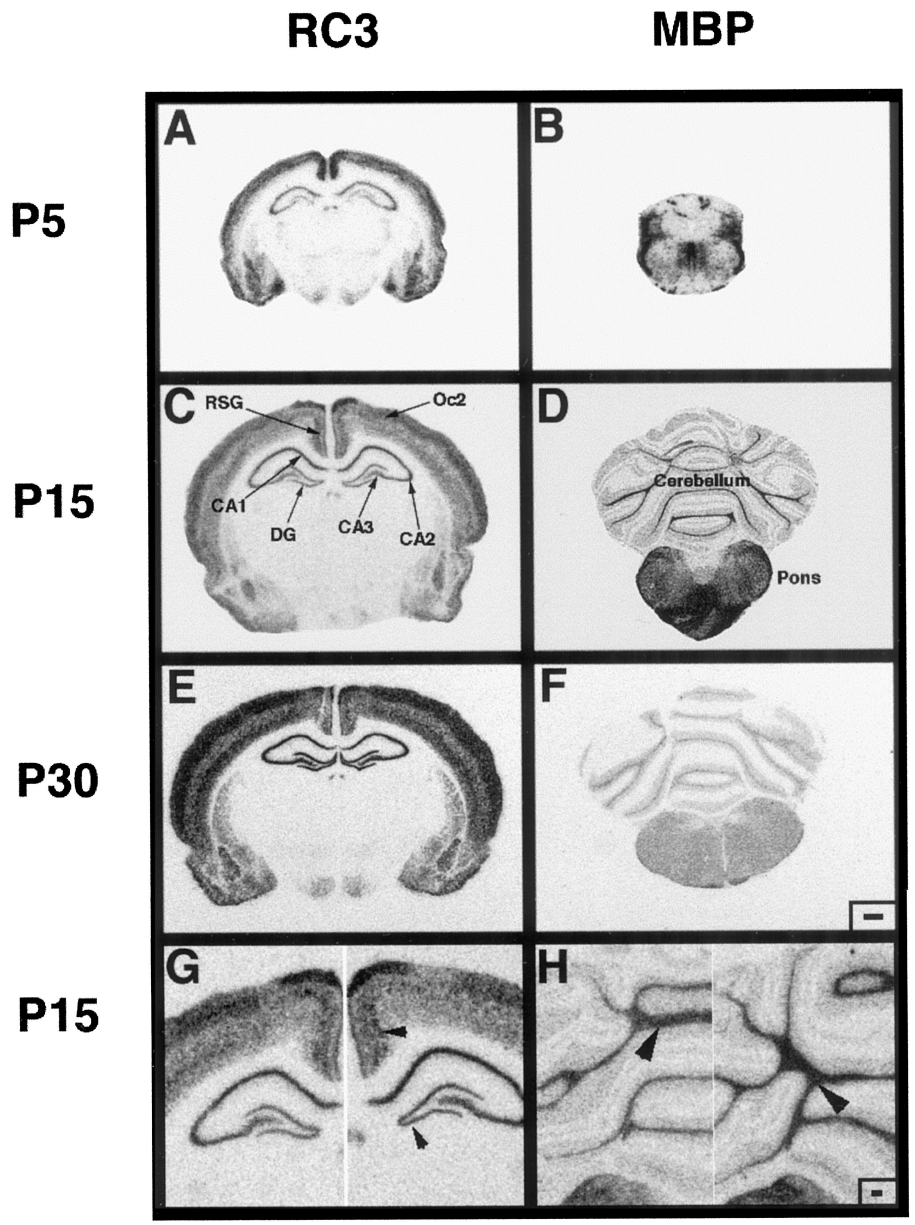

FIG. 4. Film autoradiograms after *in situ* hybridization for RC3/Neurogranin mRNA (A, C, E, and G) and MBP mRNA (B, D, F, and H). A–F were taken from control animals. G and H represents a composite to illustrate the difference in signal intensity between control animals (*left side of panel*) and those treated with 8 mg/kg A1254 (*right side*) for RC3 and MBP, respectively. *Arrowheads* identify regions that were significantly more intense in PCB-treated animals. Note that MBP mRNA was not detected in the cerebellum of P5 animals (B). *Scale bar* = 1.0 cm; A–F and G–H have the same magnification, respectively.

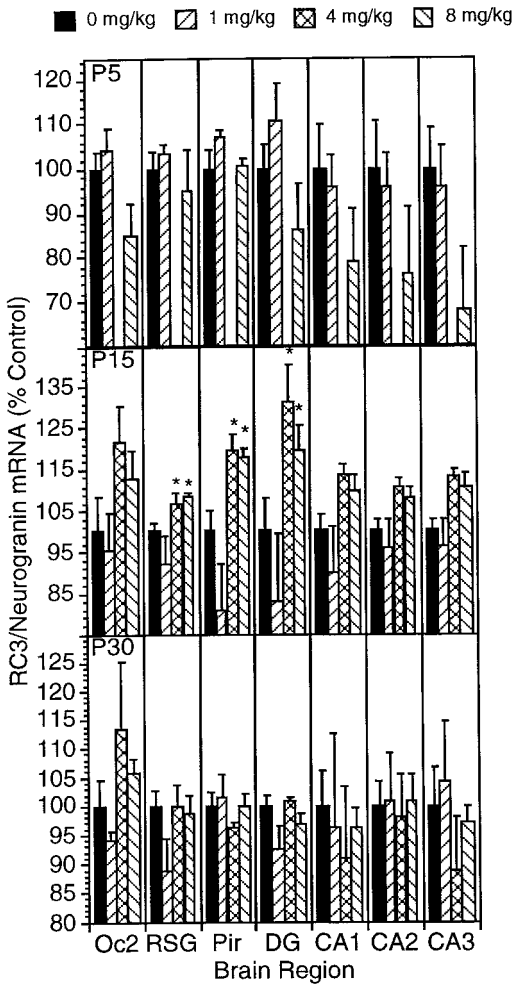

FIG. 5. Effect of A1254 exposure on RC3/Neurogranin mRNA levels in the developing brain. *Bars* represent mean ± SEM film density, presented as percent control for the purposes of illustration. Regional measurements of density and area were taken from different pups within each litter on P5, P15, and P30. There were no treatment effects on RC3 mRNA levels on P5 or P30. However, on P15, RC3/Neurogranin mRNA levels, as reflected in film density, were significantly elevated in RSG ($F_{3,25} = 3.027$; $P < 0.05$), Pir ($F_{3,24} = 3.129$; $P < 0.05$), and DG ($F_{3,26} = 3.436$; $P < 0.05$). RC3/Neurogranin expression was not affected by treatment in any other brain region. *, $P < 0.05$, compared with 0-mg/kg group, using Bonferroni's t test.

arises because PCB exposure is known to reduce circulating levels of thyroid hormone but to produce physiological or developmental effects that are not uniformly characteristic of antithyroid or thyromimetic actions (see first paragraph of text). Therefore, to directly address this question, we determined the effect of maternal PCB exposure on the expression of two genes known to be directly regulated by thyroid hormone during brain development. Our results confirmed that PCB exposure significantly reduces circulating levels of total T_4. This observation has been amply documented (14, 17, 18, 19), and others have shown that the same doses of A1254 also reduce free T_4, and total and free T_3 (18). However, despite severe reduction in circulating concentrations of thyroid hormone, our results demonstrate that on P15, when MBP and RC3/Neurogranin expression are most sensitive to thyroid hormone (43, 44), A1254 exposure elevated the expression of RC3/Neurogranin over that of controls. In addition, increasing doses of A1254 restored the expression of MBP in the developing brain despite the concomitant induction of severe hypothyroxinemia. The responses of MBP and RC3/Neurogranin expression to A1254 are clearly different from those predicted by the responses of these genes to chemical goitrogens such as propylthiouracil or methimazole (MMI). These goitrogens uniformly reduce the expression of MBP and RC3/Neurogranin in the developing brain (36, 34). Therefore, A1254 is not simply producing a hypothyroxinemic state that has predictable consequences based on studies with goitrogens.

Several aspects of the present data support the interpretation that maternal exposure to A1254 altered MBP and RC3/Neurogranin expression through the thyroid hormone signaling pathway. For example, thyroid hormone does not alter MBP expression in the cerebellum on P5 or P30, as shown by treatment with the goitrogen MMI (36). However, on P15, MMI treatment significantly reduces the expression of MBP in cerebellum. Thus, MBP expression exhibits the same temporal pattern of sensitivity to thyroid hormone and A1254. RC3/Neurogranin expression also is unaffected by MMI (45) or A1254 on P5. However, on P15, thyroid hormone regulates the expression of RC3/Neurogranin in the RSG and DG but not in CA1, CA2, CA3, or layers IV-II in the Oc2 (46). Considering that A1254 affected RC3/Neurogranin expression only in brain regions known to be sensitive to thyroid hormone, the effects of A1254 on RC3/Neurogranin expression are temporally and spatially similar to that of thyroid hormone.

The specific effects of A1254 on MBP and RC3/Neurogranin expression are different, but the general pattern is the same. For example, the relative abundance of MBP mRNA was significantly reduced by 1 mg/kg A1254, but higher doses of A1254 (4 and 8 mg/kg) restored levels to control values despite progressively more severe hypothyroxinemia. This general pattern was also observed in the response of RC3/Neurogranin expression. The 1 mg/kg dose did not affect RC3/Neurogranin expression, but higher doses (4 and 8 mg/kg) actually increased the abundance of RC3/Neurogranin mRNA. These results strongly suggest the possibility that two independent effects of A1254 exposure are operative in a dose-dependent manner. First, at lower doses, A1254 reduces circulating levels of T_4, and this effect produces consequences on the expression of some genes, such as MBP, and on developmental processes. At higher doses of exposure, specific PCB congeners may become concentrated in brain tissue to overcome or reverse the effects of the increasingly severe hypothyroxinemia, producing a thyromimetic effect.

The first effect of A1254 exposure is the obvious reduction in circulating T_4, and there is ample evidence supporting the concept that PCB-induced hypothyroxinemia directly affects brain development. For example, A1254 produces a hearing deficit in rats (18) similar to that produced by propylthiouracil treatment (47) that can be, at least partially, ameliorated with T_4 treatment (20). In addition, perinatal exposure to PCB reduces choline acetyltransferase activity in the cerebral cortex of rats, which was either partially or completely reversed by T_4 replacement, depending on brain area (21). Deficits in motor coordination, cognitive development, and

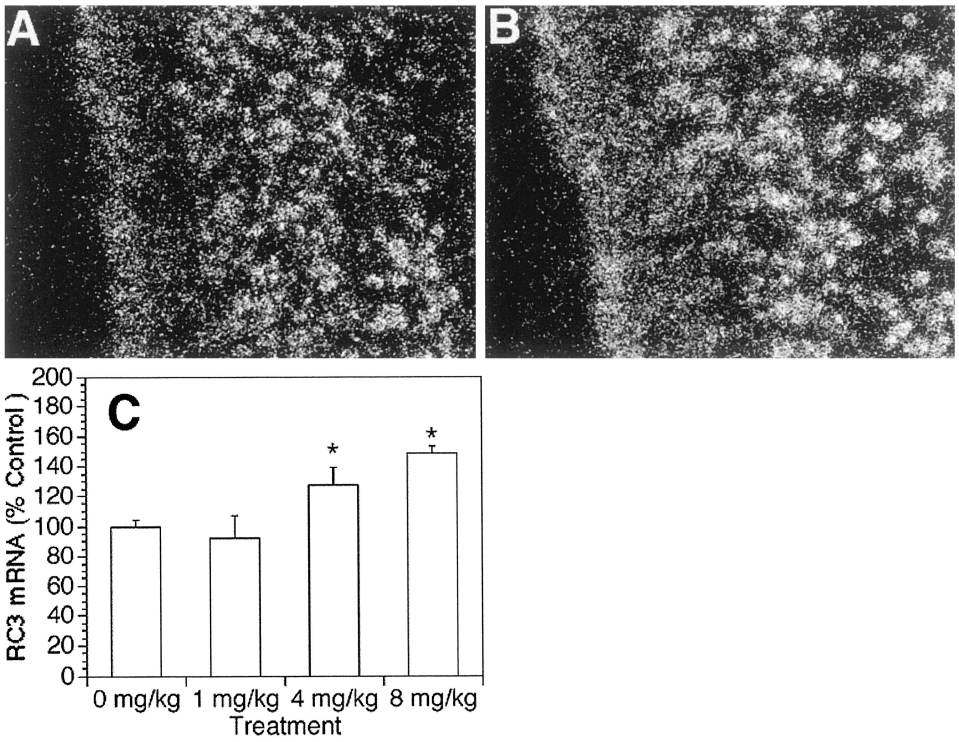

FIG. 6. Effect of A1254 exposure on cellular levels of RC3/Neurogranin mRNA in retrosplenial cortex (RSG). A, Darkfield image of RSG from representative section of pup brain, on P15, derived from control dam (0 mg/kg A1254). B, Darkfield image of RSG from representative section of pup brain, on P15, derived from a dam treated with 8 mg/kg. Magnification = 200×. C, Quantitation of single cell level of RC3/Neurogranin mRNA in RSG. *Bars* represent the mean ± SEM grain number per cell, displayed as percent control. *, $P < 0.05$, compared with 0-mg/kg group, using Bonferroni's *t* test.

muscular hypotonia in humans are some of the symptoms of congenital hypothyroidism that are correlated with background exposure to PCBs (12, 48). In Rotterdam (The Netherlands), PCB levels in women, chosen at random, were inversely related to serum total T_4 in their newborn children (49), and these PCB levels were also negatively correlated with birth size and early growth rate (50), as well as with various neurological measures (51). These observations lend credibility to the concern that PCB exposure can affect brain development by reducing circulating levels of thyroid hormone.

The second effect of developmental PCB exposure revealed by the present experiment is the thyromimetic effect that occurs at higher doses of PCB exposure. This effect was exemplified by the increased cellular expression of RC3/Neurogranin in the retrosplenial cortex. Considering that thyroid hormone increases the cellular expression of RC3/Neurogranin in the RSG by a transcriptional mechanism (46), it is possible that A1254 also is affecting both RC3/Neurogranin and MBP mRNA levels by a transcriptional mechanism. Therefore, it is possible that individual PCB congeners, or classes of congeners, can directly activate the TR, either as parent congeners or after hydroxylation or methylation. Several authors have speculated that specific PCB congeners are structurally similar enough to thyroid hormone to bind to the TR and perhaps influence thyroid hormone action (12, 13, 25, 28, 52). Individual PCB congeners can bind to several T_4-binding proteins, including transthyretin (53, 54), T_4-binding globulin (28), intracellular (55), and nuclear (52) T_4-binding sites. Cheek *et al.* (28) also have shown that specific PCB congeners can bind to the human TRβ1. Although the affinity of TRβ1 for individual PCB congeners (K_i = 32 μM) is low, the concentration of individual congeners in rat (56, 57) and human (58) brain tissue has been estimated to be as high as 50 μM (56). Though speculative, this hypothesis is consistent with the present results for both RC3/Neurogranin and MBP. In each case, the low dose of A1254 did not provide enough of these T_4-like PCB congeners to affect gene expression. However, at higher doses of A1254, thyroid hormone-like PCB congeners may have reached a concentration that restored MBP expression to normal, and increased RC3/Neurogranin expression, despite progressively more severe hypothyroxinemia.

The A1254 mixture used in the present experiment contains a large number of individual PCB congeners (59), which may account for these two effects on the thyroid axis. Some congeners are quite potent at reducing circulating levels of T_4, especially the non-*ortho*-substituted congeners that occupy a coplanar configuration such as 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) (60). However, these congeners do not become concentrated in brain tissue (57, 56, 61). In contrast, the *ortho*-substituted congeners that occupy a noncoplanar configuration, such as 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153), are not as potent at reducing circulating T_4 (60), but they do become concentrated in brain tissues of animals (56, 57, 61) and may bind to the TR (24). The observation that specific congeners, or classes of congeners, become selectively concentrated in tissues suggests that the dose and duration of exposure may interact such that a high dose/short exposure produces the same effects as low dose/long exposure.

It seems that metabolism of individual PCB congeners may be required for interactions with T_4-binding proteins. Brouwer and colleagues (19, 62) have shown that hydroxylated PCB congeners are present in fetal and neonatal rats born to dams gestationally exposed to PCBs. Moreover, hydroxy-

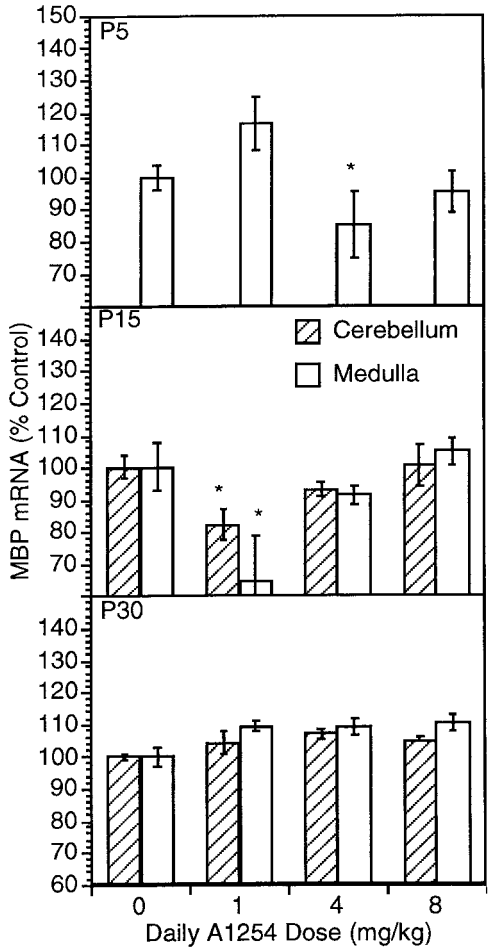

FIG. 7. MBP mRNA levels in the cerebellum and medulla of pups derived from dams treated with different doses of A1254. *Bars* represent mean ± SEM of film density, displayed as percent control. Measurements were taken from different pups within each litter on P5, P15, and P30. Treatment effects on MBP mRNA levels in the cerebellum were restricted to P15, where 1 mg/kg A1254 induced a significant decrease in MBP mRNA levels ($F_{3,23} = 3.291$; $P < 0.05$). MBP mRNA was also reduced in the medulla, at this time, by 1 mg/kg A1254 ($F_{3.23} = 4.926$; $P < 0.01$). Interestingly, only the integrated density of the MBP signal was affected in the pons/medulla. On P5, MBP expression was not detected in the cerebellum.

lated congeners exhibit a higher affinity for binding to transthyretin than their parent congeners (14). Considering the large number of 209 possible congeners present in A1254 (56, 59) and the number of possible metabolic modifications, it will be challenging to perform the kinds of *in vitro* studies required to test directly whether individual PCB congeners can bind to the TR and transactivate gene expression.

An alternate (or additional) mechanism by which PCB exposure may produce a thyroid hormone-like effect on the developing brain is by enhancing thyroid hormone uptake into tissues and increasing the conversion of T_4 to T_3. Specifically, PCB-induced hypothyroxinemia may induce cellular uptake of T_4 or T_3 and increase the expression of deiodinases responsible for intracellular conversion of T_4 to T_3. Tissue uptake of T_3 or T_4 is elevated by reduced levels of T_4 (63–65), which also increases the expression of type II deiodinase in brain (66, 67). However, these processes are affected by hypothyroxinemia caused by goitrogens or PCBs, indicating that the differences in effects of these two treatments may not arise from the induction of compensatory mechanisms alone. Clearly, further work is required to understand the role of T_4 transport and deiodination in the regulation of thyroid hormone action during periods of hypothyroxinemia caused by different agents.

In conclusion, the present study clearly demonstrates that developmental exposure to a complex mixture of PCBs produces a severe reduction in circulating levels of thyroid hormone, but thyroid hormone-like effects on the expression of two separate thyroid hormone-responsive genes. These thyroid hormone-responsive genes are expressed in different cell types; RC3/Neurogranin mRNA is expressed in neurons (45), whereas MBP mRNA is expressed in oligodendrocytes (68). Thus, A1254 exerts a similar action on two separate genes expressed in very different types of cells. These data support the concept that the effect of A1254 on MBP and RC3/Neurogranin expression is mediated by the thyroid hormone signaling system. It is possible that individual PCB congeners within the A1254 mixture interact directly with the TR. The functional consequences of the effects we have documented are presently unclear, but these data are important because neurological development can be impaired by too little (69, 70) or too much (71) thyroid hormone; and the possibility that individual PCB congeners may interact directly with the TR suggests that PCBs may produce effects on neural development that are not consistent with effects on circulating levels of thyroid hormone.

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