Perinatal Alterations of Thyroid Hormone Homeostasis and Long-term Neurochemical Alterations in Rats Following Maternal Aroclor 1254 Exposure.

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1. Introduction

Rogan et al. [1] suggested that the neurological deficits associated with prenatal polychlorinated biphenyl (PCB) exposure may be due to a mild hypothyroidism induced by these compounds. PCBs have been shown to decrease plasma thyroxine concentrations in mice, rats and marmoset monkeys [2,3,4]. Human exposure to PCBs has also been associated with alterations in thyroid function [5]. Previous research from our laboratory has shown that perinatal exposure to coplanar PCBs (3,3',4,4',5,5'-hexachlorobiphenyl and 3,3',4,4'-tetrachlorobiphenyl) results in reductions in fetal and neonatal rat plasma total thyroxine (TT4) and free thyroxine (FT4)[6]. These decreases in TT4 and FT4 were accompanied by significant increases in type II-5'-thyroxine deiodinase activity in brain homogenates, the enzyme responsible for the local deiodination of T4 to triiodothyronine (T3).
We therefore investigated if perinatal exposure to a commercial mixture of PCBs (Aroclor 1254) altered hepatic and brain thyroid hormone metabolism, plasma and brain thyroid hormone levels in fetal and neonatal offspring. We also investigated the effects of perinatal PCB exposure on the glial cell marker glial fibrillary acidic protein, and the neuronal cell marker synaptophysin in diverse brain structures in the neonatal and adult offspring. In addition, the level of calcineurin activity (localized in neurons) was investigated in the cerebella of the offspring as a marker for prenatal hypothyroidism.

2. Materials and methods:

Animals: Pregnant wistar rats were administered an oral dose of 0, 5 or 25 mg Aroclor 1254/kg bodyweight on day 10 to 16 of gestation. Pregnant animals were sacrificed on day 20 of gestation to examine fetal effects, male and female neonates were sacrificed on day 4 and 21 postpartum and adult offspring were sacrificed 90 days postpartum.
Thyroid hormones: Plasma TT4, FT4 and TT3 were analysed using a immunochemiluminesence kit, plasma TSH was determined using a radioimmunoassay specific for rat TSH, both kits were obtained from Amersham, Amersham, UK. Levels of T3 and T4 in brain tissue were determined after extraction by a highly specific RIA as described by Morreale de Escobar et al. [7].
Thyroid hormone metabolism: The activities of hepatic microsomal T4 uridine diphosphoglucuronic acid transferase (T4-UDPGT) and type II thyroxine 5'-deiodinase (5'D-II) in forebrain homogenates were determined using ^{125}I-T4 as a substrate in assays previously described [6].

Ethoxy-resorufin-O-deethylase (EROD) activity. EROD activity was measured with hepatic microsomes essentially according to Burke et al.[8] adapted for use with 96 well plates and a spectrophotometric plate reader.
Calcineurin activity. Calcineurin (a calmodulin-dependent phosphatase) activity was determined fluorimetrically in cerebellar supernatants using methylumbilleferone phosphate as a substrate according to Ruiz de Elvira et al.[9].
Glial fibrillary acidic protein (GFAP). GFAP concentrations were determined in discrete brain regions (lateral olfactory tract, prefrontal cortex, striatum, hypothalamus, hippocampus, cerebellum and brainstem) using a sandwich ELISA according to Van Den Berg and Gramsbergen [10].
Synaptophysin. Synaptophysin levels in discrete brain regions (same as used for GFAP determinations) were determined using a modification of the dot-blot method described by Brock and O' Callagan et al. [11].

3. Results

Thyroid Hormones: Fetal plasma TT4 and FT4 were significantly reduced by 50% and 80% by the low and high PCB dose, respectively. Maternal plasma TT4 and FT4 were only significantly reduced (50%) in the high dose group. Neonatal plasma TT4 and TT3 were reduced (50%) 4 days after birth by the high dose. Male neonatal plasma FT4 was unaffected by PCB treatment while female neonatal FT4 was reduced by 50% by both PCB treatments. On day 21 postpartum no effect was observed on maternal plasma TT4, FT4 or TT3 levels by both PCB treatments. Male and female neonatal plasma TT4, FT4 and TT3 were reduced by the high PCB dose (30%, 30% and 20% respectively) 21 days after birth. On day 90 postpartum no effects were observed on the plasma levels of thyroid hormones in the offspring by PCB treatment. TSH levels were not altered by PCB treatment in plasma from fetuses, neonates or adult offspring.

The concentration of T4 in fetal cerebella was reduced to below the level of detection of the assay by the highest PCB dose, while levels of T3 in the fetal cerebella were unaffected. A slight but significant reduction in T3 levels was observed in the fetal forebrain following maternal exposure to the highest PCB dose. On day 21 postpartum neonatal female concentrations of T4 in the forebrain were significantly reduced by 25% and 44% by the low and high PCB exposure, respectively, however, no reductions were observed in female neonatal forebrain T3 concentrations. Male neonatal forebrain T3 and T4 concentrations were not altered by maternal PCB exposure.

Thyroid hormone metabolism: The activity of 5'D-II was significantly induced in fetal forebrain homogenates by 35% and 99% after maternal exposure to the low and high PCB dose, respectively. In female neonatal forebrain homogenates 5'D-II activity was significantly decreased by 49% relative to controls in the lowest dose group, but was unaltered in the high dose group. A similar decrease was observed in male neonatal 5'D-II activity from the low dose group, although the difference was not significant.
T4-UDPGT activity was significantly increased by more than 400% in hepatic microsomes from dams from the high dose group, no effect was observed by the low PCB dose. In fetal hepatic microsomes T4-UDPGT activity was unaffected by the low PCB dose, however, the activity in the microsomes from the high dose group was significantly decreased by more than 50%. Male and female neonatal T4-UDPGT activities were significantly increased relative to controls by both PCB-treatments. The greatest induction was observed in hepatic microsomes from male neonates (30% and 120%, low and high dose), while in female neonates an increase of 30% was observed for both dose groups. Ninety days after birth no effects of PCB-treatment were

observed in male hepatic microsomal T4-UDPGT activity, while female T4-UDPGT activity was significantly decreased relative to controls (40%) in both PCB-treated groups.

EROD activity: Maternal hepatic microsomal EROD activity was induced 20 fold by the lowest PCB dose and more than 200 fold by the highest PCB dose. Fetal EROD activity was undetectable in microsomes from the control and low dose group, and just at the limit of detection in the high dose group (1.0 $\pm$0.2 pmol resorufin/mg*min). The induction of EROD activity in male and female neonatal hepatic microsomes was nearly identical, an induction of 50 fold and 150 fold was observed in the low and high dose groups, respectively. In male adult hepatic microsomes a slight, but significant EROD induction (20%) was observed in the high dose group, while EROD activity was significantly decreased in female hepatic microsomes by 60% and 37% in the low and high dose group, respectively.

Calcineurin activity: Effects on the level of calcineurin activity were observed only in the cerebella of the female offspring. On day 21 postpartum a significant decrease in cerebellar calcineurin activity was observed of 38% and 24% in female neonates from the low and high dose group, while in adult female offspring calcineurin activity increased 31% and 54% in the low and high dose group.

Glial Fibrillary Acidic Protein (GFAP): GFAP concentrations were significantly increased by 28% in the cerebella of male offspring exposed to the high dose of Aroclor 1254 on both day 21 and 90 postpartum. Female cerebellar concentrations of GFAP were significantly increased (32%) by the high PCB dose only on day 21 postpartum. Statistically significant increases in GFAP concentrations relative to controls were also observed in the lateral olfactory tract of PCB-exposed offspring 90 days after birth. The GFAP concentration in the lateral olfactory tract of male offspring was increased by 34% and 32% by the low and high PCB dose, while in female offspring a significant increase (51%) was observed only at the high PCB dose.
In the brainstem GFAP concentrations increased in controls between day 21 and day 90 postpartum. However, this increase was not observed in PCB-exposed offspring, which resulted in significantly lower brainstem GFAP concentrations relative to controls. For male offspring on day 90 postpartum the GFAP concentrations were 44% and 41% lower than controls on from the low and high dose group respectively. Adult female offspring exposed perinatally to the low and high dose had brainstem GFAP concentrations that were 31% and 29% lower than controls. Also in the striatum significant decreases were observed in GFAP concentrations from male and female adult offspring from the high dose group of 20% and 33%, respectively.

Synaptophysin: The brainstem was the most sensitive brain region analysed for the effects of perinatal PCB exposure on synaptophysin concentrations. Brainstem synaptophysin concentrations were significant reduced relative to controls by 29% and 18% in female neonates from the low and high exposure group, respectively, while in male neonates a significant reduction in synaptophysin concentration (38%) was observed only in the high exposure group. Differing effects of perinatal PCB exposure were observed in brainstem synaptophysin concentrations in male and female adult offspring. While synaptophysin concentrations increased significantly in the brainstem of female offspring (by 47% and 109%) in the low and high exposure groups, synaptophysin concentrations were significantly decreased by 20% and 31% in the brainstem from adult male offspring from the low and high exposure group.

4. Discussion

Perinatal Aroclor 1254 exposure results in altered thyroid hormone homeostasis in fetal and

neonatal rats. In fetal rats the decreases in plasma TT4 and FT4 in combination with increased brain 5'D-II activity resemble the effects of hypothyroidism. While forebrain or cerebellar T4 concentrations were severly depressed following maternal PCB exposure, brain T3 concentrations were slightly, but significantly decreased. While it had been expected that plasma TSH concentrations would be elevated in the PCB-exposed offspring due to decreased TT4, FT4 and TT3 concentrations, no treatment related effects were observed. Reductions in fetal plasma TT4 and FT4 levels at the low PCB dose were observed in the absence of decreases in maternal plasma TT4 or FT4 levels and in the absence of increased fetal hepatic microsomal T4-UDPGT activity, indicating that another mechanism may be involved other than increased hepatic excretion or decreased transplacental transport of T4.
The induction of maternal or neonatal EROD activity indicates the potential for significant Ah-receptor mediated effects in the exposed animals. It is noteworthy that only in adult female offspring both hepatic microsomal EROD activity and T4-UDPGT activity were significantly lower in PCB-exposed animals relative to control values following perinatal PCB-exposure.
Neurochemical alterations due to perinatal PCB exposure were found 21 and 90 days after birth. Neuronal cell markers (calcineurin activity, synaptophysin) as well as a glial cell marker (GFAP) were altered in a complex manner, dependent either on the age, sex or brain region of the animal at observation. The brainstem appeared to be the most sensitive region for PCB-induced alterations in GFAP and synaptophysin in both male and female offspring. The PCB-induced increases in cerebellar GFAP concentrations and calcineurin activity are consistent with perinatal hypothyroidism 9,12).

In conclusion, maternal exposure to Aroclor 1254 results in decreased fetal and neonatal plasma T4 levels, which in turn cause decreases in fetal and neonatal brain T4 levels. The decreases in brain T4 levels are partially compensated by an increased deiodination of T4 to T3 in the fetal brain, however significant decreases in brain T3 levels were still observed. The occurence of long term alterations observed in neural and glial biochemical markers may be the result of low brain thyroid hormone concentrations following maternal PCB exposure.

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5. References

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