Developmental neurotoxicity of single polychlorinated biphenyls in the neonatal mouse

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1. Introduction

During perinatal development of the mammalian brain, rapid brain growth occurs, known as the brain growth spurt[1]. In man, this period begins during the third trimester of pregnancy and continues throughout the first 2 years of life. In mouse and rat this period is neonatal, spanning the first 3-4 weeks of life. During this period of rapid growth, the brain undergoes several fundamental developmental phases, viz. maturation of axonal and dendritic outgrowth, establishment of neural connections, synaptogenesis, multiplication of glia cells with accompanying myelinization, and cell, axonal and dendritic death[2]. The brain growth spurt is associated with numerous biochemical changes that will transform the perinatal brain into that of the mature adult. One of the major signal substances in the CNS is acetylcholine (ACh), which acts as the transmitter in the cholinergic pathways. In rodents, this transmitter system in the CNS undergoes rapid development during the first 3-4 weeks after birth[3] when gradually increasing numbers of muscarinic and nicotinic receptors are found in the cerebral cortex and hippocampus[3,4,5]. The cholinergic transmitter system is involved in many behavioural phenomena[6] and correlates closely with cognitive functions[7].

In several reports we have shown that low-dose exposure of environmental toxic agents to mice during the neonatal period can lead to disruption of adult brain function, and also to an increased susceptibility to toxic agents at adult ages[8].

Polychlorinated biphenyls (PCBs) constitute a large category of chlorinated hydrocarbons known as persistent environmental contaminants. Human epidemiological studies indicate that perinatal exposure to PCBs can have developmental neurotoxic effects, and animal studies have shown that exposure to commercial mixtures of PCBs during fetal and postnatal development can cause behavioural aberrations and changes in brain transmitters[9,10].

In different studies we have investigated the effects of neonatal exposure to single PCB congeners such as, 2,4,4′-trichlorobiphenyl, 2,2′,5,5′-tetrachlorobiphenyl, 2,3′,4,4′,5-pentachlorobiphenyl, 2,3,3′,4,4′,5-hexachlorobiphenyl, 2,3,3′,4,4′-pentachlorobiphenyl, 3,3′,4,4′-tetrachlorobiphenyl and 3,3′,4,4′,5-pentachlorobiphenyl on: spontaneous motor behaviour, learning and memory function, cholinergic muscarinic and nicotinic receptors and biogenic amines in the CNS of the adult animal.

2. Methods

The PCB congeners 2,4,4'-trichlorobiphenyl (IUPAC 28), 2,2',5,5'-tetrachlorobiphenyl (IUPAC 52), 2,3',4,4',5-pentachlorobiphenyl (PCB 118), 2,3,3',4,4',5-hexachlorobiphenyl (IUPAC 156) 2,3,3',4,4'-pentachlorobiphenyl (IUPAC 105), 3,3',4,4'-tetrachlorobiphenyl (IUPAC 77) and 3,3',4,4',5-pentachlorobiphenyl (IUPAC 126) were generously donated by Prof. Å. Bergman (Wallenberg Laboratory, University of Stockholm, Sweden). The substances were administered orally to neonatal NMRI mice as one single dose on postnatal day 10. The amounts of the different congeners given were as follows; 2,4,4'-trichlorobiphenyl, 2,2',5,5'-tetrachlorobiphenyl, 2,3',4,4',5-pentachlorobiphenyl, 2,3,3',4,4'-pentachlorobiphenyl and 2,3,3',4,4',5-hexachlorobiphenyl, 0.7 - 14 μmol/kg body weight (roughly 0.2 - 5 mg/kg body weight, b.wt); 3,3',4,4',5-pentachlorobiphenyl, 0.14 - 1.4 μmol/kg b.wt (0.046 - 0.46 mg/kg b.wt); 3,3',4,4'-tetrachlorobiphenyl, 1.4 - 140 μmol/kg b.wt (0.41 - 41 mg/kg b.wt). Mice serving as controls received 10 ml/kg b.wt of the 20% fat emulsion vehicle in the same manner.

Spontaneous behaviour: the test measures locomotion; horizontal movement, rearing; vertical movement, and total activity; all types of vibration within the test cage, i.e. those caused by mouse movements, shaking (tremors) and grooming. The test was performed in adult male mice (about 4 months old).

Swim maze: the swim maze was of Morris water maze type. The ability of the mice to locate a submerged platform was studied for 5 days. Performance in a swim maze of Morris maze type has been suggested to be correlated to cholinergic function in the CNS. The test was performed in adult male mice (about 5 months old).

Receptor assays: Measurement of the nicotinic cholinergic receptors (NAChR) in the cerebral cortex. The proportions of high- and low affinity binding sites and their corresponding affinity constants were assayed in a competition binding study, using the tritium-labelled nicotine (L-(-)-[N-methyl-^{3}H]-nicotine and various concentrations of unlabelled (-)nicotine. Measurement of the cholinergic muscarinic (MAChR) and nicotinic receptor (NAChR) in hippocampus. The density of MAChR and NAChR was assayed by using tritium-labelled quinuclidinyl benzilate ([^{3}H]QNB) and tritium-labelled nicotine (L-(-)-[N-methyl-^{3}H]-nicotine, respectively.

The levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) in striatum were measured by high-performance liquid chromatography.

3. Results and Discussion

Neonatal exposure to lightly chlorinated ortho-substituted PCB congeners 2,4,4'-trichlorobiphenyl (PCB 28), 2,2',5,5'-tetrachlorobiphenyl (PCB 52), 2,3',4,4',5-pentachlorobiphenyl (PCB 118), 2,3,3',4,4',5-hexachlorobiphenyl (PCB 156) and 2,3,3',4,4'-pentachlorobiphenyl (PCB 105) as one single dose (0.7 - 14 μmol/kg b.wt to 10-day-old mice) produced persistent neurotoxic effects in the adult animals (4 months old) following exposure to PCB 28 or 52[11,12]. Both compounds induced permanent aberrations in spontaneous motor behaviour. Furthermore, neonatal exposure to PCB 52 also affected learning and memory functions in the adult animals. In the animals with evidently defective memory and learning functions, the cholinergic nicotinic receptors in the cerebral cortex were affected. This neonatal

exposure to PCB 52 (14 μmol (4.1 mg)/kg b.wt) also alters the spontaneous motor behaviour response to a cholinergic agent, viz. nicotine, at adult age, but not to amphetamine, an agent known to affect the dopaminergic system[13]. The effects on spontaneous motor behaviour and NAChR were observed when PCB 52 was administered on postnatal day 3 or 10, whereas there was no effect on postnatal day 19, indicating a limited period during neonatal life during which the induction of permanent changes in adult brain function takes place. Exposure to 2,3',4,4',5-pentachlorobiphenyl (PCB 118), 2,3,3',4,4'-pentachlorobiphenyl (PCB 105) and 2,3,3',4,4',5-hexachlorobiphenyl (PCB 156), mono-ortho congeners ('co-planar-like'), in the same dose range caused no significant change in spontaneous motor behaviour or swim-maze behaviour.

Neonatal exposure to the co-planar PCB 3,3'4,4',5-pentachlorobiphenyl (PCB 126) was found to induce permanent aberrations in spontaneous behaviour in the adult animal. This effect was also shown to worsen with age, as the effect was more pronounced in 4-month-old than in 2-month-old mice. Furthermore, neonatal exposure to PCB 126 (1.4 μmol (0.46 mg)/kg b.wt) affected learning and memory functions in the adult animal and in these animals the cholinergic nicotinic receptors in the hippocampus were affected[12]. In our earlier studies we have observed that neonatal exposure to the co-planar PCB, 3,3',4,4'-tetrachlorobiphenyl (PCB 77) could induce permanent aberrations in adult spontaneous behaviour and to affect cholinergic receptors (muscarinic) in hippocampus in both neonatal mice and in animals reaching an adult age of 4 months[14,15].

Considered together, these studies indicate that both lightly chlorinated ortho-substituted and co-planar PCBs can be potent inducers of behavioural aberrations when given to mice during the neonatal period of brain development. Our findings also indicate that there may be regionally specific effects in the brain of the different PCB congeners.

In the experiments mentioned, there were no significant effects on the weight development between PCB- and vehicle-treated animals. Exposure during this developmental stage of the neonatal brain development and the selected doses of PCB 28, PCB 52 and PCB 126 did not significantly alter the levels of dopamine or its metabolites in the striatum.

In recent experiments we have seen that neonatal exposure to PCB 52 (0.8 and 4.1 mg/kg b.wt) can lead to an increased susceptibility in adult mice to renewed exposure to PCB 52 (4.1 mg/kg b.wt). It was particularly interesting that immediately after the adult treatment with PCB 52, no further disturbances in spontaneous behaviour were evident. The additional changes were observed 2 months after the adult PCB 52 exposure and apparently have a delayed onset. This increased susceptibility in adults neonatally exposed to an environmentally toxic agent has been observed following neonatal exposure to DDT (1,1,1-trichloro-2,2-*bis*(p-chlorophenyl)ethane) and adult exposure to bioallethrin (pyrethroid, Type I)[16].

In conclusion, these studies have shown that neonatal development and the developing cholinergic system are suscetible to disturbances caused by lightly chlorinated ortho-substituted and co-planar PCBs, leading to permanent behavioural and cholinergic receptor changes in the adult animal. Furthermore, neonatal exposure to a lightly chlorinated PCB can induce increased susceptibility to a subsequent exposure at adult age.

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5. References

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