Developmental Neurotoxicity of Coplanar and Di-*ortho*-substituted PCBs in the Neonatal Mouse

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Introduction

Human epidemiological studies indicate that perinatal exposure to polychlorinated biphenyls (PCBs) might cause developmental neurotoxic effects, while studies in experimental animals have shown that exposure to PCBs during fetal and postnatal development can cause behavioural aberrations and changes in brain transmitters and receptors[1,2].

During perinatal development of the mammalian brain, there is a period of rapid brain growth, known as the 'brain growth spurt'[3]. In Man, this period begins during the third trimester of gestation 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[4]. The 'brain growth spurt' is associated with numerous biochemical changes that transform the feto-neonatal 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[5] when gradually increasing numbers of muscarinic and nicotinic receptors are found in the cerebral cortex and hippocampus[5,6,7]. The cholinergic transmitter system is involved in many behavioural phenomena[8] and correlates closely with cognitive functions[9].

In a series of studies we have shown that low-dose exposure to environmental agents such as DDT, pyrethroids, organophosphates, paraquat and nicotine, during the rapid development of the neonatal mouse brain, can lead to irreversible changes in adult brain function[10,11]. The induction of these disturbances occurs at doses that apparently have no permanent effects when administered to the adult animal. We have also seen that there can be a critical period during neonatal development of the mouse brain when these permanent effects are induced[11,12,13,14]. Furthermore, an increased susceptibility to toxic agents can be observed in adult animals exposed during neonatal life, indicating that neonatal exposure to toxic agents can potentiate and/or modify the reaction to adult exposure to xenobiotics[15,16].

In more recent studies we have described the developmental neurotoxic effects of neonatal exposure to single PCB congeners such as 2,4,4′-trichlorobiphenyl (IUPAC 28), 2,2′,5,5′-tetrachlorobiphenyl (IUPAC 52), 3,3′,4,4′-tetrachlorobiphenyl (IUPAC 77) and 3,3′,4,4′,5-pentachlorobiphenyl (IUPAC 126) on: spontaneous motor behaviour, learning and memory function, cholinergic muscarinic and nicotinic receptors in the CNS of the adult animal[17,18,19,20]. These studies also

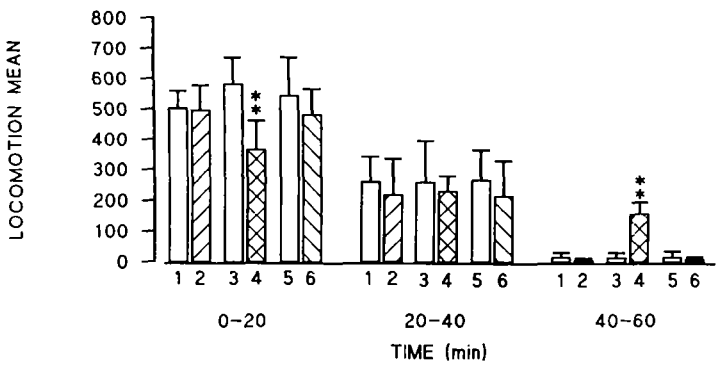

Figure 1. Spontaneous behaviour (locomotion) in 4-month-old male NMRI mice exposed to a single oral dose of 3,3',4,4'-tetrachlorobiphenyl (PCB 77, 4.1 mg), or the 20% fat-emulsion vehicle (10 ml) per kg body weight at an age of either 3-days (bars 1 and 2), 10-days (bars 3 and 4), or 19-days (bars 5 and 6). Plain bars denote controls, hatched bars denote PCB 77 treated mice (mean±SD). Motor activity, observed as locomotion, rearing, and total activity, was measured for 3 x 20 min in an automated device consisting of cages (40 x 25 x 15 cm) placed within two series of infrared beams, as described elsewhere[18]. The statistical evaluation was by ANOVA with a split-plot design, and pairwise testing with the Tukey HSD test. Statistical difference vs. control is indicated by ** $P \leq 0.01$.

indicated that there may be regionally specific effects in the brain of the different PCB congeners, where coplanar PCBs affect cholinergic receptors in hippocampus and di-*ortho*-substituted PCB can affect cholinergic receptors in the cerebral cortex. However, PCB congeners such as 2,3',4,4',5-pentachlorobiphenyl (IUPAC 118), 2,3,3',4,4',5-hexachlorobiphenyl (IUPAC 156), 2,3,3',4,4'-pentachlorobiphenyl (IUPAC 105), when given at similar doses, were not found to induce any neurotoxic effects[18,20]. Current investigations have indicated that there is a critical period in the neonatal development of the mouse brain when persistent behavioural effects are induced by a coplanar and a di-*ortho*-substituted PCB. Furthermore, neonatal exposure to PCB, di-*ortho*-substituted, can lead to an increased susceptibility in the adult mouse to a new interventive exposure to PCB, indicating that neonatal exposure to PCB can potentiate and/or modify the reaction to adult exposure to PCBs.

A defined critical time in neonatal brain development

The induction of behavioural disturbances by neonatal exposure to PCB in the mouse seems to be limited to a short period of time during neonatal development. Two different PCB congeners were studied, PCB 77 (3,3',4,4'-tetrachlorobiphenyl) and PCB 52 (2,2',5,5'-tetrachlorobiphenyl). PCB 77 has been shown to affect muscarinic cholinergic receptors in the hippocampus[17] and PCB 52 to affect nicotinic cholinergic receptors in the cerebral cortex[18]. The compounds were given as one single oral dose to mice at the age of either 3 days, 10 days, or 19 days. At the adult age of 4 months the mice were subjected to spontaneous motor behaviour tests. In neonatal mice exposed to the coplanar PCB 77 (4.1 mg/kg body weight) a significant change in spontaneous motor behaviour at the adult age of 4 months was only seen in mice given PCB 77 at the age of 10 days (Fig.1).

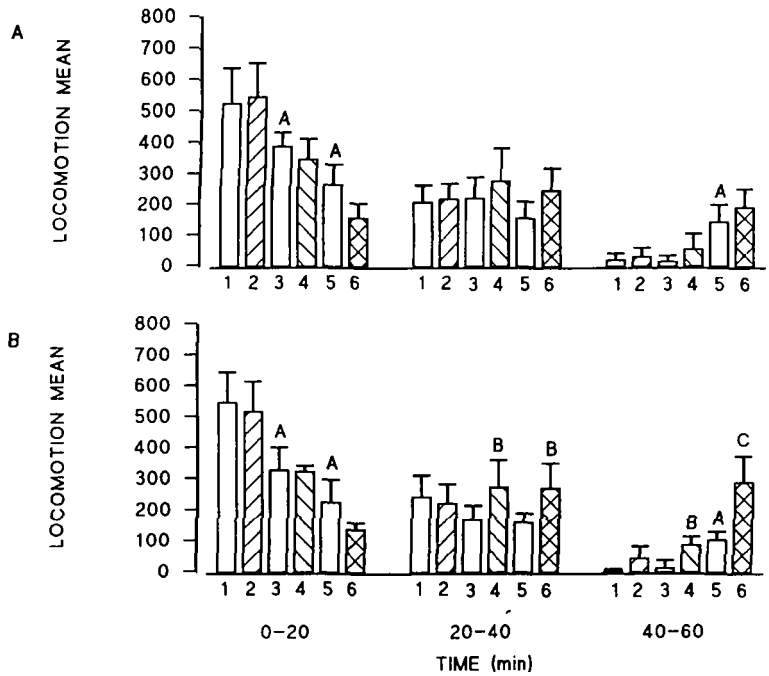

Figure 2. Spontaneous behaviour (locomotion) in 4-month-old (A) and 6-month-old (B) male NMRI mice following (i) a single oral exposure to 2,2',5,5'-tetrachlorobiphenyl (PCB 52, 0.8 or 4.1 mg/kg body weight) on day 10 after birth, and (ii) a single oral dose of PCB 52 (4.1 mg/kg body weight) at age 4 months. Controls received the 20% fat-emulsion vehicle (10 ml/kg body weight). Regarding measurement of motor activity, see Fig.1. The treatment groups are denoted as: *1*, vehicle — vehicle; *2*, vehicle — PCB 52 (4.1 mg/kg); *3*, PCB 52 (0.8 mg/kg) — vehicle; *4*, PCB 52 (0.8 mg/kg) — PCB 52 (4.1 mg/kg); *5*, PCB 52 (4.1 mg/kg) — vehicle; *6*, PCB 52 (4.1 mg/kg) — PCB 52 (4.1 mg/kg). The statistical evaluation was by ANOVA with a split-plot design, and pairwise testing with the Tukey HSD test. A = significantly different from vehicle-vehicle, $P \leq 0.01$. B = significantly different from its respective control, $P \leq 0.05$. C = significant different from its respective control, $P \leq 0.01$.

Similarly, in mice exposed to the di-ortho-substituted PCB 52 (4.1 mg/kg body weight) a significant behavioural aberration was observed in adult mice given PCB 52 at an age of either 3 or 10 days. The adult mice (4 months) displayed a non-habituating behavioural profile. They were evidently hypoactive during the first part of the 60 min test period, while toward the end of the period they became demonstrably hyperactive.

This critical window for the induction of permanent effects of PCBs shows similarities to our earlier studies. In various investigations we have observed the developing cholinergic system to be sensitive to environmental agents[11]. In a recent study we observed a critical stage in the development of cholinergic nicotinic receptor subtypes, with consequences for behavioural response at adult age. After neonatal mice had been exposed to nicotine at three different ages during the neonatal period, low-affinity nicotinic binding sites could not be found at any time, though the persistence of this effect was evident only in adult mice exposed on days 10-14. A spontaneous behaviour test

performed on adult mice (4 months) did not reveal any difference between vehicle-treated and nicotine-treated mice, whereas when challenged with nicotine and observed for sponataneous behaviour, their response to nicotine was hypoactive, though only in mice given nicotine on days 10-14. The response to nicotine by controls and the other age categories to nicotine was an increased activity.

A similar critical period for the induction of permanent changes in cholinergic muscarinic receptors and spontaneous behaviour has been observed following neonatal exposure to both DDT and diisopropylfluorophosphate[12,14]

Increased susceptibility in adults neonatally exposed to a di-*ortho*-substituted PCB

We noticed earlier that neonatal exposure to a neurotoxic agent such as DDT can lead to increased susceptibility in adults to agents such as bioallethrin[15] and paraoxon[16] (insecticides). Among the

effects observed were behavioural disturbances, and changes in cholinergic receptors. A time-response effect was also observed, as the aberrant behaviour in mice neonatally exposed to DDT and to bioallethrin or paraoxon as adults became more pronounced 2 months after the adult exposure, compared with that 24 h after adult exposure. In the present experiments it was evident that neonatal exposure to PCB 52 at both 0.8 and 4.1 mg/kg body weight can potentiate the susceptibility of adult mice on renewed exposure to PCB 52 (4.1 mg/kg body weight). It was particularly interesting that, 24 h after the adult exposure to PCB 52, there were no further disturbances in spontaneous behaviour (Fig. 2A); such additional changes were observed 2 months later (Fig. 2B). These animals became significantly more active than mice only exposed to PCB 52 during the neonatal period. This aberration in spontaneous behaviour was found to develop over time, indicating a time-response/time-dependent effect. The dose used for adult exposure had no effect on neonatally untreated animals. These results indicate that, in adult mice, susceptibility to PCB may be acquired, in differing degrees, from PCB exposure during perinatal life when the maturational processes of the developing brain and CNS are at a stage of critical vulnerability.

Concluding remarks

When considered together, these results indicate that low-dose exposure to some PCBs during the rapid development of the neonatal brain ('brain growth spurt') can lead to irreversible changes in adult brain function. The induction of these disturbances occurs at doses that apparently have no permanent effects when administered to the adult animal. Our studies have also shown that there is a critical period during neonatal development of the mouse brain when these permanent effects are induced. Furthermore, the increased susceptibility to PCB at adult age in animals exposed during neonatal life indicates that neonatal exposure to PCB can potentiate and/or modify the reaction to adult exposure to PCB.

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