Interactive effects of PCB and nicotine administered during the neonatal brain development

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Introduction

Present days environment is increasingly afflicted by vast numbers of hazardous contaminants and exposure to different environmental toxic agents can occur throughout life, even from fertilisation. This can include exposure to both persistent and non-persistent xenobiotics which can induce brain disruption when administered during a critical phase of neonatal brain development [1].

During mammalian development there are critical periods for normal maturation of the central nervous system (CNS). There is a period of rapid brain growth , known as "the brain growth spurt"[2].This period does not take place at the same time in all mammalian species, in man, this period begins during the third trimester of pregnancy and continues throughout the first 2 years of life. However, in rodents, this period is neonatal, spanning the first 3-4 weeks of life. The brain undergoes several developmental phases during this period of rapid growth, such as major axonal and dendritic outgrowth, synaptogenesis, establishment of neuronal connections, multiplication of glia cells, myelinization and numerous biochemical changes such as the development of the cholinergic transmitter system, including gradually increasing numbers of muscarinic and nicotinic receptors found in the cerebral cortex and hippocampus[3][4][5].

Earlier studies have shown that low-dose exposure to different environmental agents during the rapid development of the neonatal mouse brain can lead to irreversible changes in adult brain function[1][6].The induction of these disturbances occurs at doses that apparently have no permanent effects when administered to the adult animal. These studies also indicate that there is a defined critical period during neonatal development of the mouse brain when these persistent effects are induced [7][8][9][10].

In a series of studies, Eriksson and co-workers have shown that different PCB congeners have developmental neurotoxic effects. Among others, 2,2′,5,5′-tetrachlorobiphenyl (IUPAC 52), have been shown to cause altered spontaneous behaviour, learning and memory functions, as well as changes in cholinergic nicotinic receptors [11]. Also nicotine, a well known addictive drug and former insecticide, has been shown to cause behavioural changes as well as changes in nicotinic receptors [1][6][12].

With regard to some of the similarities in effects caused by PCB 52 and nicotine when given during neonatal life the present study was undertaken to determine the interaction effects between nicotine and 2,2′,5,5′-tetrachlorobiphenyl (PCB 52).

Methods

Ten day old male NMRI mice received orally 2,2´,5,5´-tetrachlorobiphenyl (PCB 52) dissolved in a 20% fat emulsion vehicle and s.c. injections of (-)nicotine-base dissolved in 0.9% NaCl (10 ml/kg b.w.) as follows in table 1.

Table 1. Substance, dose and age of mice used in this study.

Group	Treatment
1	**Day 10: 20% fat emulsion vehicle (10 ml/kg b.w.) per os** **Day 10-14: 0.9% NaCl (10 ml/kg b.w.) s.c. twice daily.**
2	**Day 10: 20% fat emulsion vehicle (10 ml/kg b.w.) per os** **Day 10-14: 33 μg (-)nicotine-base/kg b.w. s.c. twice daily.**
3	**Day 10: PCB 52, 4.1 mg/kg b.w. (in vehicle) per os** **Day 10-14: 0.9% NaCl (10 ml/ kg b.w.) s.c. twice daily.**
4	**Day 10: PCB 52, 4.1 mg/kg b.w. (in vehicle) per os** **Day 10-14: 33 μg (-)nicotine-base/kg b.w. s.c. twice daily.**

The PCB congener 2,2´,5,5´-tetrachlorobiphenyl was generously donated by Professor Å. Bergman, Wallenberg Laboratory, University of Stockholm, Sweden and the (-)nicotine-bi-(+)-tartrate where obtained from Sigma, St. Louis USA

Spontaneous behaviour was measured for 3 x 20 min in an automated device consisting of cages placed within two series of infrared beams (Rat-O-Matic, ADEA Elektronik AB, Uppsala, Sweden).

Spontaneous behaviour: the test was performed in male mice at an age of 4 months. 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 and grooming.

Nicotine-induced behaviour: was tested directly after the spontaneous behaviour test. The mice were given saline or nicotine s.c. (40 or 80 μg (-)nicotinebase /kg b.w.). Thereafter the animals were observed for another 60 min period [13].

Receptor assay: Nicotinic cholinergic receptors (NAChR) were measured in a P2 fraction of the cerebral cortex dissolved in Na-K-phosphate buffer, and by using unlabelled and tritium labelled α-bungarotoxin[4].

Results and Discussion

The present study showed that co-exposure to both nicotine and PCB 52 affected the development of nicotinic receptors in the cerebral cortex, measured by binding of α-bungarotoxin. This neonatal co-exposure to PCB 52 and nicotine also affected the behavioural response to nicotine at adult age. The response to nicotine were more pronounced in co-exposed animals compared to animals exposed to only PCB 52.

One of the major transmitter systems in the brain is the cholinergic system, which is associated with many physiological processes and also consciousness such as memory, learning, audition and vision[14].

In rodents, this transmitter system undergoes rapid development during the first 3-4 weeks after birth[3].

Earlier studies have shown behavioural changes in adult animals after neonatal exposure to PCB 52 [10][11]. These animals showed altered spontaneous behaviour. In spontaneous behaviour tests, information about animal's ability to habituate to a novel environment can also be obtained. Normal habituation is defined as a decrease in locomotion, rearing and total activity variables in response to the diminished novelty of the test chamber over the 60 min test period, divided into three 20 min spells. In the present study normal habituation was demonstrated in the control animals and in the animals receiving only nicotine. In the co-exposed animals and in animals receiving PCB 52, a hypoactive behaviour was displayed in the beginning of the test period, while toward the end, they became hyperactive.

When observed for nicotine induced behaviour, co-exposed animals showed additional changes. Control animals responded with an increased activity to nicotine while the opposite were observed in all the other treatment groups. However, the most pronounced response to nicotine were observed in the co-exposed animals.

Earlier studies have shown that neonatal exposure to nicotine (66 μg/kg b.w.)[12] and PCB 52 [10][11] can affect the development of nicotinic low affinity (LA) binding sites. The present study showed that effects on the development of nicotinic receptors were only seen in mice neonatally co-exposed to both PCB 52 and nicotine. α-bungarotoxin is an antagonist to acetylcholine and binds with high affinity to the nicotinic receptor constituted of the α7 subunit[15][16]. There was a significant difference in binding of α-bungarotoxin in the co-exposed animals compared to control animals.

Taken together, the present study indicates that neonatal co-exposure to PCB 52 and nicotine induce an interactive effect on behaviour and nicotinic receptors.

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References

(1) Eriksson, P., *Neurotoxicology*, 1997; 18 (3), 719-726.

(2) Davison, A.N. and Dobbing, J., *Applied Neurochemistry;* Blackwell, Oxford, 1968; 178, 253.

(3) Fiedler, E.P., Marks, M.J., Collins, A.C., *J. Neurochem.* 1987, 49, pp. 983-990.

(4) Falkeborn, Y., Larsson, C., Nordberg, A., Slanina, P., *Int. J. Dev. Neurochem.*, 1983, 1, 187-190.

(5) Slotkin, T.A., Orband-Miller, L., Queen, K.L., *J. Pharmacol. Exp. Ther.*, 1987, 233, 361-368.

(6) Eriksson, P., Ankarberg, E., Fredriksson, A., 1998, Exposure to nicotine during a defined period in neonatal life induces permanent changes in brain nicotinic receptors and response to nicotine in adult mice, (Submitted)

(7) Eriksson, P., Ahlbom, J., Fredriksson, A., *Brain Res.*, 1992, 582, 277-281.

(8) Ahlbom, J., Fredriksson, A., Eriksson, P., *Brain Res.*, 1994, 645, 318-324.
(9) Ahlbom, J., Fredriksson, A., Eriksson, P., *Brain Res.*, 1995, 677, 13-19.
(10) Eriksson, P., Fredriksson, A, *Environ. Toxicol. Pharmacol.*, 1996, 1, 217.
(11) Eriksson, P., Fredriksson, A., *Environ. Toxicol. Pharmacol.*, 1996, 1, 155.
(12) Nordberg, A., Zhang, X., Fredriksson, A., Eriksson, P., *Dev. Brain Res.*, 1991, 63: 201-207.
(13) Archer, T., Fredriksson, A., Lewander, T., Söderberg, U., *Scand. J. Psychol.*, 1987, 28, 242-249.
(14) Karzmar, A.G., In: *Cholinergic mechanisms*; ed. P.G. Waser, Raven Press, New York, 1975; p. 501.
(15) Wonnacott, S., *J. Neurochem.*, 1986, 47, 1706-1712.
(16) Orr-Urtreger, A., Göldner, F. M., Saeki, M., Lorenzo, I., Goldberg, L., De Biasi, M., Dani, J. A., Patrick, J. W., and Beaudet, A. L., *J. Neurosc.*, 1997, 17(23):9165-9171.