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LETTER TO THE EDITOR

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The olfactory system as a portal of entry for airborne polychlorinated biphenyls (PCBs) to the brain?

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Abstract Ferrets, mammalian carnivores, kept in an indoor enclosure were continuously exposed to low concentrations of polychlorinated biphenyls (PCBs) in the ambient air for 5 years. After that time PCB concentrations were quantified in the olfactory bulbs and in the remaining brain, adipose tissue and liver. The results revealed unexpectedly high PCB concentrations in the olfactory bulbs, surpassing those in the remaining brain and the peripheral tissues. The PCB congener pattern in the olfactory bulbs resembled that found in the ambient air and the less chlorinated volatile PCBs were found in higher concentrations. We, therefore, assume that airborne PCBs enter directly via the olfactory system and are transported through the axons to the olfactory bulbs where they accumulate.

Key words Airborne PCBs · Olfactory system · Axoplasmic transport · Ferret

Introduction

The widespread distribution of polychlorinated biphenyls (PCBs) in the environment has resulted in low-level exposure of many human and animal populations. The significance of these exposures is not known, but one area of concern is the potential effect on the central nervous system. Sufficient data have been gathered to determine that PCB exposure represents a risk to neurobehavioural development in children (Golub and Jacobson 1995). Behavioural studies in animals have confirmed that PCBs exert negative effects on learning, memory and motor activity in a variety of species (Tilson et al. 1990). However, it is unclear whether PCBs exert their main influence on the nervous system directly or whether nervous system dysfunction is secondary to hormonal alterations. The manifestation of PCB-induced neurotoxicity most likely depends on several factors, predominantly the specific congeners in the exposure mixture and/or the stage of development during exposure (Ness et al. 1994). The knowledge of which PCB congeners accumulate in the brain and specifically the site of accumulation in the brain is therefore critical to a better understanding of PCB-induced neurotoxicity.

Only a few animal studies have evaluated the regionalization of PCB accumulations in different brain areas after orally applied PCBs by quantifying PCB residues. No significant differences in congener profile among brain regions have been reported (Ness et al. 1994). However, the olfactory bulbs, which are rather small in most laboratory species (e.g. mice and rats), have not been analysed separately, nor, so far as we know, has any study attempted to identify the individual PCB congeners that accumulate in different brain areas following exposure to airborne PCBs. In our study we used the European ferret, the domesticated form of the European polecat (*Mustela putorius*), which has proven to be a useful animal in several neurotoxicological studies (Hoar 1984) including the olfactory system (Apfelbach et al. 1992). The ferret, a carnivorous mammal, relies heavily on olfactory cues when searching for prey (Apfelbach 1986). Not surprising, the olfactory bulbs (Fig. 1) are well developed and comprise ~5% of the total brain weight.

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Materials and methods

Animals and treatment

In our study, we determined which PCBs accumulate in the brain following long-term exposure to airborne PCBs. We examined 10 European ferrets (*M. putorius f. furo* L.). Animals, which were kept

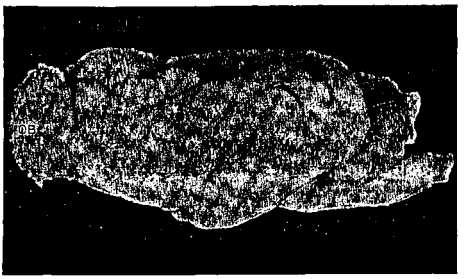

Fig. 1 The brain of the ferret with remarkably large olfactory bulbs (*OB*)

indoors in an animal care room, were chronically exposed for 5 years – approx the lifespan of a polecat in nature – to low concentrations of PCBs (sum of all PCBs, 260 ng/m^3 air) in the ambient air emitted continuously from PCB containing sealants. In the ambient air, tetra-chlorinated PCBs (tetra-CBs) dominated the congener profile with PCB-52 in highest concentrations followed by hexa- and penta-chlorinated PCBs (hexa-CB, penta-CB; Fig. 2). The food of the animals was slightly contaminated because of the large amount of fish in the diet (17 ng PCBs/g diet; daily consumption ~50 g diet/kg body weight). Control animals (n = 3) were reared and kept outdoors (sum of all PCBs < 0.05 ng/m^3 air) for 5 years and fed on the same diet.

Animals were killed under deep Nembutal anaesthesia. The brain, adipose tissue and liver were immediately removed and taken for PCB quantification. The brain was further separated into the olfactory bulbs (~0.3 g) and the remaining brain (~7 g). The olfactory nerves and the olfactory epithelia were not removed separately.

PCB analysis

Airborne PCBs were analysed according to Balfanz et al. (1993). We developed a modified analytical method for two reasons: (1) because of the small amounts of olfactory bulb and brain tissue, and (2) to avoid background pollution. For chemical analysis of PCBs in the tissues we used gas chromatography-mass spectrometry (GC-MS). All solvents were of high purity. Heptane, acetone, and toluene were of Pestanal quality (Riedel de Haen, Seelze, Germany). Dichlormethane and pentane were obtained from Promochem (Wesel, Germany) in nano grade. The $^{13}C_{12}$-labelled PCB standards were purchased from Cambridge Isotope Laboratories (Woburn, Mass., USA).

The samples were weighed and the $^{13}C_{12}$-labelled PCB standard mixtures (12 ng per each PCB congener) were added. The samples were homogenized after the addition of 3–50 ml heptane using an ultrasonic rod. The solvent with the extracted lipids was thermally treated in a capped tube for 12 h at 80 °C, dried under a stream of nitrogen and the dry weight was recorded. The clean-up procedure was started by addition of 3–5 ml heptane and 2–5 ml concentrated sulphuric acid to the dried sample. The organic-fraction was separated and cleaned-up further by heating the sample at 50 °C after the addition of 2–5 g silica gel (ICN Biomedicals, Eschwege, Germany)/44% conc. H_2SO_4 (Merck, Darmstadt, Germany).

The following cleaning procedure was performed by chromatography on 0.8 g alumina Super I (ICN Biomedicals) and 0.3 g silica gel B/44% conc. H_2SO_4. After pre-elution with 4 ml pentane the PCBs were collected with 6 ml heptane/dichloromethane (1:1). The PCB-fraction was reduced to a volume of 50 µl by a gentle stream of nitrogen, and a 2 µl aliquot was analysed by GC-MS. The isotope dilution method was used for quantification of individual congeners and of homologues. Total PCB was calculated as the sum of the tri- to decaCB.

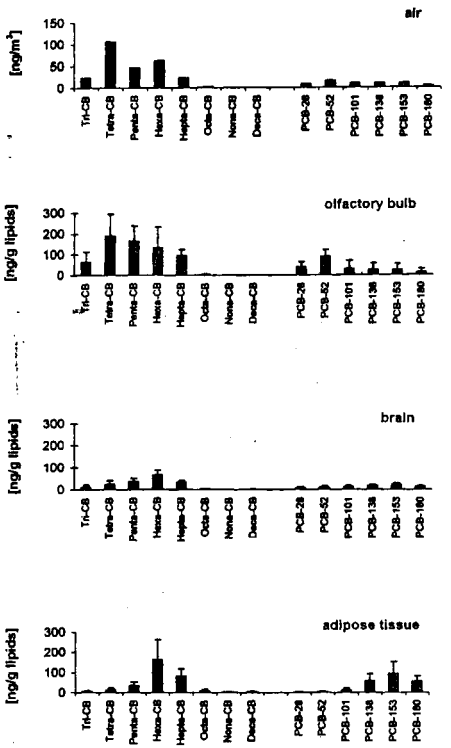

Fig. 2 Polychlorinated biphenyl (PCB) concentrations are shown in the ambient air, olfactory bulb, remaining brain, adipose tissue and liver. The biological data (mean and SD are of n = 10 chronically exposed animals. The homologue sums of tri- to decachlorbiphenyls are shown in columns to express the PCB-concentration in a sample (mono- and dichlorbiphenyls, which are normally not found in biological samples, are not shown). Additionally, the concentrations of six analytically important congeners (the so-called indicator congeners) are shown. These congeners are as follows: 2,4,4'-trichlorbiphenyl (= PCB-28); 2,2',5,5'-tetrachlorbiphenyl (PCB = 52), 2,2',4,5,5'-pentachlorbiphenyl (= PCB-101); 2,2',3,4,4',5'-hexachlorbiphenyl (= PCB-138); 2,2',3,3',5,5'-hexachlorbiphenyl (= PCB-153) and 2,2',3,4,4',5,5'-heptachlorbiphenyl (= PCB-150)

Gas chromatographic analysis was performed on a Hewlett-Packard 5890 A gas chromatograph equipped with a fused-silica capillary column (30 m × 0.32 mm i.d., 0.25 µm film thickness, DB 5, J & W Scientific, Folsol, USA). Helium was used as carrier gas at a head pressure of 20 p.s.i. The oven was temperature-programmed from 80 °C (4 min, 1 min splitless) to 150 °C at 25 °C/min, then to 300 °C at 6 °C/min, then isothermal for 15 min. A Hewlett-Packard HP MSD 5970 mass selective detector was used for the MS detection. The MS was run in the Selected Ion Monitoring mode. The organochlorine residue levels in the various tissues are expressed on a lipid basis.

Results and discussion

In the brains of outdoor control animals, low PCB-levels were detected (PCB sum, 110.15 ng/g lipid); the PCB concentrations in the olfactory bulbs remained at the background level (sum tri-CB, 0.00 ng; sum tetra-CB, 0.00 ng; sum penta-CB, 0.76 ng; sum hexa-CB, 1.40 ng; sum octa-CB, 0.23 ng). In the exposed group we found significantly elevated concentrations of PCBs in the olfactory bulbs (PCB sum, mean ± SD 642.46 ± 330.54 ng/g lipid) compared to the remaining brain (Fig. 2) (PCB sum, mean ± SD 169.54 ± 66.62 ng/g lipid, $P < 0.002$; Mann Whitney U-test, two-tailed). The concentrations of the more volatile lower chlorinated tetra-CB congeners (PCB-52) in the olfactory bulb samples paralleled the concentrations of these congeners in the air and were considerably greater than those found in the other tissues. In fact, for each category of PCB, even the less volatile ones, the bulbs had a considerably higher concentration than seen in any of the other tissues. Whereas there were relatively higher concentrations of the less volatile hexa- and hepta-CB congeners in the remaining brain, adipose tissue (PCB sum, mean ± SD 302.9 ± 148.7 ng/g lipid) and liver (PCB sum, mean ± SD 202.2 ± 103.72 ng/g lipid), we found that the total concentrations of these less volatile PCBs in these tissues were still lower than those in the bulbs (Table 1).

Under ordinary circumstances food is thought to be the main source of PCB intake (DFG report 1988), and in our studies some of the airborne PCBs we administer must settle on the food and be ingested by the animals. Because PCBs are lipid soluble it is reasonable to assume that orally ingested PCBs will be distributed by the blood stream among the different body compartments in proportion to their fat content. The sole exception, however, is the brain (Aguilar 1985). When expressed on the basis of lipid content, as reported for a variety of different mammalian species, the levels of organic chlorine residue in the brain are considerably lower than in the rest of the body, generally in a ratio of 1:10 (Hayes 1975, Aguilar 1985). The suggested explanation for this considerable difference has been that the blood-brain barrier partially blocks the passage of the pollutants into the brain (Walker 1975).

In contrast to the data of others reporting an equal distribution within the brain we found in the exposed group that the total PCB concentrations and the concentrations of individual PCB congeners are significantly higher in the olfactory bulbs than in the rest of the brain. It is entirely conceivable that the results can be explained by the anatomy of the olfactory system. Each bipolar sensory neuron in the olfactory epithelium has an apical dendritic ending on the surface of the epithelium in the nasal cavity and projects an axon from its basal end to the olfactory bulb. Our data suggest that inhaled pollutants pass into the dendrites of olfactory sensory neurons and are transported via olfactory axons directly to the bulbs where they accumulate. Support for our argument can be derived from the evidence that the olfactory portal of entry to the central nervous system is used by heavy metals, e.g. cadmium (Gottofrey and Tjälve 1991), and solvents such as toluene, xylene or styrene (Ghantous et al. 1990). It is unlikely that the unusual distribution of PCB residues in the central nervous system can be explained by differential distribution to the olfactory bulb via the blood stream after ingestion or by way of the pulmonary system, and it is unlikely that special metabolic pathways in different brain areas could account for these large differences.

In conclusion, we believe that airborne PCBs enter olfactory sensory neurons directly and are transported to the olfactory bulbs where they accumulate. This hypothesis is supported by our data showing drastically elevated PCB concentrations in the olfactory bulbs compared to those in the rest of the brain. Moreover, the homologue-profiles in the olfactory bulbs show a trend towards the less chlorinated and more volatile congeners and resemble the profiles of these compounds found in the air. We believe that this is the first report describing this route of entry for PCB intake in mammals. Since in all mammalian species, including human, the anatomy and physiology of the olfactory system follows the same basic construction and mechanisms (for review see Farbman 1992), it is fair to assume that even in humans this route of PCB intake may play an important role. However, present guidelines on indoor air pollution for humans do not take into account the possibility that PCBs may enter the central nervous system directly via the olfactory pathway and therefore may underestimate the hazardous potential of these compounds.

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Table 1 Polychlorinated biphenyl (PCB) sum (ng/g lipids) in different organs of control and exposed ferrets

	Control animals	Exposed animals	P
Liver	184.1	202.2	NS
Adipose tissue	276.0	302.9	NS
Brain	110.1	169.5	0.05
Olfactory bulb	Below background level	642.4	0.02

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