Behavioral, Neurochemical, and Hormonal effects of Aroclor 1254 in Adult Rats

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1. Introduction

Polychlorinated biphenyls (PCBs) are widespread environmental contaminants[1]. USEPA banned the usage of PCBs after the two major accidental PCB poisoning incidents ("Yusho" in Japan and "YuCheng" in Taiwan) where more than 1000 people were diagnosed as having Yusho ("Oil disease" in Japanese) and suffered with chloracne, numbness, weakness in the limbs and decreased peripheral nerve conduction velocities[2]. Children exposed *in utero* showed clinically evident developmental delays and cognitive deficits[3]. In laboratory animals, developmental PCB exposure has been reported to alter cognitive and motor functions[4,5]. With regard to neurochemical effects, PCBs alter brain neurotransmitter levels preferentially dopamine[6,7,8] and the decreased dopamine levels were attributed to the selective accumulation of *ortho*-substituted congeners in brain[9]. However, the basic cellular mechanism by which these PCB congeners cause neurological deficits is not known.

The role of signal transduction mechanisms including Ca^{2+}-homeostasis and inositol phosphates in neurotoxicolgy is currently under investigation by several laboratories. We have explored the possible effects of PCBs on these processes in an attempt to understand the cellular mechanism for neurotoxicity of PCBs. The distribution of Ca^{2+} within the cell is complex[10] and normal physiological function of the cell is regulated by changes in intracellular free Ca^{2+} ($[Ca^{2+}]_i$), which ranges from 0.1 to 0.3 μM. This low concentration is regulated by energy-requiring transport systems located in plasma membrane, endoplasmic reticulum (ER) and mitochondria[10]. Also, binding of an agonist to membrane receptors leads to the generation of second messengers such as diacylglycerol (DAG) and inositol trisphosphate (IP_3) from membrane phospholipid hydrolysis[11]. DAG activates protein kinase C (PKC) which, in turn, catalyzes the phosphorylation of a variety of cellular proteins. On the other hand, IP_3 releases Ca^{2+} from ER and regulates several processes including translocation and activation of PKC[12]. Perturbations in Ca^{2+}-homeostasis as well as sustained activation of PKC have been reported to be involved in the pathogenesis of neuronal injury[13,14].

Our initial studies with 2,2'-dichlorobiphenyl (-DCB), a non-coplanar congener, increased intracellular $[Ca^{2+}]_i$, effectively inhibited $^{45}Ca^{2+}$-uptake by mitochondria and microsomes, altered inositol phosphate production, and caused protein kinase C translocation at concentrations of 5-50 μM[15,16]. 2,2'-DCB was cytotoxic only at higher

concentrations and required longer exposure periods (>100 μM). 3,3',4,4',5-Pentachlorobiphenyl, on the other hand, had little or no effect on signal transduction events and was not cytotoxic. In agreement with previous reports[17], our SAR studies indicated that the activity of PCB congeners was associated with chlorination favoring non-coplanarity while those with chlorination favoring coplanarity were less active[18,19]. This is further supported by the observation that polychlorinated diphenyl ethers (PCDE), in which coplanarity is difficult to achieve, are active in neuronal preparations[20]. In summary, all these *in vitro* studies suggest that the signal transduction system is a possible target for non-coplanar PCBs. In the present study, we have examined the *in vivo* effects of PCBs on signal transduction mechanisms in different brain regions by treating adult rats repeatedly with a commercial mixture, Aroclor 1254.

2. Methods

Adult male Long Evans Hooded rats (250-300 g) were dosed orally with Aroclor 1254 in corn oil (2 ml/kg). The selected dosages were 0, 10 or 30 mg/kg/day. The rats were dosed 5 times a week for four weeks. At 24 hr after the last dose, rats were tested for motor activity[21] in a photocell device for 30 min, then immediately sacrificed for the determination of circulating thyroid hormone levels, neurochemical analysis, and toxicokinetic distribution of PCB congeners.

Freshly isolated cerebellum, frontal cortex and striatum were fractionated to obtain different subcellular fractions. Intracellular Ca^{2+} buffering was determined by measuring $^{45}Ca^{2+}$-uptake by microsomes and mitochondria as outlined by Moore et al.[22]. Total and membrane-bound PKC were determined by measuring the incorporation of ^{32}P from γ-[^{32}P]ATP into neurogranin[23]. Circulating thyroid hormones (total T_4, free T_4, total T_3 and free T_3) were determined using the standard radioimmunoassay kits (Diagnostic Products, Inc., Los Angeles, CA). Blood was collected and allowed to clot on ice for about an hour. The blood samples were centrifuged at 2500 rpm for 15 min. Serum samples were stored at -80°C until radioimmunoassay. Congener-specific analysis of PCBs was performed using a graphitized carbon and high-resolution gas chromatography with electron capture detection[24].

3. Results and Discussion

Behavioral effects: Body weight gain in both the treated groups was not altered until after 10 days of treatment. In the 10 mg/kg dose group, body weight gain was similar to that of control rats. However, body weight gain of rats from the high-dose group was significantly lower than the control and low-dose groups starting 12-14 days of treatment. Motor activity (horizontal but not vertical) was significantly lower in rats dosed with 30 mg/kg Aroclor 1254. Motor activity in the 10 mg/kg dose group was not significantly different from control group. These results suggest that Aroclor 1254 at 30 mg/kg dose causes hypoactivity in rats following repeated exposure.

Neurochemical effects: Ca^{2+} buffering by microsomes was significantly lower in all three brain regions from the 30 mg/kg group. In the same dose group, mitochondrial Ca^{2+} buffering was affected in cerebellum but not in cortex or striatum. Similarly, total PKC was decreased significantly while membrane bound PKC was elevated significantly in cerebellum at 10 and 30 mg/kg. Total as well as membrane-bound PKC was not altered in cortex or striatum in any of the dose groups. These results suggest that *in vivo* treatment with a PCB mixture produces neurochemical changes similar to those observed after *in vitro* exposure of neuronal cell cultures. It is preliminary to extrapolate these changes to any neuronal deficits caused by PCBs.

Circulating thyroid hormone levels: Total as well as free T_4 and T_3 were determined in serum samples. Circulating T_4 (total and free) concentrations were drastically reduced following Aroclor 1254 treatment. The reduction was >95% in both the 10 and the 30 mg/kg dose groups. Circulating T_3 concentrations were also reduced following Aroclor 1254 treatment; however, the reductions were smaller when compared to T_4. The reduction in T_3 concentrations were 29-42% in treated rats. These results are indicative of a severe hypothyroid state in Aroclor 1254-exposed rats.

TABLE 1. *Behavioral, neurochemical changes and circulating thyroid hormone levels in rats treated repeatedly with Aroclor 1254.*

Measurement	Control	Aroclor 1254 10 mg/kg	Aroclor 1254 30 mg/kg
Motor activity (counts/30 min session):			
Horizontal Activity	4714 ± 374	4181 ± 268	2939 ± 395*
Vertical Activity	352 ± 39	313 ± 25	248 ± 45
Microsomal $^{45}Ca^{2+}$-uptake (pmol/mg protein/min):			
Cerebellum	51.6 ± 2.5	38.9 ± 5.4	38.2 ± 0.9*
Frontal cortex	38.2 ± 2.2	39.9 ± 0.8	23.7 ± 4.0*
Striatum	16.9 ± 1.6	10.5 ± 2.3	7.6 ± 2.3*
Mitochondrial $^{45}Ca^{2+}$-uptake (pmol/mg protein/min):			
Cerebellum	12.9 ± 0.3	12.1 ± 0.4	10.6 ± 0.3*
Frontal cortex	8.5 ± 0.5	8.9 ± 0.3	8.7 ± 0.3
Striatum	4.0 ± 0.1	5.1 ± 0.6	5.2 ± 0.4
Total Protein Kinase C (PKC) activity (nmol/mg protein/min):			
Cerebellum	1.79 ± 0.05	1.57 ± 0.03*	1.43 ± 0.04*
Frontal cortex	1.36 ± 0.06	1.41 ± 0.07	1.27 ± 0.04
Striatum	0.79 ± 0.05	0.86 ± 0.05	0.78 ± 0.03
Membrane associated PKC (% of total):			
Cerebellum	31.9 ± 1.7	42.5 ± 1.5*	47.8 ± 1.8*
Frontal cortex	37.7 ± 2.6	43.3 ± 2.1	40.5 ± 1.4
Striatum	50.1 ± 2.1	42.7 ± 1.6	46.3 ± 3.8
Circulating Thyroid hormone levels:			
Total T_4 (ng/ml)	60.5 ± 3.1	2.1 ± 1.2*	2.2 ± 0.9*
Free T_4 (pg/ml)	21.2 ± 2.8	0.7 ± 0.1*	0.8 ± 0.3*
Total T_3 (ng/ml)	0.86 ± 0.08	0.61 ± 0.03*	0.69 ± 0.03*
Free T_3 (pg/ml)	2.53 ± 0.25	1.45 ± 0.13*	1.18 ± 0.18*

Values are mean ± SEM of 4-12 rats. *Significantly different from control at $p < 0.05$.

Toxicokinetics: Our previous *in vitro* studies indicate that some of the PCBs (non-coplanar in nature) at concentrations of 5-50 μM perturb intracellular signal transduction mechanisms in neuronal cultures[15,16]. It is not clear whether such concentrations are achievable in brain *in vivo*. We have now conducted PCB congener-specific analysis in different brain regions as well as in blood, liver and fat tissue. While PCB concentrations in control rat brain regions were less than 0.02 ppm, total PCB congeners in treated animals accumulated to ppm levels. Total PCB congeners were greater in frontal cortex (15.9 ± 0.3 ppm) and cerebellum (13.1 ± 1.7 ppm) compared to striatum (0.64 ± 0.19 ppm) suggesting differential accumulation of PCBs in some brain regions. The levels of PCBs in fat were high (555 ± 41 ppm). The circulating levels of PCBs in the blood were 1.55 ± 0.01 ppm. Among 99 different PCB congeners analyzed, hexachlorinated biphenyls constituted about 50% of the total. Predominant congeners detected in different brain regions were: 2,2',4,4',5- (PCB 99) and 2,3',4,4',5- (PCB 118) pentachlorobiphenyls; 2,2',4,4',5,5'- (PCB 153), 2,2',3,3',4,6'- (PCB 132), 2,2',3,4,4',5'- (PCB 138), 2,3,3',4,4',5- (PCB 156) and 2,3,3',4',5,6- (PCB 163) hexachlorobiphenyls; and 2,2',3,3',4,4',6- (PCB 171) heptachlorobiphenyl. PCB concentrations observed in brain are equivalent to approximately 2 to 50 μM, which are similar to those used in earlier *in vitro* studies.

In summary, these results suggest that *in vivo* treatment with a PCB mixture produces neurochemical changes similar to those observed after *in vitro* exposure of neuronal cell cultures. Concentrations that inhibited signal transduction processes *in vitro* are achievable *in vivo* in brain. Additional studies are needed to extrapolate this information to the observed neurobehavioral changes following exposure to PCBs.

4. References

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