# THE EFFECT OF POLYCHLORINATED BIPHENYLS AT THE NEUROTRANSMITTER RECEPTOR LEVEL IN THE BRAIN

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## Introduction

It is well established that PCB has an effect on the nervous tissue, particularly on learning and memory [1,2]. We have previously shown that the *ortho*-substituted PCBs (*o*-PCBs) have an effect on neurotransmitter uptake and storage [3,4]. The *o*-PCBs inhibit the vesicular uptake of biogenic amines by competitive inhibition of the so-called VMAT-2 transporter. Further *o*-PCBs inhibit the uptake of the neurotransmitters dopamine, glutamate and GABA into synaptosomes. The physiological consequence of the inhibition of uptake is a high dopamine level in the cytoplasm of the nerve terminals with subsequent leakage of dopamine. Further inhibition of the uptake into synaptosomes may lead to high concentrations of dopamine, glutamate and GABA in the synaptic cleft. This could lead to toxic effects due to prolonged stimulation and a subsequently desensitisation of the receptors. In addition since *o*-PCBs affect uptake, it could also interfere directly with the membrane or the receptors. We have therefor studied the effect of PCBs on receptors *in vitro* and in addition studied the effect on glutamatergic cerebellar granule cells.

## Materials and Methods

*Chemicals*

2,2'-DCB (PCB 4, IUPAC), 3,3',4,4',5-PCB (PCB 126, IUPAC), 2,2',4,4',5,5'-HCB (PCB 153, IUPAC) were purchased from Dr. Ehrenstorfer (Germany) and AccuStandard Inc (USA). The PCB mixtures Aroclor 1254 (A1254) and Aroclor 1242 (A1242) were purchased from AccuStandard Inc (USA). Cyclosporin A (CSA), (±) α-tochopherol (vitamin E) and NBQX were purchased from Sigma-Aldrich Co (St. Louis, USA). Basal Eagle's medium (BME) was purchased from GibcoBRL (Norway).

*Membrane preparation for receptor binding*

We have used similar, but not identical methods for membrane preparations. For muscarinic, NMDA and AMPA receptors we have used a procedure previously described [5]. For dopamine 1 and 2 receptors we have followed the procedure as described [6,7].

In principle male Wistar rats (~200 g) were decapitated. The cerebrum was taken out and homogenised in Tris-HCl buffer with an Ultra-Turax. The homogenate was centrifuged for 10 min at 1000g and the supernatant centrifuged again at 22500 g for 20 min. This pellet was rehomogenised and incubated for 30 min at 37°C to remove endogenous ligands. The pellet was collected after recentrifugation and rehomogenised in 10 volumes incubation buffer. Regarding glutamate receptor binding studies the pellet was washed several times to remove endogenous glutamate.

*Assay for receptor binding*

Dopamine D1 and D2 receptor binding was studied by incubating membranes (~0.3mg) with 50μM PCB for 15 min followed by the addition of 1nM [$^3$H]-SCH23390 (D1-receptor) or 1nM [$^3$H]-raclopride (D2-receptor) in a total volume of 500 μl. The samples were incubated for 30 min at 25°C.

NMDA receptor binding was studied by incubating membranes (~0.06 mg protein) with 10 μM glutamate, 10 μM glycine and 4μM [$^3$H]-MK801 in a total volume of 400μl as described above.

Muscarine receptor binding was studied by incubating membranes with 1 nM [$^3$H]-QNB in a total volume of 400μl as described above.

The reactions were stopped by adding ice-cold saline buffer to the membrane preparation followed by filtration through filter.

*The effect of PCBs on cerebellar granule cells*

Cerebellar granule cells were prepared from 6-7 days old Wistar rats [8]. The granule cells were grown on 55-mm plastic dishes for 6-8 days before being used. The growth media (BME with fetal bovine serum) was then removed from the plated granule cells and replaced by 4 ml prewarmed BME without fetal bovine serum containing PCB and/or neurprotective substances and exposed to PCBs up to 24 hours. Protection of the granular cells were carried out with 3μM MK-801, 10μM NBQX and 0.5μM cyclosporine A. Neurone survival was determined by using the trypan blue exclusion assay.

**Results and Discussion**

The results show that there were no or only small effects of PCBs on the receptor binding *in vitro* of the dopamine-, glutamate and muscarine receptor ligands (Table 1 and 2).

Table 1. The effect of PCBs on dopamine receptors. The results are presented as percent binding of ligand ± SEM (n = 3-6).

| Receptor | Ligand | 50 μM A1254 | 50 μM A1242 |
|---|---|---|---|
| Dopamine (D1) | SCH23390 | 91 ± 4 | 84 ± 7 |
| Dopamine (D2) | Raclopride | 83 ± 7 | |

Table 2. The effect of PCBs on glutamate and muscarine receptors. The results are presented as percent binding of ligand ± SEM (n = 3-6).

| Receptor | Ligand | 50μM PCB 4 | 50 μM PCB 153 | 50 μM PCB 126 |
|---|---|---|---|---|
| NMDA | MK-801 | 81 ± 6 | 111 ± 7 | 94 ± 9 |
| Muscarine | QNB | 98 ± 1 | 96 ± 5 | 93 ± 2 |

Surprisingly there was a significant effect of *o*-PCBs on cerebellar granule cell survival. After 24 h of exposure, 10μM and 30μM of A1254 and A1242 induced approximately 50% cell death respectively. The coplanar PCB 126 did not have any effect on cell survival at 50 μM even

after 24 h of exposure. In contrast 8 μM of the PCB congener 153 gave approximately 50% cell death after 24 h of exposure.

The NMDA receptor antagonist (3 μM) and the antioxidant vitamin E (10μM) protected against the PCB-induced cell death. Also the AMPA receptor antagonist NBQX and the mitochondrial permeability pore blocker cyclosporine A gave significant although less protection (Table 3). The results show that, although there was no effect of PCB on the NMDA receptor binding, the *o*-PCBs kill granular cell by excitotoxicity. This must mean that *o*-PCBs have an effect on the energy status of the cell, which lower the threshold for calcium penetration through the ionic channel of NMDA[9]. Previously it has been shown that *o*-PCBs disturb the calcium homeostasis in cerebellar granule cells[10,11]. It is further interesting that NBQX has a protective effect, since it underlines that the AMPA receptor is also involved, although to a lesser extent. The protection offered by vitamin E clearly shows the involvement of free radical formation in the neurone. We have also shown that free radical formation in granule cells by incubation with Arochlor 1254, assayed by the oxidation of 2,6-dichlorofluorescin, is inhibited by the same compounds as shown in Table 3.

Table 3. The effect of A1254 on cerebellar granule cells, after 6 h exposure, in combination with neuroprotective agents. The results are presented as percent cell death, mean ± SEM.

| | |
|---|---|
| Control | 6.8 ± 0.5 |
| Aroclor 1254 (15μM) | 59.8 ± 3.9 |
| Aroclor 1254 (15μM) + MK 801 (3μM) | 8.9 ± 0.5 |
| Aroclor 1254 (15μM) + Vitamin E (10 μM) | 26 ± 4.9 |
| Aroclor 1254 (15μM) + Cyclosporin A (0.5μM) | 31.7 ± 3.1 |
| Aroclor 1254 (15μM) + NBQX (10μM) | 33.8 ± 3.1 |

In conclusion *o*-PCBs may be neurotoxic because they can kill neurones by an excitotoxic mechanism involving calcium entry into the cell, activation of the NMDA receptor and production of free radicals.

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