# The effect of ortho-chlorinated biphenyls on the high affinity uptake of the neurotransmitters L-glutamate, GABA and dopamine into rat brain synaptosomes

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## Introduction

Polychlorinated biphenyls (PCBs) are lipophilic and resistant to biological decomposition and can accumulate in higher trophic levels through the food chain. PCBs have neurotoxic effects, particularly on learning and memory, but the mechanism of action remains uncertain (1). Several studies have indicated that PCBs may lead to changes in the brain amines, particularly the dopaminergic system and the serotonergic system. In our laboratory we have recently established that ortho-chlorinated PCBs are potent inhibitors of the vesicular uptake of dopamine and serotonin (2). Others have suggested that PCBs inhibit the synthesis of dopamine (3,4). Rosin and Martin (5), however, found that a mixture of PCBs inhibited the uptake and stimulated the release of catecholamines in isolated mouse brain synaptosomes. In this study we have examined the potential of selected PCB congeners in inhibiting the uptake of L-glutamate, GABA and dopamine into rat brain synaptosomes. Inhibition of the uptake will automatically lead to an apparent increased release of neurotransmitters. The effect of different PCB congeners on the uptake of L-glutamate, GABA and dopamine into synaptosomes has not previously been examined, and are of particular interest since glutamergic signaling are involved in memory. Further, we discuss the observed effects on synaptosome uptake with our previous results on PCBs effect on uptake into rat brain vesicles .

## Materials and Methods

*Chemicals*

2,2'-DCB, 2,2',6-TCB, 2,2',4,5-TCB, 2,2',4,6-TCB, 2,2',6,6'-TCB, 2,2',4,5',6-PCB, 3,3'-DCB, 4,4'-DCB, 3,3',4,4'-TCB and 3,3',4,4',5-PCB were purchased from Dr. Ehrenstorfer (Germany) and Cambridge Isotopes Laboratories (USA).

*Preparation of synaptosomes*

Rat brain synaptosomes were prepared by the method of Gray and Whittaker (6). Male Wistar rats (150-200g) were killed by decapitation and the brains were quickly removed and kept on ice. The brains were homogenised (~5% wt/vol) in 0.32 M sucrose and the homogenate was centrifuged for 10 min (1000g, Sorvall SS-600 rotor). The supernatant was then mixed with 1.3 M

sucrose to obtain a 0.8 M suspension to remove myelin on further centrifugation. The supernatant was centrifuged for 30 min (20 000 g) to get a crude synaptosomal pellet (P2). The synaptosomal pellet was gently resuspended in 0.32 M sucrose and used in the experiment on the same day.

*Assay for uptake of L-glutamate, GABA and dopamine into synaptosomes.*

High affinity uptake of L-glutamate and GABA into synaptosomes was carried out by the method of Meldahl and Fonnum (7). High affinity uptake of dopamine was determined by method of McNaught *et al* (8). Synaptosomes (8µg protein/ml for L-glutamate and GABA uptake, 32µg protein/ml for dopamine uptake) were preincubated at 25°C for 15 minutes in absence or presence of PCB in Tris-Krebs buffer. For uptake of dopamine the Tris-Krebs buffer contained in addition 1.7 mM ascorbic acid and 80 µM pargyline. The uptake was started by adding substrate containing either 100 nM L-3H-glutamate (1.0 µCi), 50 nM 3H-GABA (1.0 µCi), or 100 nM 3H-dopamine (0.5µCi). The mixtures (500 µl final volume) were incubated for 3 minutes (L-glutamate and GABA) or 10 minutes (dopamine) and the reaction was terminated by filtration with a solution of 0.15 M NaCl and 0.05 % (w/v) bovine serum albumin in a cell harvester (Skatron) onto a glass fiber filtermat. The filters were dissolved in 10 ml of Filter Count (Packard) and counted for retained radioactivity in a liquid scintillation spectrophotometer (Packard Tri-Carb 300).

**Results and Discussion**

The uptake of L-glutamate, GABA and dopamine into synaptosomes were inhibited by the ortho-chlorinated biphenyls in a concentration dependent manner. The effect of PCBs on the uptake of the neurotransmitters into synaptosomes was found to be dependent upon the amount of synaptosomal material present. The $EC_{50}$-values (concentration with 50% inhibition) of the L-glutamate, GABA and dopamine uptake were between 5µM and 20µM for all the ortho-chlorinated PCBs tested with the exception of 2,2',6,6'-TCB which did not inhibit the uptake. Apart from 3,3'-dichloro biphenyl the nonortho-chlorinated biphenyls showed no effect on the uptake. This is in agreement with previous observations on other neurotoxic effects of the different congeners (1). One interesting feature is that L-glutamate, GABA and dopamine uptake is inhibited in the same concentration range. Since the protein content is higher in the dopamine experiment, the uptake of dopamine is probably more effectively inhibited than the other. This is similar to the observed effect on the vesicular uptake, where the uptake of dopamine was much more inhibited than the uptake of L-glutamate and GABA (2). The vesicular uptake of dopamine was inhibited by PCB concentrations as low as 10 µM and the uptake of dopamine into synaptosomes indicate similar range of inhibitor concentrations. The amount of protein when measuring vesicular uptake of dopamine is slightly higher than for measuring synaptosomal uptake of dopamine. This means that at least for some PCB congeners the effect on synaptosomal uptake is similar to the effect on vesicular uptake. PCBs were stronger inhibitors of synaptosomal uptake of L-glutamate and GABA than for vesicular uptake. This could even be the case when the difference in protein concentration is taken into consideration. Based on the present experiments, however, it seems clear that inhibition of dopamine uptake is more important than the effect on L-glutamate and GABA.

Our results are in agreement with previous experiments on the content and release of catecholamines in cell culture and brain preparations. Chishti *et al* (9) showed that the PCB mixtures Aroclors 1254 and 1260 reduce the dopamine concentration and elevate the level of the dopamine metabolite dihydroxyphenyl acetic acid (DOPAC) in rat striatal slices. They also

showed that PCBs increase the concentration of dopamine and the DOPAC into the medium. They suggested that PCBs may alter the vesicular packaging of newly synthesised dopamine in the rat brain. Messeri *et al* (10) showed that PCB could induce spontaneous release of catecholamines from chromaffin granula, which could indicate a PCB mediated effect on the reuptake of catecholamines. Seegal *et al* (11) showed that *in-vitro* exposure of PCBs to rat striatal slices reduce tissue concentration and elevate media concentration of dopamine. They suggested that the increased media concentration of dopamine were due to either enhanced release or a PCB-induced inhibition of the uptake of dopamine.

As a conclusion both the vesicular and the synaptosomal uptake are inhibited by PCBs at concentration well below 20 µM and can therefore have physiological consequences. The synaptosome membranes are the first to encounter PCBs and even if the uptake into synaptosomes should be less affected than the vesicular uptake it may be exposed to higher PCB concentrations. This indicate that inhibition of synaptosomal uptake of neurotransmitters may be involved in the neurotoxicity of PCBs.

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