The effect of 2,2'-dichloro biphenyl and 4,4'-dichloro biphenyl on the vesicular uptake of glutamate and dopamine.

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Introduction

Through human activity polychlorinated biphenyls (PCB) have in the past 30 years developed to be a global environmental problem. The different PCB compounds have complex toxicological properties. The co-planar PCBs (non-ortho PCBs) has an effect mediated through the Ah-receptor, and leads to cytochrome P450 1A1 induction, hepatomegaly, thymic atrophy and reduced body weight gain (1, 2). The ortho substituted PCB congeners, which are the most abundant in environment, may give toxic effects like cytochrome P450 2B induction, hepatotoxicity, carcinogenicity, immunotoxicity and neurotoxicity (3, 4, 5, 6). The finding that PCB may impair human cognitive functions has led to an intensive research on the effects of different PCBs on brain neurotransmitters, and on other biological systems that may contribute to their activity in the nervous system (7). There has been found that exposure to commercial mixtures of PCB gives a regional specific decrease of the concentration of dopamine in the rat brain (8,9). Maier *et al* (10) have shown that 2,2'-dichloro biphenyl inhibits Mg-ATPase activity in different rat brain regions. They also demonstrated that the 3,3',4,4',5-pentachloro biphenyl had no effect. The structure activity relationship of potentially neurotoxic PCB congeners in rat have recently been reviewed by Kodavanti and Tilson (7).

The object of this investigation is to describe the neurotoxic properties of different PCB congeners, with emphasis on the ortho-substituted PCB congeners. The lipid solubility of PCBs made us to look into their effect on membranes and transport mechanisms. In the present communication we have compared the effect of a neurotoxic (2,2'-dichloro biphenyl) and a non-neurotoxic (4,4'-dichloro biphenyl) PCB on the uptake of the neurotransmitters dopamine and glutamate into synaptic vesicles.

Materials and methods

Preparation of synaptic vesicles

Synaptic vesicles were isolated as described by Fykse and Fonnum (11) from brains of male Wistar rats. The vesicles were isolated from a crude synaptosomal fraction by hypotonic shock and subjected to a sucrose density gradient. The vesicles show a Mg-ATPase dependent uptake of neurotransmitters (dopamine, glutamate, GABA), but no sodium dependent uptake which indicate no plasma membrane contamination.

Assay for vesicular uptake

Vesicles (0.1 mg protein/ ml) were preincubated at 30°C for 15 minutes in absence or presence of PCB. The uptake of neurotransmitters was started by adding substrate containing ^{3}H-glutamate or ^{3}H-dopamine, and ATP. The mixtures were incubated for 3 minutes and the reaction was stopped by adding ice cold KCl followed by filtration. The filters were counted for retained radioactivity in a liquid scintillation spectrophotometer.

Assay for measurement of ΔpH

The ΔpH was determined by measuring the fluorescence quenching of acridine orange using a luminescence spectrometer (12). Experiments were performed at 30°C in a medium containing vesicles (0.05 mg protein/ ml) and acridine orange. The reaction was started by addition of ATP. Inhibitors were added 1 minute after addition of ATP. The specific protonophore FCCP was added at the end of the experiment to dissipate the pH gradient.

Results

The uptake of dopamine and glutamate in synaptic vesicles were inhibited by 2,2'-dichloro biphenyl in a concentration dependent manner. The EC_{50}-values (concentration with 50% inhibition) of the dopamine and glutamate uptake was 17.5 μM (± 3.5, SD) and 65 μM (± 37, SD) respectively. 4,4'-dichloro biphenyl had no effect on either the vesicular dopamine or glutamate uptake up to its solubility limit.

2,2'-dichloro biphenyl showed a rapid concentration dependent destruction of the pH gradient with a EC_{50}-value of 10 μM (± 2.5, SD). 4,4'-dichloro biphenyl had no effect on the vesicular pH gradient up to its solubility limit.

Discussion

The results show that different chlorinated biphenyls may have different toxicological effects. 2,2'-dichloro biphenyl is a potent inhibitor of the uptake of dopamine in synaptic vesicles. The effect on the uptake of glutamate is less. 2,2'-dichloro biphenyl also destroys the pH gradient at the same concentration range needed to inhibit the uptake of dopamine. The uptake of dopamine in vesicles is predominantly dependent on a high pH gradient while the uptake of glutamate is predominantly dependent on a high electrochemical gradient (13). The findings by Maier *et al* (10) that 2,2'-dichloro biphenyl is a potent

inhibitor of Mg-ATPase activity in rat brain, should indicate equal effect of both glutamate and dopamine uptake. Since this is not the case the PCB probably has a larger effect on ΔpH than on the Mg-ATPase. The results may also explain the lower brain dopamine concentration in rat exposed to mixtures of PCB. Further we now investigate different PCB congeners. This may reveal structure activity relationship of the neurotoxicity of the PCB congeners (lipid solubility, chlorination, chlor position, biphenyl rotation).

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