ADDITIVE BINDING OF PCBs AND 2,3,7,8-TCDD TO THE Ah RECEPTOR

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Abstract

The purpose of this work was to determine whether the antagonism between 2,3,7,8-TCDD and PCBs previously reported for teratogenicity in this mouse strain may be a mass-law effect. Mixtures of 2,3,7,8-TCDD and PCBs were assayed by competitive binding of the mixture and a fixed aliquot of radiolabelled [^{3}H]-TCDD with a fixed amount of Ah receptor protein from C57BL/6 mouse liver. The mixtures studied behaved additively in the assay at several different ratios. Expression of the mixtures as TCDD-equivalences suggested that the protective effects of PCBs previously observed are more than simple mass law effects.

Introduction

The teratogenic effects of PCBs and 2,3,7,8-TCDD, by themselves and in combination, have been studied for the C57BL/6 mouse (1,2). Administration of 2,3,7,8-TCDD alone caused the development of cleft palate in 62% of the offspring of female mice when the dose of TCDD was 20 μg/kg. The percentage of affected offspring fell to < 10% when the TCDD was coadministered with either 270 mg/kg of 2,2',4,4',5,5'-hexachlorobiphenyl or 244 mg/kg of Aroclor 1254. In the sense that the PCBs exerted a "protective" effect against the development of cleft palate, they were described as being antagonistic towards TCDD.

Many toxic responses of PCDDs and related compounds, including teratogenicity, are believed to be mediated through the intracellular Ah receptor protein, which binds planar, non-polar molecules such as 2,3,7,8-TCDD with high affinity (3). Following entry of the toxicant into the cell and binding to the Ah receptor, the receptor/ligand complex translocates into the nucleus, where it associates with "dioxin responsive elements" on the DNA.

Several studies on mice have shown the intensity of response to TCDD to correlate with the occupancy of the Ah receptor by TCDD, with the cellular levels of the Ah receptor and, within the PCDD family, with the binding affinity and toxicity (4). These findings suggest that the intensity of response is determined, at least in part, by the intracellular concentration of receptor/ligand complexes. In the case of the mouse teratogenicity experiments, PCBs were found to cause no cleft palates when administered alone at the doses indicated above. Therefore, if TCDD and PCB compete for a limited number of Ah receptor sites, it might be expected that the severity of the toxic response would decline in parallel with the proportion of receptor sites that were occupied by TCDD. According to this hypothesis, the "protective" effect of PCBs against teratogenicity could be a "mass law" effect, in chemical terminology, in the sense that two substances, one biologically active and one inactive, compete for a fixed number of Ah receptor sites. The purpose of

the experiments described here was to test this hypothesis.

In earlier work we have developed a rapid assay for PCDDs, PCDFs, and other stereochemically similar compounds, based upon the strength of binding of the toxicants in an environmental sample to a fixed amount of Ah receptor (5). Because the Ah receptor is thermally unstable, the C57BL/6 mouse happens to be the best candidate so far discovered as a source of the hepatic cytosol receptor, since its Ah receptor is stable for long periods near 4 ^{o}C (6). The methodology employed was to carry out competitive binding experiments as follows: the sample competes with a fixed quantity of a radiolabelled TCDD for a fixed quantity of the Ah receptor. In the absence of any competitor, a maximal amount of radiolabelled Ah receptor-TCDD complex is formed; as the concentration of the unlabelled competitor increases, the concentration of the radiolabelled complex falls. The choice of [^{3}H]-TCDD as the radiolabelled substance is convenient because TCDD is one of the most strongly binding PCDD congeners; competition experiments are only feasible if the radiolabelled compound is more strongly binding than the competitor (7).

Methodology and Results

A competition curve was prepared for each individual substance by allowing a fixed amount of [^{3}H]-2,3,7,8-TCDD and varying amounts of unlabelled competitor to compete for a fixed amount of the Ah-receptor. The amount of radiolabelled TCDD-Ah receptor complex corresponding to each concentration of unlabelled TCDD was obtained by the use of the hydroxylapatite adsorption assay (8). EC_{50} values (the concentration of the competitor which causes a 50% reduction in the amount of radiolabelled TCDD-Ah receptor complex) for the substances studied are shown in Table 1.

TABLE 1: EC_{50}-values for single competitors

Compounds	EC_{50}-Values (mol L^{-1})
2,3,7,8-TCDD	1.1×10^{-9}
2,2',4,4',5,5'-HCB	2.4×10^{-5}
Aroclor 1254	5.4×10^{-6} *a*

a molar mass of Aroclor 1254 taken as 323 g mol^{-1}.

For concentrations other than the EC_{50}'s, the concentrations of the individual competitors can be expressed as TCDD-Equivalence concentrations (TCDD-EqC) based on the relationship [1].

$$[1] \qquad \text{(TCDD-EqC)} = \frac{(EC_{50}\text{-value of 2,3,7,8-TCDD}).(\text{concentration of competitor})}{(EC_{50}\text{-value of competitor})}$$

Competition curves were then obtained for mixtures of the substances in Table 1. The TCDD-EqC of each mixture was expressed as the sum of the TCDD-EqC's of the individual components. These experiments, which employed the substances in Table 1 in various combinations, are shown in Table 2.

TABLE 2: *TCDD-Equivalence Concentrations for several mixtures*

Mixture	Mixing-ratio	TCDD-Equivalence Concentration x 10^{-9} (mol L^{-1})
2,2',4,4',5,5'-HCB/TCDD	10000:1	9.3×10^{-10}
2,2',4,4',5,5'-HCB/TCDD	1000:1	1.3×10^{-9}
2,2',4,4',5,5'-HCB/TCDD	1:1	1.7×10^{-9}
Aroclor 1254/TCDD	10000:1	9.8×10^{-10}
Aroclor 1254/TCDD	1000:1	1.3×10^{-9}
Aroclor 1254/TCDD	1:1	1.4×10^{-9}

As was shown in Table 1, the EC_{50} value for TCDD alone is 1.1×10^{-9} mol L^{-1}. Since the concentrations of 2,2',4,4',5,5'-HCB and Aroclor 1254 have been expressed in terms of their TCDD-equivalence to produce Table 2, it follows that the TCDD Equivalence Concentration of any mixture should likewise be 1.1×10^{-9} mol L^{-1} if the competitors behave additively. Table 2 confirms that this is indeed the case within the precision of the assay. From this we can conclude that PCBs and TCDD compete for the same site(s) on the Ah receptor.

By the use of eq. [1] with concentration units of mol/kg rather than mol/L, we can calculate the TCDD-equivalences of the substances used in the teratogenicity experiments. The TCDD-equivalence of 2,2',4,4',5,5'-HCB (750 μmol/kg) was 3.4×10^{-8} mol/kg and of Aroclor 1254 (244 mg/kg) was 1.5×10^{-7} mol/kg, compared with 6.2×10^{-8} mol/kg of TCDD itself. If we can assume that these amounts of toxicants lead to intracellular concentrations that are greatly in excess of the Ah receptor concentration, we would conclude that the proportions of receptor molecules occupied by TCDD and HCB in the TCDD/HCB experiment were 65:35, with 29:71 the corresponding ratio for the TCDD/Aroclor mixture. In other words, only about half of the receptor sites have TCDD "displaced" from them. This suggests that the protective effect of PCBs against the teratogenicity in the C57BL/6 mouse is probably more than a simple mass law effect, and may in fact be antagonism as originally proposed.

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