POLYCHLOROBIPHENYLS THAT INDUCE δ-AMINOLEVULINIC ACID SYNTHETASE INHIBIT UROPORPHYRINOGEN DECARBOXYLASE IN CULTURED CHICK EMBRYO LIVER CELLS

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1. Introduction

Polychlorobiphenyls (PCBs) are toxic industrial chemicals which are widely distributed in the environment and have been identified in birds, fish and humans [1]. Oral administration of a commercial PCB mixture to chickens caused a hepatic-type porphyria characterized by hepatic accumulation and urinary excretion of uroporphyrin [2,3]. We studied the structural requirement of synthetic PCBs for inducing porphyrin accumulation in cultured chick embryo liver cells and found that 3,4,3′,4′-tetrachlorobiphenyl and 3,4,5,3′,4′,5′-hexachlorobiphenyl were most active [4]. To clarify the mechanism of the porphyrinogenic activity of these PCBs, we examined the relationship between induction of ALA synthetase and inhibition of uroporphyrinogen decarboxylase 3,4,3′,4′-Tetrachlorobiphenyl and 3,4,5,3′,4′,5′-hexachlorobiphenyl, which specifically induce ALA synthetase, also inhibit uroporphyrinogen decarboxylase to a comparable extent.

2. Materials and methods

Several synthetic PCBs were the generous gift from Dr T. Mizutani, Kyoto Prefectural University, Kyoto. Chick embryo liver cell cultures were prepared as in [4] except that phenol red was omitted from Eagle's minimum essential medium. Cultured cells in Falcon dishes (2 ml) were incubated with 4 μg of an individual PCB dissolved in 4 μl dimethylsulfoxide (DMSO) for 1 h. Then 0.2 μmol ALA was added and incubation was continued for up to 6 h. Incubation was terminated at the times indicated and total porphyrins were extracted from separated cells and medium, then quantified in a Hitachi MPF-4 fluorescence spectrophotometer [4]. For characterization, the isolated porphyrins were adsorbed on talc, esterified with methanol–sulfuric acid (19:1, v/v), and then separated by high-pressure liquid chromatography (HPLC) using a Hitachi chromatograph (model 638–50) with a Lichrosorb S1-100 column (250 × 4 mm) and quantified with a 650-10 LC spectrofluorometric detector (excitation 404 nm, emission 620 nm). The eluent was ethylacetate:*n*-hexane (1:1, v/v). Uroporphyrin–ester isomers were separated on a μ-Porasil column (300 × 39 mm) using *n*-heptane:glacial acetic acid:acetone:water (600:300:200:1, by vol.) [5,6]. Uroporphyrin I and III octamethylesters were kindly supplied by Dr T. K. With, Central County Hospital, Svendborg. Coproporphyrin III tetramethylester was prepared from diphteria toxoid [7]. Protoporphyrin IX dimethylester was prepared by Ramsey's method [8]. Hepta-, hexa- and pentacarboxylic porphyrins were isolated from hemoglobin-free chicken erythrocyte hemolysates incubated with ALA [9]. Uroporphyrinogen decarboxylase was purified from chicken erythrocytes as in [10]. The enzymatic activity was determined by measuring the amount of coproporphyrinogen formed from uroporphyrinogen as in [11]. Uroporphyrinogen was prepared by reducing uroporphyrin solution with freshly prepared 3% sodium amalgam. The assay system contained 277 pmol uroporphyrinogen, 5 μmol glutathione, 0.5 μmol EDTA, 355 μmol potassium phosphate (pH 7.0), 4.4 μg enzyme and 50 μg PCBs dissolved in 50 μl DMSO in a total volume of 5 ml. Incubation was carried out anaerobically at 37°C for 1 h in the dark. After that, the reaction mixture was oxidized with quinhydrone [7,12], then porphyrins

Table 1
Effect of PCBs on porphyrin formation from exogenous ALA in cultured chick embryo liver cells

PCB added	Porphyrins (pmol/mg protein)		Prevalent type of porphyrin
	Cells	Medium	
None	49 ± 4	240 ± 13	Proto
2,3,2′,3′-Tetrachlorobiphenyl	54 ± 4	230 ± 14	Proto
2,4,2′,4′-Tetrachlorobiphenyl	53 ± 9	230 ± 10	Proto
2,5,2′,5′-Tetrachlorobiphenyl	54 ± 6	230 ± 8	Proto
2,6,2′,6′-Tetrachlorobiphenyl	51 ± 4	240 ± 14	Proto
3,4,3′,4′-Tetrachlorobiphenyl	210 ± 9	89 ± 6	Uro
3,5,3′,5′-Tetrachlorobiphenyl	130 ± 10	150 ± 16	Uro + Proto
2,3,4,2′,3′,4′-Hexachlorobiphenyl	65 ± 9	240 ± 17	Proto
2,4,6,2′,4′,6′-Hexachlorobiphenyl	58 ± 4	250 ± 9	Proto
3,4,5,3′,4′,5′-Hexachlorobiphenyl	190 ± 9	91 ± 4	Uro

Chick embryo liver cells were cultured in 2 ml Falcon dishes in Eagle's minimum essential medium containing 10% fetal calf serum for 20 h. After the original medium was replaced, various PCBs in 4 μl DMSO (final conc. 2 μg/ml) were added and the mixture was incubated for 1 h. 0.2 μmol ALA was then added and the incubation continued for another 3 h. Total porphyrin content of cells and medium was quantified and the major porphyrins were characterized by HPLC. Porphyrin content of cells and medium was expressed/mg cell protein. Values represent the mean ± SD of 3–6 individual experiments

were separated as in [11,13] and spectrofluorometrically quantified. The protein concentration was determined following [14].

3. Results

When cultured chick embryo liver cells were supplemented with ALA, a large amount of protoporphyrin accumulated, primarily in the medium (table 1). A small amount of uroporphyrin, usually <10% of total porphyrins and some coproporphyrin also were formed. Addition of a strong inducer such as 3,4,3′,4′-tetrachlorobiphenyl or 3,4,5,3′,4′,5′-hexachlorobiphenyl resulted in accumulation of a large amount of uroporphyrin, while protoporphyrin accumulation was greatly reduced. Moreover, much of the uroporphyrin remained intracellular (table 1). With a moderate inducer, 3,5,3′,5′-tetrachlorobiphenyl, the increase in uroporphyrin and the decrease in protoporphyrin were more moderate. Addition of a weak inducer such as 2,3,2′,3′-, 2,4,2′,4′-, 2,5,2′,5′- or 2,6,2′,6′-tetrachlorobiphenyl and 2,3,4,2′,3′,4′- or 2,4,6,2′,4′,6′-hexachlorobiphenyl was with little or no effect on porphyrin accumulation after 3 h incubation (table 1), but elongation of the incubation time led to accumulation of uroporphyrin to some extent over the control in the cells (not shown).

The time course of porphyrin accumulation with 3,4,3′,4′-tetrachlorobiphenyl is shown in fig.1.

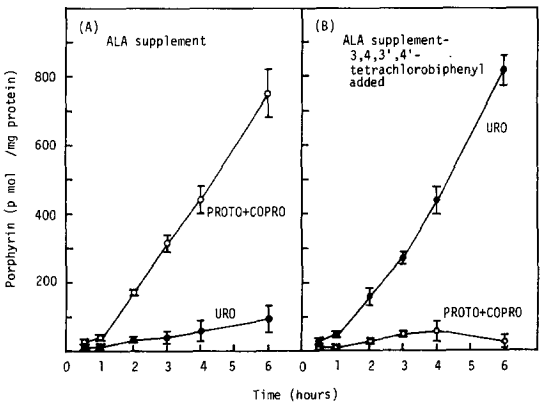

Fig.1. Time course of porphyrin formation from exogenous ALA by cultured chick embryo liver cells (A) in the presence of 3,4,3′,4′-tetrachlorobiphenyl (B). Experimental conditions were as in table 1, except that after addition of ALA, incubations were terminated at various times, from 0.5–6 h. Values represent the mean ± SD of 3 individual experiments. Uro-(porphyrin) reflects the sum of penta-, hexa-, hepta- and octacarboxylic porphyrins.

Table 2
Effect of PCBs on inhibition of uroporphyrinogen decarboxylase

PCB added	Coproporphyrinogen formed (pmol)	Inhibition (%)
None	112	–
2,3,2′,3′-Tetrachlorobiphenyl	65	42
2,4,2′,4′-Tetrachlorobiphenyl	62	44
3,4,3′,4′-Tetrachlorobiphenyl	18	84
3,4,5,3′,4′,5′-Hexachlorobiphenyl	9.4	92

The assay system contained 277 pmol uroporphyrinogen, 5 μmol glutathione, 0.5 μmol EDTA, 355 μmol potassium phosphate (pH 7.0), 4.4 μg partially purified uroporphyrinogen decarboxylase and 50 μg PCBs dissolved in 50 μl DMSO in a total volume of 5 ml. Assays were done anaerobically at 37° C for 1 h in the dark

Whereas ALA supplementation alone (A) resulted mainly in protoporphyrin accumulation, addition of 3,4,3′,4′-tetrachlorobiphenyl (B) reversed the pattern in that mostly uroporphyrin was formed at the expense of proto- and coproporphyrin. The uroporphyrin formed was almost entirely the isomer type III. Typical inhibitory effects of PCBs on partially purified uroporphyrinogen decarboxylase are shown in table 2, i.e., 3,4,3′,4′-tetrachlorobiphenyl and 3,4,5,3′,4′,5′-hexachlorobiphenyl almost completely inhibited coproporphyrinogen formation from uroporphyrinogen, whereas other PCBs partially inhibited the enzyme activity.

4. Discussion

We had established that the porphyrinogenic effect of PCBs in cultured chick embryo liver cells exhibits a sharply defined structure-activity relationship in that only 3,4,3′,4′-tetrachlorobiphenyl and 3,4,5,3′,4′,5′-hexachlorobiphenyl produced a marked accumulation of uroporphyrin [4]. We have now demonstrated that in ALA supplemented cultures, these same compounds lead to accumulation of a large amount of uroporphyrin III, whereas with other PCBs, which were weak inducers of porphyrin synthesis [4], the accumulated porphyrin was mostly protoporphyrin released into the medium. 3,5,3′,5′-Tetrachlorobiphenyl, found to moderately induce porphyrin formation [4], produced a mixture of uro- and protoporphyrin (table 1). We have also succeeded in demonstrating the inhibitory effects of PCBs on uroporphyrinogen decarboxylation using partially purified enzyme preparations. These results indicate that the same PBCs which most actively induce porphyrin synthesis also inhibit uroporphyrinogen decarboxylase. We had suggested a possibility of direct action of PCBs at transcriptional level [4]. Although this finding does not contradict that possibility, it indicates that porphyrinogenic PCBs inhibit primarily uroporphyrinogen decarboxylase leading to depletion of heme from which an increase of a synthesis of ALA synthetase results.

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