Decreased activity of uroporphyrinogen decarboxylase caused by 2,4,5,3′,4′-pentabromobiphenyl in chick embryo hepatocyte cultures

Difference in activity in intact or homogenized cells

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Uroporphyrinogen decarboxylase activity was investigated in cultures of chick embryo liver by two different methods: (1) analysis of porphyrin composition following incubation of intact cells with δ-aminolevulinic acid; and (2) a more conventional direct enzymic assay of cell homogenates. Activity was detectibly decreased following exposure of cells to 100 ng/ml 2,4,5,3′,4′-pentabromobiphenyl using the first method, but not the second. This decrease in activity was reversed by homogenizing the cells treated with 100 ng/ml pentabromobiphenyl. It is concluded that the direct homogenate assay of the enzyme may miss or underestimate decreases in its in vivo activity.

Porphyria *Uroporphyrin* *Polybromobiphenyl* *Aminolevulinic acid* *Liver*

1. INTRODUCTION

Activity of uroporphyrinogen decarboxylase (EC 4.1.1.37) (URO-D) is decreased in liver homogenates from animals treated for several weeks with hexachlorobenzene [1] or 2,3,7,8-tetrachloro-*p*-dibenzodioxin [2]. The activity is also decreased in humans with the liver disease, porphyria cutanea tarda (PCT) (see ref. in [3]). Both the chemically induced porphyria and PCT are characterized by similar patterns of porphyrin accumulation and excretion (see reviews [4,5]).

Chick embryo liver cells in culture have been reported to exhibit an apparent decrease in activity of URO-D after treatment for only one day with some polyhalogenated hydrocarbons [6]. We have recently extended these studies using various congeners of polybrominated biphenyls [7]. Other workers have shown specific effects on URO-D in the culture by planar congeners of polychlorinated biphenyls [8,9] and have investigated some aspects of the mechanism [10].

In all these studies, accumulation of uroporphyrin (URO) by intact hepatocytes incubated with δ-aminolevulinate (ALA) has been used as an indicator of decreased URO-D activity [6–10]. However, this accumulation of URO, with or without prior incubation with ALA, has not been correlated with direct measurements of URO-D activity on cell homogenates using a conventional enzyme assay.

We now report that low concentrations of 2,4,5,3′,4′-pentabromobiphenyl (PBB) decrease URO-D activity in intact cells assayed by incubation with ALA. This concentration of PBB is much lower than that required to decrease URO-D activity assayed directly in homogenates.

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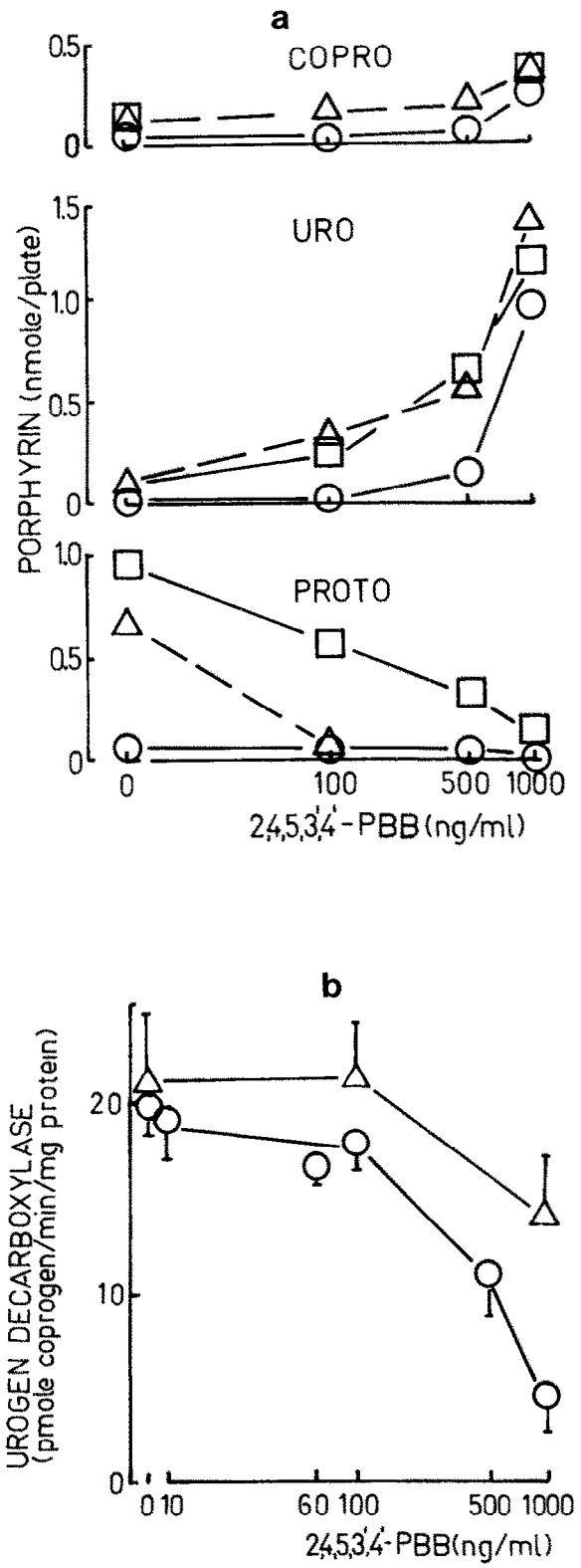

2. MATERIALS AND METHODS

2,4,5,3′,4′-PBB was isolated from Firemaster, a mixture of polybrominated biphenyls by Drs G. Dannan and S. Aust, Michigan State University. Porphyrin standards were from Porphyrin Products (Logan, UT). PBB was added to cultures in dimethylsulfoxide (2 μl/ml). Synthetic pentacarboxylporphyrin III and URO III were gifts from Dr A. Jackson, University College, Cardiff.

Chick embryo hepatocyte cultures were prepared and maintained as in [11]. URO, protoporphyrin (PROTO) and coproporphyrin (COPRO) in cultures and homogenates were determined by the spectrofluorometric method in [12] after extraction with an equal volume of 0.5 N perchloric acid in methanol. Porphyrin composition was also determined by thin-layer chromatography [13] and by high pressure liquid chromatography (HPLC) [14]. Cells and medium from 10-cm diam. dishes were lyophilized separately before preparation of methyl esters of the porphyrins [13]. URO-D was assayed in homogenates as described using either pentacarboxyporphyrinogen III or uroporphyrinogen III [15].

3. RESULTS

Figure 1 shows the dose–response curves for the effect of PBB on URO-D activity using either porphyrin formation from ALA by intact cells (fig.1a) or the rate of decarboxylation of penta-

Fig.1. Decreased activity of uroporphyrinogen decarboxylase after exposure of cultured hepatocytes to 2,4,5,3′,4′-pentabromobiphenyl (PBB). (a) Porphyrin composition after exposure of intact cells to ALA. Cultures were prepared in 6-cm diam. dishes as described in section 2 and exposed to PBB for 20 h. To one set of cultures, DES (100 μg/ml) was then added. After 4 h of further incubation, 150 μM ALA was added and porphyrin composition determined after 90 min of further incubation. Each point is the mean of triplicate dishes (values agreed within 10%). (○) PBB only; (△) PBB and ALA; (□) PBB, DES and ALA. (b) Rate of decarboxylation of pentacarboxyporphyrinogen III by homogenates of cultured hepatocytes. Homogenates were prepared after 20 h PBB treatment and enzyme activity determined as in section 2. Each point is the mean ± SD for 3 separate dishes. Results from the two separate experiments are shown (○,△).

carboxyporphyrinogen III by cell homogenates (fig.1b), as indicators of URO-D activity. Intact cells exposed to PBB produced more URO and less PROTO when incubated with ALA than did control cells (fig.1a). Addition of desferrioxamine (DES), a powerful iron chelator, to prevent formation of heme from PROTO, had no effect on URO accumulation but enhanced PROTO formation at all concentrations of PBB. These results show that PROTO formation is progressively inhibited by PBB at concentrations from 100–1000 ng/ml. The inhibition is accompanied by accumulation of URO, but not COPRO (fig.1a), suggesting that these changes are produced by progressive inhibition of URO-D activity.

In contrast, measurement of URO-D activity in homogenates showed no decrease in activity until a concentration of 500 ng PBB/ml was reached (fig.1b) by which concentration the intact cells accumulated porphyrin (shown by HPLC to be mainly URO and heptacarboxylic porphyrin). Almost identical results were obtained when uroporphyrinogen III was used as substrate, thus excluding the possibility that PBB selectively decreases the decarboxylation of pentacarboxyporphyrinogen. A similar dose–response curve was obtained when cultured cells were sonicated before incubation with ALA (fig.2). Again, there was no evidence for a decrease in URO-D activity at 100 ng PBB/ml.

The cellular and medium porphyrins in cultures treated with PBB and then incubated with ALA (fig.1a) were fractionated by thin-layer chromatography. The patterns were similar at 100 ng PBB/ml and 1000 ng PBB/ml for both cells and medium. Cells contained mainly URO (58% type III isomer at 100 ng/ml; 42% at 1000 ng/ml) and heptacarboxylic porphyrin (100% type III isomer; accounting for 23% and 17% of the total porphyrin at 100 ng/ml and 1000 ng/ml, respectively). At both concentrations, the medium contained URO and porphyrins with 7 to 4 carboxyl groups, including a porphyrin of the isocoproporphyrin series, and PROTO. URO and 7-carboxylporphyrin was almost entirely retained within the cells as reported originally [16] and recently confirmed [9].

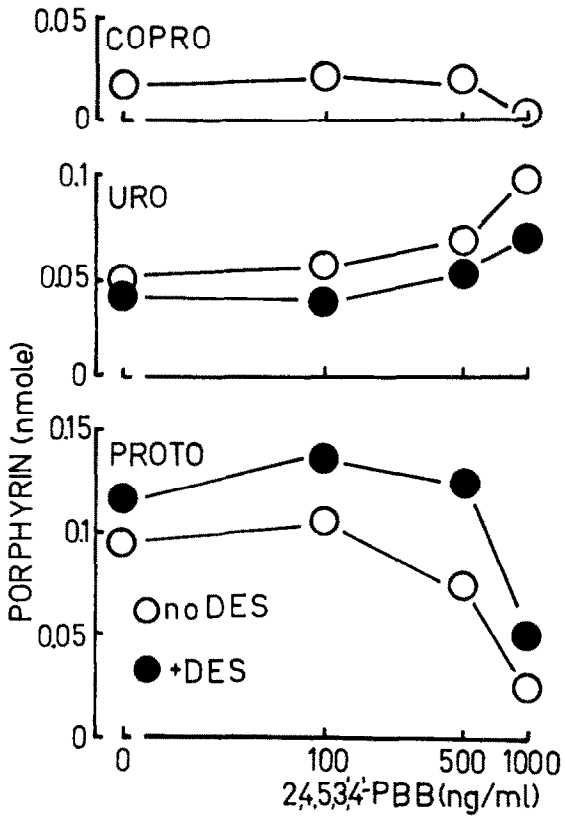

Fig.2. Composition of porphyrins generated from ALA by sonicates of cultures treated with 2,4,5,3′,4′-pentabromobiphenyl. Cultures were prepared as described in section 2 in 10-cm diam. dishes. Cultures were exposed to PBB for 20 h and then rinsed with saline, harvested in 1.1 ml 50 mM Tris (0.25 M sucrose) 1 mM ALA (pH 7.4) at 4°C, sonicated and 0.2 ml incubated at 37°C for 90 min with (●—●) and without (○—○) DES (0.75 mg/ml). Porphyrin composition was determined as described in section 2, and was corrected for porphyrin present before incubation of sonicates.

4. DISCUSSION

Previously, it has been shown only by indirect methods that the activity of URO-D is apparently decreased after exposure of chick embryo cultured hepatocytes to several different polyhalogenated aromatic hydrocarbons [6–10]. We have now shown that the decrease in URO-D is also detected in homogenates of the cells by the more conventional direct assay used previously to measure URO-D in homogenates of mammalian liver (fig.1b) [1,3,15]. The decrease in URO-D, when determined by direct measurement in cell homogenates (fig.1b), occurred at PBB concentrations that cau-

sed URO accumulation by the cultures in the absence of added ALA (fig.1a). Such a relationship between URO-D activity and hepatic porphyrin concentration has been observed in the experimental porphyrias caused by halogenated hydrocarbons in rodents [1,17].

An unexpected finding was that loading intact hepatocytes with ALA caused increased URO and decreased PROTO accumulation at a PBB concentration (100 ng/ml) which was much lower than that required to produce either a decrease in URO-D activity, when assayed in cell homogenates, or an accumulation of porphyrin in the absence of ALA (fig.1). The pattern of porphyrin production from ALA by intact cells after treatment with 100 ng PBB/ml closely resembled the patterns found with 1000 ng/ml and those which accompany decreased hepatic URO-D in intact animals and humans with PCT [1,4,5]. The cells treated with PBB retained most of the porphyrin (mainly URO), as does intact liver. The composition of medium porphyrins resembled that of the excreted porphyrins (urine and feces) of the intact animal. These similarities suggest that ALA loading uncovers a URO-D defect in the intact cells at 100 ng PBB/ml. This decrease in URO-D in the intact cells after treatment with 100 ng PBB/ml was reversible by breaking the cells as detected by composition of porphyrins accumulated from ALA (fig.2).

Two possible explanations for differences between the dose–response curves of intact cells and homogenates are: (1) that there is a small decrease in activity of URO-D (perhaps less than 10%) that is not detected by the homogenate assays; (2) that there is inhibition of URO-D in the intact cells (perhaps as much as 50%) that is relieved when the cells are broken. A major difficulty in comparing the dose–response curves is that the ALA loading technique does not give the % decrease in URO-D in the intact cells directly. However, if one assumes that DES prevented conversion of PROTO to heme, then the amount of PROTO accumulated in the presence of DES (fig.1a) provides an estimate of the relative decrease in URO-D. A 50% decrease in PROTO was caused by 100 ng PBB/ml and 500 and 1000 ng PBB/ml caused further decrease in PROTO.

Nevertheless, we cannot rule out the second explanation, i.e., decreased URO-D inhibition upon cell breakage and dilution. One might postulate two phases of the inhibition, a reversible one at 100 ng PBB/ml and an irreversible one at 1000 ng PBB/ml. This explanation is consistent with a metabolite of PBB inhibiting URO-D [6,10] and dissociation of the metabolite from the enzyme upon dilution. Currently there are few data in favor of the metabolite mechanism of URO-D inhibition. Furthermore, very rapid change in porphyrin accumulation from PROTO to URO in cells treated with 3,4,3′,4′-tetrachlorobiphenyl has been reported in [9], a finding we have reproduced (unpublished). Any inhibitory metabolite would have to have been very rapidly produced.

Our results show that direct homogenate assays of URO-D may not reflect in vivo activity of the enzyme. Thus, in animals treated for long periods with halogenated hydrocarbons [1], URO-D may have been decreased earlier than detected by the homogenate assay. Similarly, in human PCT, where an approximate 40% decrease in hepatic URO-D is detected in homogenates (e.g., [18]), it is possible that the degree of decrease is greater and that small decreases in URO-D activity may have been missed by exclusive use of the homogenate assay.

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