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XENOBIOTIC MEDIATED INHIBITION OF HEPATIC UROPORPHYRINOGEN DECARBOXYLASE ACTIVITY IN 17-DAY-OLD CHICK EMBRYO LIVER CELLS IN CULTURE*

MARTHA E. LYON, JAMES A. OWEN† and GERALD S. MARKS‡
Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada K7L 3N6

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Abstract—Uroporphyrin, heptacarboxylic acid porphyrin and coproporphyrin were the major porphyrins to accumulate when phenobarbital, nifedipine, 3,5-diethoxycarbonyl-2,4,6-trimethylpyridine (Ox-DDC) and 3,3′,4,4′-tetrachorobiphenyl (TCBP) were added to chick embryo hepatocyte culture. This pattern of porphyrin accumulation is consistent with the demonstration that these chemicals inhibit uroporphyrinogen decarboxylase (UROG-D). The degree of UROG-D inhibition observed was: TCBP (39%), Ox-DDC (39%), nifedipine (25%) and phenobarbital (50%). Since significant UROG-D inhibition was observed when the bulk of the porphyrins in the crude enzyme preparation was removed by gel filtration, it is unlikely that porphyrins produce the enzyme inhibition. When succinylacetone, a potent inhibitor of δ-aminolevulinic acid dehydratase, was coadministered with Ox-DDC, phenobarbital, TCBP and nifedipine, UROG-D inhibition was not observed. These results suggest that heme biosynthesis must proceed in order for xenobiotic mediated UROG-D inhibition to occur.

Several polyhalogenated aromatic hydrocarbons, e.g. hexachlorobenzene and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, when administered to rodents decrease hepatic uroporphyrinogen decarboxylase (UROG-D) activity [1]. Treatment of 17-day-old chick embryo liver cell cultures with 3,3′,4,4′-tetrachlorobiphenyl (TCBP) has been shown to cause the accumulation of uroporphyrin and heptacarboxylic acid porphyrin [2] and to inhibit UROG-D [3]. However, although the pyridine, 3,5-diethoxycarbonyl-2,4,6-trimethylpyridine (Ox-DDC), produces a pattern of porphyrin accumulation similar to that produced by TCBP, Swain [4] was unable to demonstrate UROG-D inhibition. The method used [4] to monitor UROG-D activity [3], and which was successful with TCBP, involved gel filtration of a crude UROG-D enzyme preparation to remove porphyrins which would have interfered with the enzyme assay. It was possible that the difficulty in observing UROG-D inhibition with Ox-DDC might have been due to the removal by gel filtration of "inhibitory" substances. We proposed therefore to reinvestigate the possibility that Ox-DDC and other porphyrogenic chemicals inhibit UROG-D activity in chick embryo liver cells in culture. In this study pentacarboxylporphyrinogen I (penta'gen I) was utilized as the substrate for UROG-D rather than uroporphyrinogen III to simplify the measurement of UROG-D activity.

The following methods were used: (1) Succinylacetone, a potent and specific inhibitor of δ-aminolevulinic acid (ALA) dehydratase [5], was added to the chick embryo hepatocyte culture concomitantly with Ox-DDC and other chemicals to prevent porphyrin formation. By this means it was thought that UROG-D inhibition would occur and be demonstrable without the need to remove porphyrins by means of a Sephadex G-25 column prior to the enzyme assay. (2) The UROG-D assay was carried out with the omission of the gel filtration procedure to preclude the possibility of removing possible enzyme inhibitory substances in this step.

In a previous study we showed [6] that, when nifedipine is added to chick embryo hepatocyte culture, uroporphyrin, heptacarboxylic acid porphyrin and coproporphyrin accumulate, suggesting that nifedipine may act as a UROG-D inhibitor. For this reason the effect of nifedipine on UROG-D activity in chick embryo liver cells was studied. Phenobarbital has been reported to cause the accumulation of uroporphyrin and heptacarboxylic acid porphyrin when added to chick embryo hepatocyte culture and was reported to inhibit UROG-D [7]. We have therefore reinvestigated the porphyrin pattern produced by phenobarbital and its effect on UROG-D activity.

MATERIALS AND METHODS

Chemicals

Nifedipine was a gift from Dr. D. J. Triggle (Buffalo, NY). Partisil 5 was purchased from Whatman (Clifton, NY). Ox-DDC was synthesized previously in our laboratory according to the method of Loev

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† Supported by an Ontario Ministry of Health Career Scientist Award.
‡ To whom correspondence should be sent.

and Snader [8]. Sodium phenobarbital was purchased from Allen & Hanbury Ltd. (Toronto, Ontario). 3,3′,4,4′-Tetrachlorobiphenyl (dioxin and furan free) was previously synthesized and purified in our laboratory and was shown to be greater than 99% pure [9]. Succinylacetone (4,6-dioxoheptanoic acid) was purchased from the Sigma Chemical Co. (St. Louis, MO). PD-10 Sephadex G25M columns were obtained from Pharmacia (Dorval, Quebec).

Preparation of uroporphyrinogen decarboxylase from chick embryo liver cell culture

A modification of the procedure of Swain *et al.* [3] was used. Details of the cell culture technique have been described previously [10]. In brief, chick embryo liver cells were maintained in 10-cm diameter plastic Petri dishes containing 15 ml of serum-free Waymouth MD 705/1 medium, supplemented with penicillin G sodium, streptomycin sulfate, insulin and L-thyroxine sodium pentahydrate. After an initial incubation period of 24 hr, the medium was discarded and replaced with fresh medium (15 ml). The porphyrogenic agents were dissolved in either 95% ethanol, acetone or distilled water and then administered to the cell culture medium in a volume not exceeding 30 μl; controls received the solvent alone. Twenty-four hours after the addition of the porphyrogenic agents, the medium was discarded and 3 ml of ice-cold 0.1 M sodium phosphate buffer, pH 6.0, containing 0.1 mM EDTA and 5 mM dithiothreitol (henceforth known as UROG-D assay buffer) was added to each dish. All procedures thereafter were carried out on ice. The cells were then scraped from the Petri dishes using a rubber policeman, and the contents of two identically treated plates were combined into one tube. The cells were centrifuged at 950 *g* for 5 min, the supernatant fluid was discarded, and the pellet was resuspended in 3 ml of UROG-D assay buffer. This suspension was homogenized using a glass Potter–Elvehjem homogenizer and a teflon motor-driven pestle (6 up and down strokes) and then centrifuged at 10,000 *g* for 20 min. The postmitochondrial supernatant fluid was used as the source of the enzyme. Fifty microliters of this supernatant fluid was retained for protein determination.

Uroporphyrinogen decarboxylase assay

Pentacarboxylic acid porphyrin I, in dilute alkaline solution, was reduced with 3% sodium amalgam to pentacarboxylic acid porphyrinogen I (penta'gen I) and was diluted to a concentration of 6.67 nmol/ml in UROG-D assay buffer.

Ten nanomoles per 1.5 ml of penta'gen I in UROG-D assay buffer was placed into the side arm of a Thunberg tube. The UROG-D assay buffer (1.5 ml) and the enzyme preparation (1 ml) were placed in the bottom of the Thunberg tube. To achieve an anaerobic atmosphere, the tube was repeatedly evacuated (water pump) and flushed with nitrogen (five times). The reaction was begun by tipping the contents of the side arm into the bottom of the tube and mixing vigorously. The tubes were then incubated for 45 min in a shaking water bath (37°) (90 oscillations/min). The reaction was terminated by adding 200 μl of 0.1% iodine in ethanol to the tubes. Mesoporphyrin IX (0.25 nmol), the internal standard for the HPLC porphyrin assay, was then added to the tubes, and the contents were then poured into 50-ml round bottom tubes and lyophilized overnight. Five milliliters of 5% concentrated sulfuric acid/methanol (v/v) was added to each tube, and the contents were allowed to stand for 24 hr at −15°. The methyl esters were extracted into dichloromethane, washed with 5% sodium bicarbonate and then distilled water, dried over anhydrous sodium sulfate, and then evaporated to dryness under nitrogen at 37°. The dried sample was redissolved in 200 μl of HPLC mobile phase:dichloromethane (1:1). Ten microliters of this sample was injected onto the HPLC column. Enzyme activity was expressed as picomoles coproporphyrin (COPRO) formed per 45 minutes per milligram of protein. Proteins were determined according to the method of Lowry *et al.* [11] as modified by Miller [12].

Selection of conditions for UROG-D assay

UROG-D activity was found to be proportional to protein concentration over the range 0.04 to 0.16 mg/ml. The protein concentration selected for use in the enzyme assay was 0.12 mg protein/ml. UROG-D activity was shown to be linear when studied between 10 and 45 min. The concentration of penta'gen used in our determination of UROG-D activity was 2.5 μM. This concentration is considerably larger than the K_m value of 0.03 μM determined by the Eadie–Hofstee plot, thus ensuring saturating conditions. Each data point shown for UROG-D activity represents the mean ± SD of at least five determinations.

The reproducibility of the UROG-D assay is dependent upon the reproducibility of the HPLC assay since UROG-D activity is determined by monitoring coproporphyrin I formation in the post-mitochondrial supernatant. The within day and between day coefficients of variation in recovering 250 pmol of coproporphyrin III or penta'gen I were, respectively, 4%, 3% (within day) and 10%, 5% (between day). The within day and between day coefficients of variation were similar when recovering the porphyrins from the post-mitochondrial supernatant.

High-pressure liquid chromatography system

Quantitation of pentacarboxylic acid porphyrin and coproporphyrin was carried out by means of a high-pressure liquid chromatographic system capable of separating and determining porphyrins as their methyl esters in the picomole range. The method was a modification of that used by Zelt *et al.* [13] and employed mesoporphyrin IX as an internal standard. The mobile phase [14] consisted of heptane–ethyl acetate–dichloromethane–methanol (60:25:14:1) and microparticle silica gel, Partisil 5, was used as the stationary phase. Standard curves were prepared by adding a series of concentrations (0.01, 0.05, 0.10, 0.25, 0.50, 1.00 nmol) of the free acid porphyrins (pentacarboxylic acid porphyrin I and coproporphyrin III) plus the mesoporphyrin IX internal standard to an untreated postmitochondrial supernatant. The porphyrin methyl esters were recovered as described above. Standard curves were constructed

by measuring the peak heights of the esterified porphyrins using an electronic integrator and expressing the data as the ratio *R/IS* where *R* is the peak height of the specific porphyrin and *IS* is the peak height of mesoporphyrin IX (0.25 nmol), the internal standard. For each biological sample, the amount of each porphyrin was determined by calculating the porphyrin to internal standard peak height ratio and then interpolating from the standard curve.

Approaches taken to monitor UROG-D activity

Gel filtration of the post-mitochondrial supernatants. In one series of experiments an attempt was made to remove the porphyrins prior to the enzyme assay by passing 1 ml of the enzyme preparation (post-mitochondrial supernatant) through a Sephadex G-25M column (PD10; 4.8 cm × 1.6 cm internal diameter) and eluting with UROG-D assay buffer. The absorbance of 1-ml fractions collected was monitored at 276 nm (protein) and 405 nm (porphyrins). The major protein peak was pooled and used as the enzyme source. Controls were treated in an identical fashion.

Succinylacetone studies. In another series of experiments, the formation of porphyrins in the cells was inhibited by the addition of succinylacetone (200 μg/ml), an inhibitor of δ-aminolevulinic acid dehydrase (ALA-dehydratase). The succinylacetone was dissolved in 95% ethanol and administered concomitantly with the porphyrogenic agents (total volume did not exceed 30 μl). The volume of solvent in controls was suitably adjusted.

No removal of porphyrins from the enzyme preparation. To determine whether porphyrins could contribute to the inhibition of UROG-D activity, we monitored UROG-D activity in the absence of succinylacetone and without removing the porphyrins from the enzyme preparations via gel filtration. To calculate the COPRO'GEN I formed from penta'gen I during the UROG-D assay, it was necessary to determine and subtract the quantity of COPRO'GEN I present in the enzyme preparation prior to incubation. Controls were treated in an identical fashion.

Determination of porphyrin profiles

Chick embryo liver cells were maintained in 6-cm diameter plastic dishes containing 5 ml of the medium. Drugs were dissolved in 95% ethanol, acetone or distilled water (maximal total volume: 15 μl). Twenty-four hours later, the contents of each dish were lyophilized and the porphyrins were esterified, extracted into dichloromethane, and evaporated to dryness as described above. The residue was dissolved in 200 μl of dichloromethane : mobile phase (1:1), and 10 μl was injected onto the column. To quantitate the individual porphyrins, several concentrations (10–1000 pmol) of uroporphyrin III, heptacarboxylic acid porphyrin I, hexacarboxylic acid porphyrin I, pentacarboxylic acid porphyrin I, coproporphyrin III, and, where appropriate, mesoporphyrin IX were added to cells and medium. After esterification and extraction of these standards, a series of standard curves were constructed. The porphyrin concentration of each biological sample was interpolated from the appropriate standard curve, and the results were expressed as picomoles of porphyrin per milligram of protein.

Statistical analysis

Comparisons were made among various groups: (1) control group (no drug or vehicle present), (2) vehicle control group (no drug present), (3) experimental group (drug and vehicle present). An analysis of variance was used to determine significant differences between groups ($P < 0.05$). Significantly different groups were identified using the non-parametric Newman–Keul's test ($P < 0.05$).

RESULTS AND DISCUSSION

The porphyrin patterns previously reported for phenobarbital [7], nifedipine [6], Ox-DDC and 3,3′,4,4′-TCBP [2] were confirmed (Fig. 1). Thus, uroporphyrin and heptacarboxylic acid porphyrin were the major porphyrins to accumulate in response to 3,3′,4,4′-TCBP (Fig. 1b), and uroporphyrin, heptacarboxylic acid porphyrin and coproporphyrin were the major porphyrins to accumulate in response to phenobarbital (Fig. 1a), nifedipine (Fig. 1c) and Ox-DDC (Fig. 1d). In the case of TCBP, uroporphyrin and heptacarboxylic acid porphyrin account for almost all of the porphyrins that accumulate; this would be anticipated if inhibition of UROG-D were the sole mechanism of action of this compound. In the case of phenobarbital, nifedipine and Ox-DDC, the accumulation of considerable quantities of coproporphyrin in addition to uroporphyrin and heptacarboxylic porphyrin suggests that these compounds may exert an effect in addition to UROG-D inhibition.

The results in Fig. 2 show that, in the presence of succinylacetone (200 μg/ml), none of the chemicals examined, viz. Ox-DDC, nifedipine, phenobarbital and TCBP, inhibited UROG-D. There are at least two possible interpretations of these results: (1) Succinylacetone prevents heme synthesis required for combination with apocytochrome P-450 to form cytochrome P-450. Cytochrome P-450, in turn, might be required for formation of metabolites from the porphyrogenic chemicals that inhibit UROG-D [3, 7, 15, 16]. Alternatively, cytochrome P-450 might be required for interaction with the porphyrogenic chemicals resulting in the formation of reactive oxygen species that may inactivate UROG-D [17]. (2) Succinylacetone prevents porphyrin formation and the porphyrins play a role in the inhibition of UROG-D. Evidence that porphyrins inhibit UROG-D has been provided by the studies of Smith and Francis [18].

In our next series of experiments designed to assess UROG-D inhibition, succinylacetone was omitted from the chick embryo liver cell culture. No attempt was made to remove the porphyrins from the crude UROG-D preparation prior to the assay. For this reason it was necessary to determine the amount of coproporphyrin in the UROG-D preparation prior to conducting the enzyme assay so that this amount could be subtracted from the total coproporphyrin detected in the assay procedure. The porphyrins present in the crude UROG-D enzyme preparation are shown in Fig. 3. Uroporphyrin and heptacarboxy-

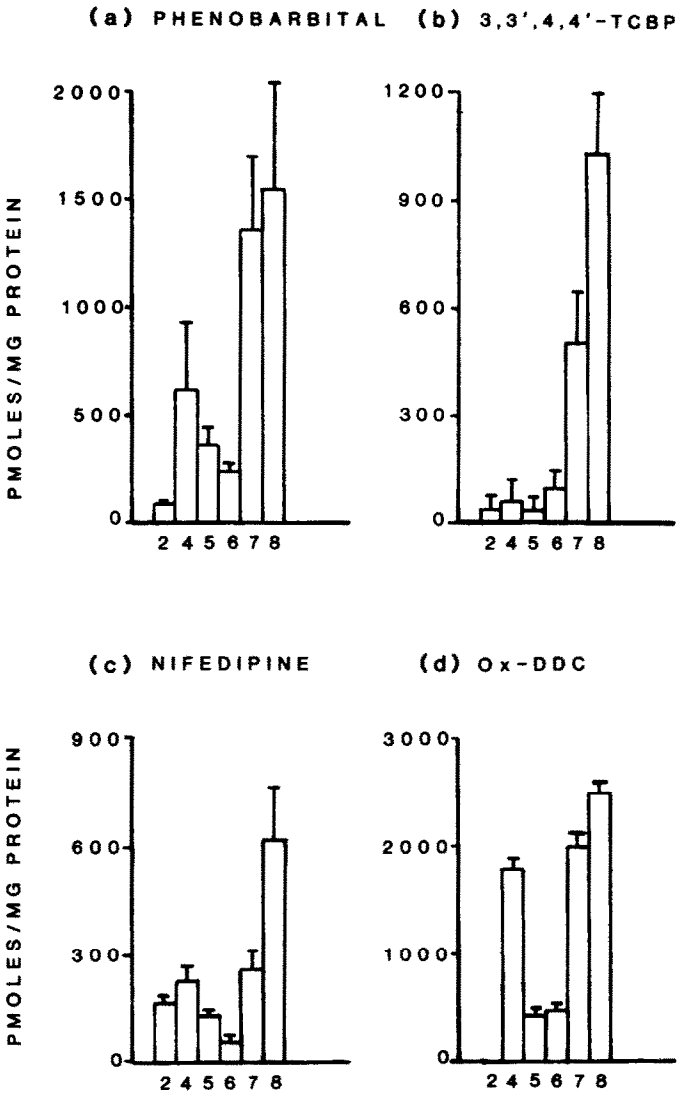

Fig. 1. Porphyrin patterns obtained 24 hr after the addition of (a) sodium phenobarbital (232 μg/ml of medium); (b) 3,3',4,4'-TCBP (10 μg/ml medium); (c) nifedipine (25 μg/ml medium) and (d) Ox-DDC (100 μg/ml medium) to chick embryo liver cell cultures. The porphyrins shown represent the total porphyrins in the cells and medium of each dish. Numbers under the bars indicate the number of carboxyl groups (8 = uroporphyrin, 7 = heptacarboxylic porphyrin, 6 = hexacarboxylic porphyrin, 5 = pentacarboxylic porphyrin, 4 = coproporphyrin, 2 = protoporphyrin). Each bar represents the mean (±SD) of five determinations. Solvent control [95% ethanol in (c) and (d), water in (a) and acetone in (b)] was 50–60 pmol porphyrin/mg protein; coproporphyrin was the major porphyrin accompanied by protoporphyrin.

lic acid porphyrin were the major porphyrins detected together with a small amount of coproporphyrin. A significant reduction in UROG-D activity was observed (Fig. 4) with TCBP (39%), Ox-DDC (39%), nifedipine (25%) and phenobarbital (50%). The results obtained confirm the previous findings with TCBP [3], and with phenobarbital [7]. Recently, Schoenfeld *et al.* [19] demonstrated that nifedipine inhibits UROG-D in chick embryo liver cells.

To determine the contribution of porphyrins to the inhibition of UROG-D the experiments were repeated with the inclusion of a gel (Sephadex G25M) filtration step. The results obtained (Fig. 5) show a significant inhibition of UROG-D with TCBP (18%), Ox-DDC (41%) phenobarbital (49%) and nifedipine (31%). The 18% inhibition observed with TCBP is in agreement with a previously reported result [3]. The introduction of a gel filtration step reduced the inhibition of UROG-D observed with TCBP from 39%, without gel filtration, to 18% with gel filtration, suggesting that the porphyrins contributed to the inhibition observed when the gel filtration step was omitted. With the other chemicals, however, inclusion of a gel filtration step did not alter UROG-D inhibition. This observation suggested that porphyrins do not play a role in the UROG-D inhibition observed with these chemicals.

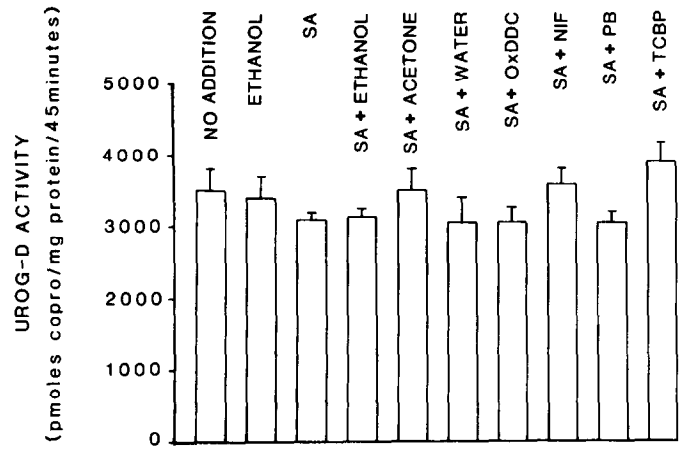

Fig. 2. UROG-D activity in chick embryo liver cell culture 24 hr after concomitant administration of succinylacetone (SA, 200 μg/ml) with Ox-DDC (100 μg/ml) or nifedipine (NIF, 25 μg/ml) or phenobarbital (PB, 232 μg/ml) or 3,3',4,4'-TCBP (TCBP, 10 μg/ml). Each bar represents the mean (±SD) of at least five determinations.

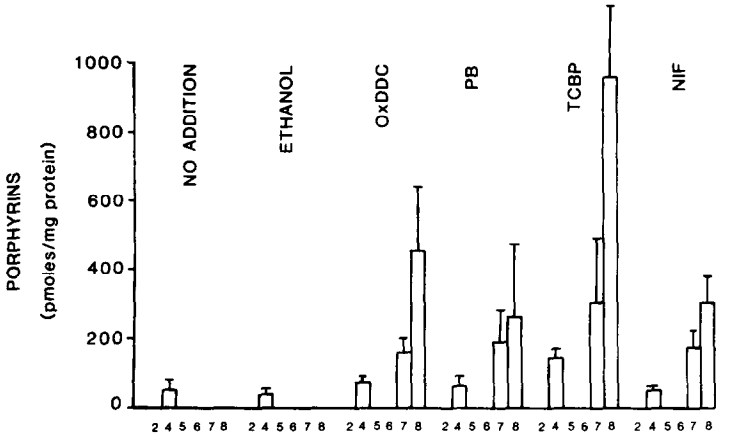

Fig. 3. Porphyrin patterns in the postmitochondrial supernatant obtained from chick embryo hepatocyte culture 24 hr following administration of Ox-DDC (100 μg/ml), nifedipine (NIF, 25 μg/ml), phenobarbital (PB, 232 μg/ml) and 3,3',4,4'-TCBP (TCBP, 10 μg/ml). Numbers under the bars indicate the number of carboxyl groups. Each bar represents the mean (±SD) of at least five determinations.

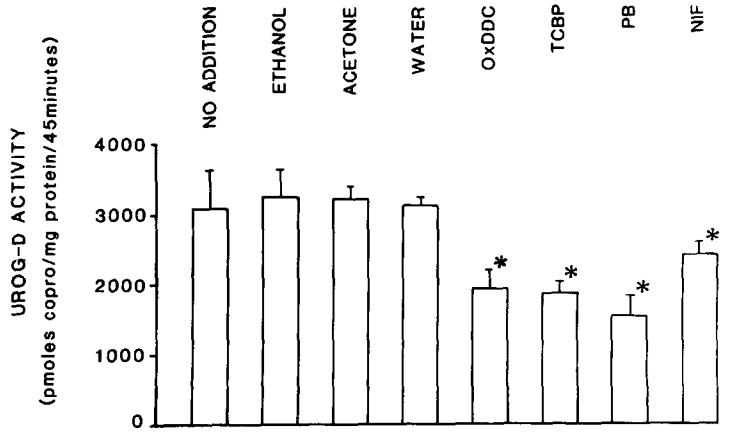

Fig. 4. UROG-D activity 24 hr following administration to chick embryo liver cell culture of Ox-DDC (100 μg/ml), nifedipine (NIF, 25 μg/ml), phenobarbital (PB, 232 μg/ml) and 3,3',4,4'-TCBP (TCBP, 10 μg/ml) without gel filtration of the postmitochondrial supernatants. Ox-DDC and nifedipine were dissolved in ethanol, TCBP in acetone, and sodium phenobarbital in water. Key: (*) significantly different from control, $P < 0.05$. Each bar represents the mean (±SD) of at least five determinations.

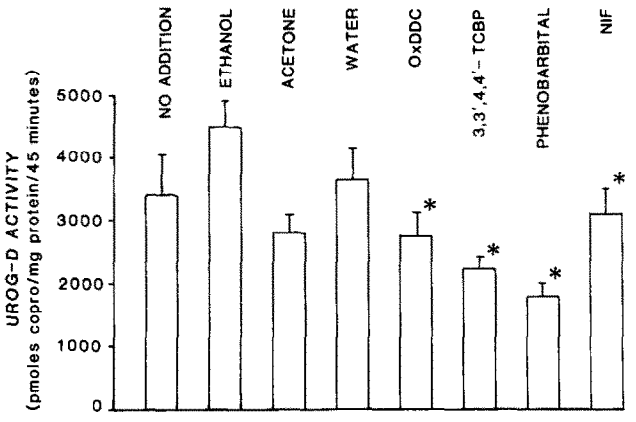

Fig. 5. UROG-D activity following gel filtration of the postmitochondrial supernatants 24 hr after administration to chick embryo liver cell cultures of Ox-DDC (100 μg/ml), nifedipine (NIF, 25 μg/ml), phenobarbital (232 μg/ml) and 3,3',4,4'-TCBP (10 μg/ml). Ox-DDC and nifedipine were dissolved in ethanol, TCBP in acetone, and sodium phenobarbital in water. Key: (*) significantly different from control, $P < 0.05$. Each bar represents the mean (±SD) of at least five determinations.

A possible explanation for the above results might have been retention after gel filtration of porphyrins in the crude UROG-D enzyme preparation following treatment with Ox-DDC, phenobarbital or nifedipine but not after treatment with TCBP. To examine this possibility the crude UROG-D enzyme preparation was examined for porphyrin content following gel filtration. The results (Fig. 6) show that this explanation is incorrect since comparable amounts of porphyrin (uroporphyrin plus coproporphyrin) were retained in each of the gel-filtered enzyme preparations. An alternative explanation for the difference observed with TCBP, before and after gel filtration, might be the removal by gel filtration of UROG-D inhibitory metabolites formed from TCBP. The following conclusions were reached: (1) it is unlikely that porphyrins play a significant role in UROG-D inhibition and (2) the accumulation of uroporphyrin and heptacarboxylic acid porphyrin as

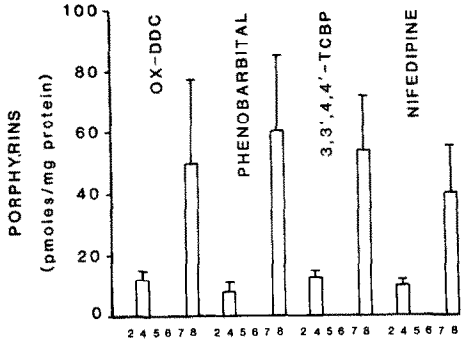

Fig. 6. Porphyrin patterns following gel filtration of the postmitochondrial supernatants obtained 24 hr after administration to chick embryo liver cell cultures of Ox-DDC (100 μg/ml), nifedipine (25 μg/ml), phenobarbital (232 μg/ml) and 3,3',4,4'-TCBP (10 μg/ml). Numbers under the bars indicate the number of carboxyl groups. Porphyrins were not detectable in any of the controls (cells alone, 95% ethanol, distilled water and acetone) following gel filtration. Each bar represents the mean (±SD) of at least five determinations.

the major porphyrins in response to Ox-DDC, phenobarbital, TCBP and nifedipine (Fig. 1) is consistent with the fact that these chemicals inhibit UROG-D.

The next question that arises is: What is the mechanism by which a variety of structurally unrelated xenobiotics cause inhibition of UROG-D? Some observations that might illuminate this problem are the following. In polyhalogenated aromatic hydrocarbon-induced porphyria, hepatic UROG-D activity is decreased without a fall in the concentration of immunoreactive enzyme protein [1]. The suggestion has been made that metabolites of polyhalogenated aromatic hydrocarbons may react with an essential catalytic site in UROG-D forming a covalently bonded enzyme–metabolite conjugate [1]. A second hypothesis is based on the fact that polyhalogenated aromatic hydrocarbons and other xenobiotics enhance formation of NADPH-dependent hydrogen peroxide formation and will therefore presumably also enhance the formation of the superoxide anion and hydroxyl radicals; it is suggested that interaction of the radicals and/or hydrogen peroxide with UROG-D results in inactivation of the enzyme. Recently, it was shown that hexachlorobenzene enhances the production of reactive oxygen species by the microsomal mixed-function oxidase system [20] and that UROG-D is susceptible to inactivation by reactive oxygen species. It has been suggested that porphyrinogens may be oxidized by peroxide to the corresponding porphyrins [17]. This may account, at least in part, for the accumulation of coproporphyrin in response to Ox-DDC, nifedipine and phenobarbital (Fig. 1). It may also account, at least in part, for the large accumulation of uroporphyrin and heptacarboxylic porphyrin (Fig. 1) despite the fact that UROG-D is only partially inhibited by these compounds.

The second hypothesis, which attributes inactivation of UROG-D to interaction with reactive oxygen species, generated by interaction of xenobiotics with the mixed-function oxidase system, is attractive in that it explains the fact that compounds

of widely differing chemical structure share the ability to inhibit UROG-D. A property that xenobiotics, which enhance production of reactive oxygen species, appear to share is that they are metabolized slowly [20]. In addition, these compounds are also lipophilic. It has been noted previously that two of the characteristic properties of porphyrogenic chemicals are lipophilicity and resistance to rapid metabolism to compounds of lower lipophilicity [21]. We would like to suggest the following hypothesis: (1) Chemicals that are readily biotransformed by hydrolytic enzymes, the mixed-function oxidase system, or phase II reactions (e.g. glucuronidation) will not exhibit UROG-D inhibition or porphyrogenicity. (2) The group of porphyrogenic chemicals that inhibit UROG-D are chemicals that are restricted to metabolism by the mixed-function oxidase system but are biotransformed less readily and, as a result, enhance the production of reactive oxygen species by this system; interaction of the reactive oxygen species with the active site of UROG-D will lead to inhibition. This hypothesis is currently being investigated in our laboratory. It is noteworthy that the porphyrogenic Ox-DDC has been shown to be slowly metabolized (half-life = 8–24 hr) in chick embryo liver cell culture, whereas the structurally related non-porphyrogenic 3,5 - diethoxylcarbonyl - 2,6 - dimethylpyridine is metabolized rapidly (0.15 to 0.6 hr) [22, 23]. Moreover, the corresponding porphyrogenic dihydropyridine, DDC, is also metabolized slowly (half-life = 10 hr) [23]. It is therefore likely that in the chick embryo liver cell system the dihydropyridine, nifedipine, which is structurally related to DDC, will also be metabolized slowly.

REFERENCES

1. G. H. Elder and D. M. Sheppard, *Biochem. biophys. Res. Commun.* **109**, 113 (1982).
2. G. S. Marks, S. B. Follows, D. T. Zelt and S. P. C. Cole, *Can. J. Physiol. Pharmac.* **61**, 546 (1983).
3. M. G. Swain, S. B. Follows and G. S. Marks, *Can. J. Physiol. Pharmac.* **61**, 105 (1983).
4. M. G. Swain, *M.Sc. Thesis.* Queen's University at Kingston (1983).
5. N. Schoenfeld, Y. Greenblat, O. Epstein and A. Atsmon, *Biochim. biophys. Acta* **721**, 408 (1982).
6. G. S. Marks, D. R. Goldman, S. A. McCluskey, E. P. Sutherland and M. E. Lyon, *Can. J. Physiol. Pharmac.* **64**, 438 (1986).
7. H. DeVerneuil, S. Sassa and A. Kappas, *Biochem. J.* **214**, 145 (1983).
8. B. Loev and K. M. Snader, *J. org. Chem.* **30**, 1914 (1965).
9. K. Nakatsu, J. F. Brien, H. Taub, W. J. Racz and G. S. Marks, *J. Chromat.* **191**, 181 (1982).
10. R. O. Morgan, P. W. F. Fischer, J. K. Stephens and G. S. Marks, *Biochem. Pharmac.* **25**, 2609 (1976).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
12. G. L. Miller, *Analyt. Chem.* **31**, 954 (1959).
13. D. T. Zelt, J. A. Owen and G. S. Marks, *J. Chromat.* **189**, 209 (1980).
14. A. Seubert and S. Seubert, *Analyt. Biochem.* **124**, 303 (1982).
15. F. M. H. Debets, W. J. H. M. B. Hamers and J. J. T. W. A. Strik, *Int. J. Biochem.* **12**, 1019 (1980).
16. L. Cantoni, D. D. Fiume, M. Rizzardini and R. Ruggieri, *Toxic. Lett.* **20**, 211 (1984).
17. A. Ferioli, C. Harvey and F. DeMatteis, *Biochem. J.* **244**, 769 (1984).
18. A. G. Smith and J. E. Francis, *Biochem. J.* **195**, 241 (1981).
19. N. Schoenfeld, J. Aelion, Y. Beigel, O. Epstein and A. Atsmon, *Clin. Sci.* **69**, 581 (1985).
20. G. H. Elder, A. G. Roberts and A. J. Urquhart, in *Colloque INSERM* (Ed. Y. Nordmann) Vol. 134, p. 147. John Libbey Eurotext Ltd., London (1986).
21. G. S. Marks, in *Handbook of Experimental Pharmacology* (Eds. F. De Matteis and W. N. Aldridge), Vol. 44, p. 201. Springer, New York (1978).
22. W. J. Racz and J. A. Moffat, *Biochem. Pharmac.* **23**, 215 (1974).
23. W. J. Racz, L. Hunter, R. G. F. Wanless, M. V. McDonald and J. A. Moffat, *Can. J. Physiol. Pharmac.* **55**, 552 (1977).