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EFFECTS OF METYRAPONE AND NORHARMANE ON MICROSOMAL MONO-OXYGENASE AND EPOXIDE HYDROLASE ACTIVITIES

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Abstract—This study was undertaken to examine the possibility that metyrapone and norharmane stimulate epoxide hydrolase and inhibit mono-oxygenase activities by binding to a cytochrome P-450 component of a stable complex containing the two enzymes. The concentration of metyrapone and norharmane which inhibited mono-oxygenase activities of hepatic microsomes from untreated and diethylnitrosamine treated rats was lower than that required to stimulate epoxide hydrolase of the same microsomes. The ability of metyrapone and norharmane to stimulate epoxide hydrolase in these microsomes was not inhibited by the addition of carbon monoxide and reductant. Epoxide hydrolase activity was inhibited by detergents but the enzyme was still stimulated by metyrapone and norharmane under conditions of total membrane disaggregation. When microsomes were solubilized, epoxide hydrolase could be quantitatively recovered by immunoprecipitation. The immunoprecipitate contained no detectable cytochrome P-450 but was stimulated by metyrapone and norharmane. A purified epoxide hydrolase was stimulated by metyrapone but not by norharmane. The response of the enzyme to norharmane was not restored by the inclusion of cytochrome P-448. These findings suggest that metyrapone and norharmane act at separate sites on both cytochrome P-450 and epoxide hydrolase.

The microsomal-oxygenase containing cytochrome P-450 and epoxide hydrolase (EC 3.3.2.3) catalyse sequential steps in the metabolism of certain xenobiotics [1]. These enzymes transform hydrophobic aromatic compounds to more hydrophilic transdihydrodiols via epoxide intermediates. The cytotoxic, mutagenic and carcinogenic potential of these epoxides is generally eliminated by the activity of epoxide hydrolase. However, during the metabolism of benzo[*a*]pyrene a more potent carcinogen is produced by hydrolysis of epoxides in the bay region [2].

Recently Griffin and co-workers have drawn attention to a number of compounds which affect the activity of both mono-oxygenase and epoxide hydrolase [3, 4]. Among these compounds are norharmane, ellipticine and metyrapone, each of which inhibit mono-oxygenase activity but stimulate epoxide hydrolase activity. The ability of ellipticine to stimulate epoxide hydrolase was lost when microsomes were disrupted with detergents, and the compound was also ineffective with a purified form of the enzyme. It was suggested that these effects could be explained if the compounds bound to a cytochrome P-450 present in the microsomal membrane as a stable, multi-enzyme complex which also contained epoxide hydrolase [3]. The existence of such a complex has been previously suggested based on spectral [5] and kinetic evidence [5–7].

This study was undertaken to investigate the effects of metyrapone and norharmane on microsomal mono-oxygenase and epoxide hydrolase. In this study microsomes were prepared from untreated rats and rats pre-treated with diethylnitrosamine, an inducer of epoxide hydrolase [8]. The effects of detergents on microsomal epoxide hydrolase were investigated in the presence and absence of stimulators.

MATERIALS AND METHODS

Chemicals. Reagents were of the highest grade available and were obtained from the sources indicated. Diethylnitrosamine, norharmane, metyrapone, 7-ethoxycoumarin, $NADH.Na_2$, $NADPH.Na_3$ and bovine serum albumin, fraction V, from Sigma (Poole, Dorset, U.K.); benzil from BDH (Poole, Dorset, U.K.); 7-hydroxycoumarin and styrene oxide from Aldrich Chemical Co. (Gillingham, Dorset, U.K.); [7(n)-^{3}H]styrene oxide from Amersham International (Bucks., U.K.).

Animals and microsomal preparation. Male Wistar rats weighing 200–250 g were used. Where appropriate, animals received 300 mg/kg diethylnitrosamine i.p. 3 days prior to sacrifice. Treated and untreated animals were starved overnight prior to sacrifice. Microsomes were prepared from the livers of at least four animals in 50 mM Tris–HCl (pH 7.5 at 5°), 1.15% (w/v) KCl and 1 mM EDTA by centrifugation of the post-mitochondrial supernatant. The resulting pellets were resuspended in 1.15% (w/v) KCl, 10 mM EDTA and recentrifuged. Microsomes were resuspended in 0.2 M potassium phosphate, pH 7.0, at a protein concentration of 30 mg/ml. Mono-oxygenase activities were performed on the day of microsomal preparation. Results obtained for epoxide hydrolase were unaffected by storage of microsomes at −20°.

Enzyme assays. Epoxide hydrolase activity was

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determined using styrene oxide as a substrate by a modification of the method of Lu and Levin [9]. The incubation mixtures, which omitted Tween 80 [9], contained 0.1 ml Tris–HCl (pH 8.7 at 37°), 0.1 ml H_2O and 0.1 ml microsomal suspension (1 mg protein diluted in H_2O). When required, metyrapone was added in 0.01 ml acetonitrile while norharmane was dissolved in acetic acid and also added in 0.01 ml. An equivalent volume of solvent was added to those incubations carried out in the absence of these agents. Mixtures were preincubated at 37° for 6 min prior to initiation of reaction by the addition of substrate in 0.02 ml acetonitrile to give a final substrate concentration of 4.9 mM. Under these conditions, time courses were linear for 20 min. After incubation, unreacted styrene oxide was extracted (3×) with petroleum ether (40–60°). Styrene glycol was determined in an aliquot of the aqueous fraction using Triton X-100–toluene (1:1, v/v) containing PPO (5 g/l.) as the scintillation mixture. This modification omits the use of ethyl acetate to extract styrene glycol from the aqueous fraction. The efficiency of such an extraction was found to be sensitive to the presence of detergents. The rate of non-enzymic conversion was determined in incubations containing boiled, inactive microsomes.

Ethoxycoumarin-7-*O*-de-ethylase was determined as described by Greenlee and Poland [10] and aniline hydroxylase by the method of Schenkman *et al.* [11] using freshly distilled aniline. Cytochrome P-450 was measured by the method of Omura and Sato [12].

Epoxide hydrolase was purified from the livers of diethylnitrosamine-treated rats by the method of Knowles and Burchell [13] and detergent was removed as described [14]. The purified enzyme contained no spectrally detectable cytochrome P-450 and was used to raise antisera in the New Zealand White rabbit. The resulting antisera (0.5 ml) were incubated with 0.1 ml of cholate solubilized membrane (see Table 2) for 16 hr at 4°. Control immunoprecipitations were carried out by a double antibody technique in which preimmune serum replaced specific antiserum. A precipitate was then obtained by the addition of antiserum to rabbit IgC produced in the donkey (a gift from the Scottish Antibody Production Unit). Precipitates were recovered by centrifugation and washed three times in a buffer containing 50 mM sodium phosphate, pH 8.0, 0.9% (w/v) NaCl, 1 mm EDTA, 0.5% (w/v) sodium cholate, 0.1% Lubrol and 10% (v/v) glycerol. Washed precipitates required for the determination of epoxide hydrolase were resuspended by sonication in 0.25 Tris–HCl (pH 8.7 at 37°) while those for cytochrome P-450 were resuspended in 0.1 M sodium phosphate, pH 7.4, 1 mM EDTA 20% (v/v) glycerol. Pellets required for electrophoresis were resuspended in

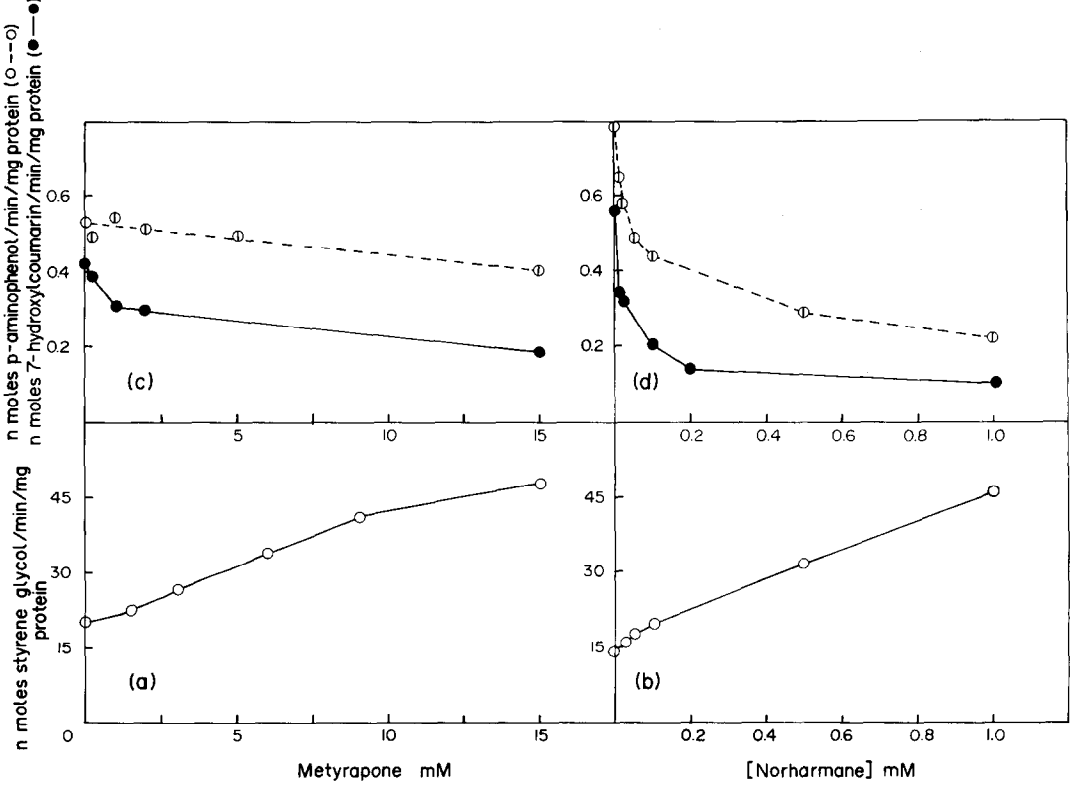

Fig. 1. Effects of metyrapone and norharmane on microsomal epoxide hydrolase and mono-oxygenase of diethylnitrosamine-treated rats. Epoxide hydrolase (a and b), aniline hydroxylase (①—①) and ethoxycoumarin-*O*-de-ethylase (●—●) were determined as described in Materials and Methods. The effects of metyrapone (a and c) and norharmane (b and d) are shown using microsomes prepared from diethylnitrosamine-treated rats. The results were obtained from a single microsomal preparation and show the mean of duplicate determinations. Similar results were obtained in two other experiments using different microsomal preparations.

H_2O and an equal volume of 100 mM Tris–HCl (pH 6.8 at 20°), 6% (w/v) SDS, 20% (w/v) glycerol, 10% (v/v) and 10% (v/v) mercaptoethanol added. SDS-polyacrylamide gel electrophoresis was carried out by the method of Laemmli [15].

Cytochrome P-448 (fraction B2) was purified by the method of Guengerich and Martin from the livers of 3-methylcholanthrene-treated rats [16]. Detergent was removed by absorption to calcium phosphate gel, repeated washings with 0.05 M potassium phosphate buffer, pH 7.7, and final desorption with 0.5 M potassium phosphate buffer, pH 7.7, as described [17]. The cytochrome had a specific content of 16.5 nmole/mg protein λ_{max} for reduced carbon monoxide difference spectrum at 447 nm, was homogenous as judged by SDS-polyacrylamide gel electrophoresis, and had no detectable epoxide hydrolase activity.

Protein was determined by the method of Lowry *et al.* [18] using bovine serum albumin as the standard.

RESULTS

The effects of metyrapone and norharmane on microsomal epoxide hydrolase and mono-oxygenase were determined over a wide range of effector concentration. Mono-oxygenase activity was determined by the rate of ethoxycoumarin demethylation and by aniline hydroxylation. In these experiments microsomes from untreated and diethylnitrosamine-treated rats were used. As previously reported [8], diethylnitrosamine treatment caused a 2- to 3-fold increase in epoxide hydrolase activity. This treatment also decreased mono-oxygenase activities to approximately 80% of the activity of untreated animals. Metyrapone and norharmane stimulated epoxide hydrolase and inhibited mono-oxygenase activities with both microsomal preparations, although metyrapone was relatively inefficient as an inhibitor of aniline hydroxylase. No significant difference was found between microsomes from untreated or diethylnitrosamine-treated animals in the concentration of effector necessary to cause 50% of the observed maximal effect for a given enzyme activity. For this reason, results are presented in Fig. 1 for microsomes only from diethylnitrosamine-treated rats. No obvious correlation was found between the concentration of effectors as stimulators of epoxide hydrolase and as inhibitors of mono-oxygenase activity. Generally both compounds produced 50% of observed maximal inhibition of mono-oxygenase activities at a concentration less than that producing 50% of observed maximal stimulation of epoxide hydrolase. The exception to this was metyrapone which had similar potency as an inhibitor of aniline hydroxylase and a stimulator of epoxide hydrolase. The difference in potency of the compounds towards the two enzyme systems may result from measuring mono-oxygenase activities at pH 7.4 and epoxide hydrolase at pH 8.7. However, when epoxide hydrolase activity was determined at pH 7.4, the specific activity was decreased but the concentration of metyrapone or norharmane producing 50% maximal observed stimulation was the same as at pH 8.7.

Metyrapone and carbon monoxide bind to common sites at haem iron of cytochrome P-450 and inhibit mono-oxygenase activity [19]. Thus the metyrapone-induced type II difference spectrum is abolished by prior treatment of microsomes with carbon monoxide in the presence of reductant. In this study we have confirmed these observations. The effect of metyrapone on epoxide hydrolase has been investigated in the presence and absence of carbon monoxide and reductant. Results of this experiment are shown in Table 1. When microsomes were treated with carbon monoxide and reductant, the epoxide hydrolase activity was still activated by metyrapone. Similar findings were made with norharmane as activator.

An investigation was made of the effects of detergents on the ability of metyrapone and norharmane to stimulate epoxide hydrolase. Enzyme activity was determined over a range of detergent concentrations in the presence and absence of stimulator. Results of an experiment using sodium cholate are shown in Fig. 2. This ionic detergent inhibited activity in the presence and absence of metyrapone or norharmane. However, stimulation was still apparent with these

Table 1. The effect of carbon monoxide and reductant on the stimulation of epoxide hydrolase by metyrapone and norharmane in microsomes from diethylnitrosamine-treated rats

Stimulator	Epoxide hydrolase (nmoles/min per mg protein) Microsomes	Microsomes + CO + sodium dithionite
None	14.10 ± 1.93 (1.00)	14-00 ± 0.84 (1.00)
Metyrapone (15 mM)	47.17 ± 0.07 (3.37)	39.47 ± 0.81 (2.82)
Norharmane (1 mM)	41.59 ± 0.25 (2.95)	36.79 ± 0.23 (2.63)

The cytochrome P-450 content of a sample of microsomes was converted to the carbon monoxide complex by the addition of carbon monoxide and sodium dithionite. The epoxide hydrolase activity of these microsomes and that of microsomes not treated with carbon monoxide were determined as described in Materials and Methods. Metyrapone and norharmane were included at the concentrations indicated. Results shown are the means of duplicate determinations, and figures in parentheses show the fold stimulation. Similar results were obtained in three separate experiments.

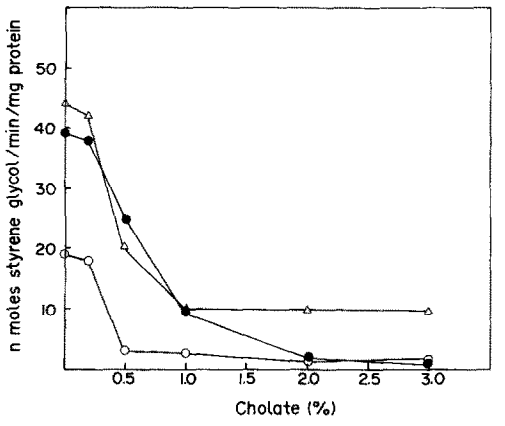

Fig. 2. Effect of sodium cholate on microsomal epoxide hydrolase in the presence or absence of metyrapone or norharmane. Microsomes from diethylnitrosamine-treated rats were preincubated for 6 min with sodium cholate in the absence (○) or presence of metyrapone (●) or norharmane (△). Epoxide hydrolase was determined as described in Materials and Methods. Results are mean values of duplicate determinations. Similar results were obtained with two other microsomal preparations.

compounds. Similar results were obtained with the non-ionic detergent Lubrol PX. Other workers [20] have reported complex effects of detergents on microsomal epoxide hydrolase. These include an activation of activity at low detergent concentration and an inhibition at high detergent concentration. During this study no detergent activation was observed at any concentration used.

It is apparent from Fig. 2 that epoxide hydrolase can be activated by metyrapone and norharmane at concentrations of cholate (1%, w/v) where the membrane is solubilized. This may indicate that the cholate-treated microsomes still contain complexes of epoxide hydrolase and cytochrome P-450 which are independent of an intact lipid bilayer. To investigate this possibility, microsomes were treated with cholate (1 mg/mg protein) and centrifuged at 100,000 *g* to remove material resistant to solubilization. An antiserum to epoxide hydrolase was added to aliquots of the resulting supernatant, and the recovery of epoxide hydrolase and cytochrome P-450 determined in the immunoprecipitate. The results of this experiment are shown in Table 2 and Fig. 3. As noted previously, the presence of cholate inhibited epoxide hydrolase but did not prevent stimulation by norharmane or metyrapone. Cholate treatment also caused a small decrease in the content of spectrally detectable cytochrome P-450. Under the conditions employed, a high proportion of epoxide hydrolase and cytochrome P-450 was recovered in the 100,000 *g* supernatant after cholate solubilization. The epoxide hydrolase activity of this supernatant could be recovered during specific immunoprecipitation and this activity was still significantly stimulated

Table 2. Recovery of epoxide hydrolase and cytochrome P-450 in immunoprecipitates formed by antiserum to epoxide hydrolase and a 100,000 *g* supernatant of cholate-solubilized microsomes

Fraction	Epoxide hydrolase		Cytochrome P-450†
	Addition	Activity*	
Microsomes	None	301 ± 11 (1.00)	9.89 ± 0.22
	Metapyrone	668 ± 15 (2.22)	
	Norharmane	655 ± 17 (2.18)	
Cholate microsomes	None	242 ± 2 (1.00)	9.01 ± 0.22
	Metyraprone	503 ± 4 (2.08)	
	Norharmane	549 ± 4 (2.26)	
Cholate supernatant	None	222 ± 10 (1.00)	8.90 ± 0.11
	Metyrapone	484 ± 11 (2.18)	
	Norharmane	562 ± 22 (2.53)	
Immunoprecipitate	None	221 ± 9 (1.00)	n.d. (<0.10)
	Metyrapone	445 ± 24 (2.01)	
	Norharmane	364 ± 19 (1.65)	

* nmoles styrene glycol/min per ml.
† nmoles/ml.
n.d., Not detected.

Microsomes from diethylnitrosamine-treated rats were resuspended in 0.1 M potassium phosphate, pH 7.4, or this buffer containing 10 mg/ml sodium cholate and 20% (w/v) glycerol. Final protein concentration was 10 mg/ml. A sample of the cholate-treated microsomes was centrifuged at 100,000 *g* for 1 hr and the resulting cholated supernatant aspirated. Antiserum to epoxide hydrolase was added to aliquots of this supernatant, and the resulting immunoprecipitates were recovered and resuspended as described in Materials and Methods.

The epoxide hydrolase activity of the immunoprecipitates was determined in the presence of potassium phosphate, cholate and glycerol at the same final concentration employed for cholate-treated fractions. Epoxide hydrolase activity was determined in the presence and absence of metyrapone (15 mM) or norharmane (1 mM) at the final concentrations indicated. The results show the means of duplicate determinations from one experiment. Figures in parentheses show the fold stimulation. Similar results were obtained with two other microsomal preparations.

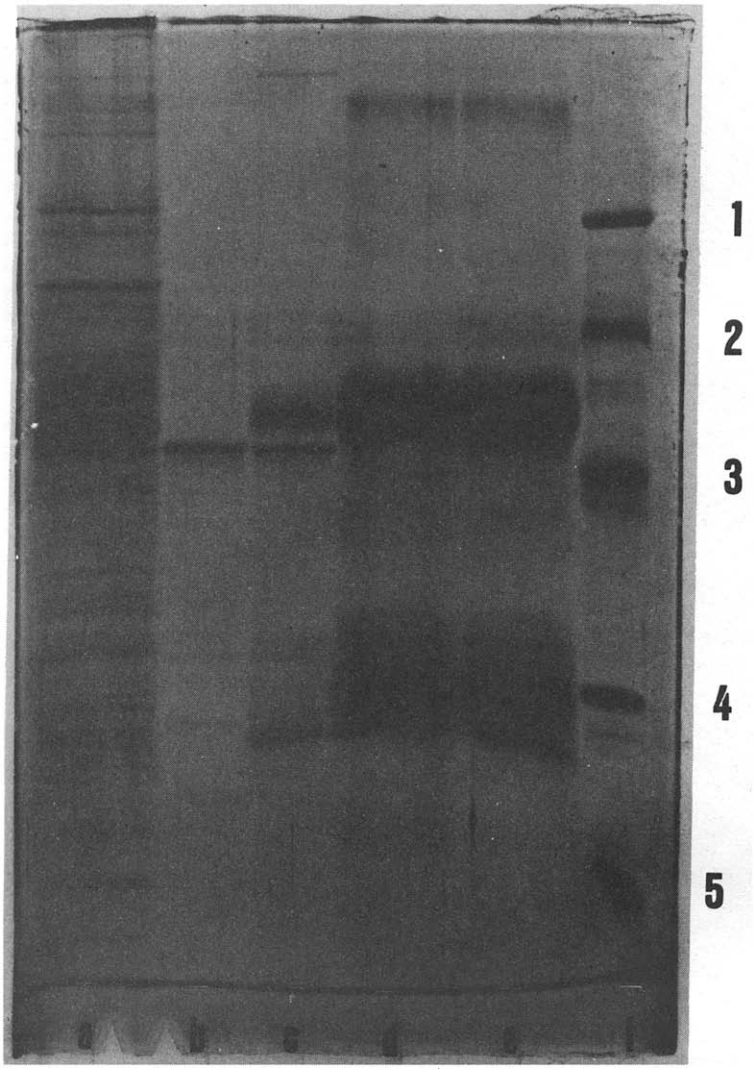

Fig. 3. SDS-polyacrylamide gel electrophoresis of immunoprecipitates formed by antiserum to epoxide hydrolase and a 100,000 *g* supernatant of cholate-solubilized microsomes. Immunoprecipitates were obtained as described in Materials and Methods and the legend to Table 2. The gel shows: channel (a) supernatant of cholate-solubilized microsomes from diethylnitrosamine-treated animals (50 μg); (b) purified epoxide hydrolase (3.5 μg); (c) immunoprecipitate formed between antiserum to epoxide hydrolase and cholate-solubilized microsomes (equivalent to 75 μg of microsomal protein); (d) as in c but using preimmune serum and a double antibody technique (equivalent to 150 μg of microsomal protein); (e) as in d but omitting solubilized microsomes; (f) molecular weight markers: (1) phosphorylase b, 94,000; (2) albumin, 567,000; (3) ovalbumin, 43,000; (4) carbonic anhydrase, 30,000; (5) trypsin inhibitor, 20,100.

by metyrapone and norharmane. However, the immunoprecipitates contained no spectrally detectable cytochrome P-450. These findings were confirmed during SDS-polyacrylamide gel electrophoresis of the immunoprecipitates. As can be seen in Fig. 3, the immunoprecipitates contained a prominent band which co-migrated with purified epoxide hydrolase. The other bands present in the immunoprecipitate formed by antiserum to epoxide hydrolase can be attributed to the precipitating antiserum. Thus when preimmune serum replaced specific antiserum and was precipitated by antiserum to rabbit IgG, an identical pattern of bands was obtained other than the absence of the epoxide hydrolase band. Neither epoxide hydrolase nor cytochrome P-450 could be detected in these immunoprecipitates.

The experiment described above indicates that activation of epoxide hydrolase by metyrapone and norharmane is independent of the presence of cytochrome P-450. The effects of metyrapone and norharmane on purified epoxide hydrolase were also investigated using a preparation which contained no detectable cytochrome P-450. The results, Fig. 4, show that the purified enzyme is markedly stimulated

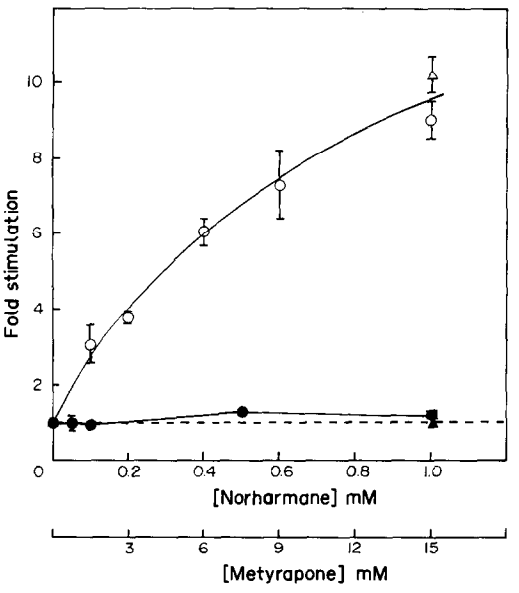

Fig. 4. Effect of metyrapone and norharmane on purified epoxide hydrolase in the presence and absence of purified cytochrome P-448. Epoxide hydrolase and cytochrome P-448 were purified as described in Materials and Methods. Epoxide hydrolase activity of the purified preparation was 120 nmol styrene glycol/min per mg protein when determined in the absence of activators or their solvents. Epoxide hydrolase activity is expressed as the fold stimulation when determined in the presence of metyrapone (—○—) or norharmane (—●—). This is the ratio of activity in the presence of activator compared to the activity of control incubation containing the solvent used as vehicle for activator. Each incubation contained 1.0 μM epoxide hydrolase. Epoxide hydrolase was also determined in incubations to which 0.6 μM cytochrome P-448 and 45 μM dilauryl-glyceryl-3-phosphorycholine were added as described [5]. These incubations contained either metyrapone (△) or norharmane (▲). The results show the means of duplicate determinations.

by metyrapone while norharmane had little effect. The addition of cytochrome P-448 to the assay system under conditions where cytochrome P-448 and epoxide hydrolase form a functionally interactive complex [5] did not alter the results. Thus the addition of cytochrome P-448 did not alter the epoxide hydrolase activity whether measured in the absence or presence of metyrapone or norharmane (Fig. 4).

DISCUSSION

Spectral [5] and kinetic evidence [5–7] has been presented to support a model in which cytochrome P-450 and epoxide hydrolase form a stable, functionally interactive complex within the microsomal membrane. Such complexes would be of major importance to toxicology and carcinogenesis. It has been suggested that some compounds, such as metyrapone and norharmane, may stimulate epoxide hydrolase and inhibit mono-oxygenase by binding to cytochrome P-450 in a complex of the two enzymes [3, 4]. These compounds would therefore provide excellent probes for the investigation of the putative complexes.

If metyrapone and norharmane exert their effects by binding to a site solely on cytochrome P-450, it might be expected that the effective concentration of these compounds would be similar for inhibition of mono-oxygenase and stimulation of epoxide hydrolase. However, no such simple relationship was found. Thus, as can be seen in Fig. 1, the effective concentration of both compounds as inhibitors of mono-oxygenase was found to be lower than that required for the stimulation of epoxide hydrolase. These findings must be interpreted with caution since neither metyrapone nor norharmane saturated their binding sites as inhibitors of mono-oxygenase or stimulators of epoxide hydrolase. It is probable that metyrapone and norharmane will bind to different forms of cytochrome P-450 with different affinities [21]. The inhibition curves obtained (Fig. 1) may thus be a composite of many interactions some of which need not involve forms of cytochrome P-450 associated with epoxide hydrolase. Such heterogeneity of interaction may mask the binding of the compounds to forms of the cytochrome which are associated with epoxide hydrolase.

While the results of comparing effective concentration range to the two enzymes may be ambiguous, other studies provide evidence that metyrapone and norharmane act at distinct sites on both cytochrome P-450 and epoxide hydrolase. Metyrapone inhibits cytochrome P-450 by binding to haem iron at a site which is common with carbon monoxide [19]. Under conditions where metyrapone binding to cytochrome P-450 is blocked by the formation of a carbon monoxide complex, metyrapone still stimulates epoxide hydrolase. Similar results were obtained with norharmane.

The effects of detergents on the ability of metyrapone and norharmane to stimulate epoxide hydrolase activity have also been investigated. Sodium cholate and Lubrol were found to be inhibitory when enzyme activity was measured in the presence or absence of stimulator (Fig. 2). However, stimulation was still apparent at detergent concentrations where cytochrome P-450 and epoxide hydrolase can be resolved by chromatographic techniques [16]. These results differ from those of Vaz *et al.* [3] who found that preincubation of microsomes with 2.1% sodium cholate did not inhibit enzyme activity but resulted in a loss in response to ellipitcine. However, in the study of Vaz *et al.* [3] the cholate concentration present in the enzyme assay was considerably lowered by transfer from the preincubation mixture, to a value at which little inhibition is observed. In other respects the assay system used by Vaz *et al.* [3] was similar to that employed here so it is not obvious as to why preincubation with cholate should have caused the enzyme to lose responsiveness to ellipticine. The results presented here also differ from those of Burchell *et al.* [20] who found that low concentrations of detergent stimulate epoxide hydrolase. Ganu and Alworth have also reported the lack of a detergent-mediated activation [22]. In both the study of Ganu and Alworth [22] and that presented here, salt-washed microsomes were employed while in the study of Burchell *et al.* [20] microsomes were prepared in isotonic sucrose. The differences may therefore arise from the different preparative techniques

and it is interesting that similar differences have been found in the response of UDP-glucuronyl transferase to detergents with microsomes prepared in these different media [23].

Although epoxide hydrolase could be stimulated by metyrapone in the presence of high concentrations of detergent, this appears to be independent of a complex involving cytochrome P-450. Thus epoxide hydrolase can be specifically and quantitatively removed from a cholate solubilized microsomal supernatant by immunoprecipitation (Table 2 and Fig. 3). The immunoprecipitates contained no spectrally detectable cytochrome P-450 and SDS-polyacrylamide gel electrophoresis revealed no microsomal proteins other than epoxide hydrolase. However, the epoxide hydrolase activity of this immunoprecipitate was stimulated by both metyrapone and norharmane.

The effects of these stimulators on a purified epoxide hydrolase which contained no cytochrome P-450 have also been investigated. As found by Levin *et al.* [24], metyrapone produces a marked stimulation of the activity of purified epoxide hydrolase (Fig. 4). Curiously norharmane was without effect on this same preparation. The lack of response of the purified enzyme to norharmane was not restored by the inclusion of cytochrome P-448. This cytochrome P-450 isoenzyme forms a functionally interactive complex with epoxide hydrolase under the conditions employed [5]. The lack of response to norharmane by the purified enzyme preparation is in contrast to the ability of this agent to stimulate purified epoxide hydrolase obtained by immunoprecipitation. The evidence presented above discounts the hypothesis that this difference results from the presence or absence of cytochrome P-450. The difference may result from a protein modification which occurs during the purification process, but not during immunoprecipitation, and which affects only the responsiveness to norharmane. Alternatively, epoxide hydrolase may exist in several forms, some of which may not respond to norharmane. The concept of multiple forms of epoxide hydrolase is contentious [14, 25] but finds some support from the findings reported here and other observations made in this laboratory. Thus two fractions of epoxide hydrolase have been obtained by chromatographic techniques. Epoxide hydrolase in both fractions can be precipitated by the anti-epoxide hydrolase serum used in this study but only one of the fractions is stimulated by norharmane (N.J. Bulleid and J. A. Craft, manuscript in preparation).

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REFERENCES

1. A. Y. H. Lu and G. T. Miwa, *A. Rev. Toxic.* **20**, 513 (1980).
2. D. M. Jerina, R. E. Lehr, H. Yagi, O. Hernandez, P. M. Dansette, P. G. Wislocki, A. W. Wood, R. L. Chang, W. Levin and A. H. Conney, in In Vitro *Metabolic Activation and Mutagenesis Testing* (Eds. F. J. deSeires, J. R. Fouts, J. R. Bend and R. M. Philpot), pp. 159–177. Elsevier-North Holland Biomedical Press, Amsterdam (1976).
3. A. D. Vaz, V. M. Fiorica and M. J. Griffin, *Biochem. Pharmac.* **30**, 651 (1981).
4. A. D. Vaz, T. Noguchi, V. M. Fiorica and M. J. Griffin, *Fedn Proc.* **39**, 1950 (1980).
5. L. S. Kaminsky, M. W. Kennedy and F. P. Guengerich, *J. biol. Chem.* **256**, 6359 (1981).
6. F. Oesch and J. Daly, *Biochem. biophys. Res. Commun.* **46**, 1713 (1972).
7. J. Capdevila, Y. Saeki, R. A. Prough and R. W. Eastabrook, in *Carcinogenesis: Fundamental Mechanisms and Environmental Effects, 13th Jerusalem Symposium on Quantum Chemistry and Biochemistry* (Eds. B. Pullman, P. O. P. Ts'o and M. V. Gelboin), D. Reidel, Dordrecht, Holland (1980).
8. R. N. Sharma, H. L. Gurtoo, E. Farber, R. K. Murray and R. G. Cameron, *Cancer Res.* **41**, 3311 (1981).
9. A. Y. H. Lu and W. Levin, in *Methods in Enzymology* (Eds. S. Fleisher and L. Packer), pp. 193–200. Academic Press, New York (1978).
10. W. F. Greenlee and A. Poland, *J. Pharmac. exp. Ther.* **205**, 596 (1978).
11. J. B. Schenkman, H. Remmer and R. W. Estabrook, *Mol. pharmac.* **3**, 113 (1967).
12. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2379 (1964).
13. R. G. Knowles and B. Burchell, *Biochem. J.* **163**, 381 (1977).
14. F. P. Guengerich, P. Wang, M. B. Mitchell and P. S. Mason, *J. biol. Chem.* **254**, 12248 (1979).
15. U. K. Laemmli, *Nature, Lond.* **227**, 680 (1970).
16. F. P. Guengerich and M. V. Martin, *Archs Biochem. Biophys.* **205**, 365 (1980).
17. J. A. Craft, M. B. Cooper, M. R. Estall, D. E. Rees and B. R. Rabin, *Eur. J. Biochem.* **96**, 379 (1979).
18. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1957).
19. S. Orrenius, B. J. Wilson, C. Bahr and J. B. Schenkman, *Biochem. Soc. Sympos.* **34**, 55 (1972).
20. B. Burchell, P. Bently and F. Oesch, *Biochim. biophys. Acta* **444**, 531 (1976).
21. A. Y. H. Lu and S. B. West, *Pharmac. Rev.* **31**, 277 (1980).
22. V. S. Ganu and W. L. Alworth, *Biochemistry* **14**, 2876 (1978).
23. A. B. Graham and G. C. Wood, *Biochim. biophys. Acta* **311**, 45 (1973).
24. W. Levin, P. E. Thomas, D. Korzeneiowski, H. Seifried, D. M. Jerina and A. Y. H. Lu, *Molec. Pharmac.* **14**, 1107 (1978).
25. F. P. Guengerich, P. Wang, P. S. Mason and M. B. Mitchell, *J. biol. Chem.* **254**, 12255 (1979).
26. G. C. DuBois, E. Appella, D. E. Ryan, D. M. Jerina and W. Levin, *J. biol. Chem.* **257**, 2708 (1982).