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SHORT COMMUNICATIONS

Effect of phenobarbital and 3-methylcholanthrene administration on epoxide hydrase levels in liver microsomes

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Enzymatic oxidation of aromatic double bonds of polycyclic hydrocarbons is believed to proceed through an initial epoxidation reaction, catalyzed by the microsomal mixed function oxidase system [1–6]. Such was first proved to be the case when naphthalene 1,2-oxide was identified as a metabolite of naphthalene [7] and has been confirmed, subsequently, with several other polycyclic aromatic hydrocarbons (reviewed in Refs. 8–10). Formation of arene oxides of polycyclic aromatic hydrocarbons is of great importance in view of their increased biological activity in producing malignant transformation of cells *in vitro* as compared to the parent compounds [11–13]. Indeed, evidence has been presented which implicates arene oxides not only in carcinogenicity but in toxicity as well (reviewed in Refs. 9 and 10). Although the application of K-region arene oxides of polycyclic hydrocarbons to mice has not led to marked tumor production [14–17], the *in situ* formation is associated with increased tumor yield [17, 18]. Considerable evidence has since accumulated which suggests that the formation of non-K-region arene oxides may be critical to the carcinogenic process [19–21]. Thus, benzo[a]pyrene 7,8-oxide, a non-K-region arene oxide of benzo[a]pyrene (BP), has been found to be a potent carcinogen on mouse skin, although weaker than the parent hydrocarbon [20], and the 7,8-dihydrodiol of BP exhibited marked tumorigenic properties on mouse skin [21].

These literature citations amply indicate the importance of the enzymatic systems which are responsible for the maintenance of steady state levels of arene oxides, i.e. the balance between formation of arene oxides by the mixed function oxidases and "detoxication" by glutathione *S*-transferases and epoxide hydrase. We have been investigating the factors which influence the intracellular activity of these enzymes, and in particular, we have examined the effects of prior administration to rodents of 3-methylcholanthrene (3-MC) and phenobarbital (PB) upon epoxide hydrase. Our results indicate that PB administration does in fact elevate the hydration of a number of oxide substrates, while 3-MC is generally weak and variable in this regard.

[^{3}H]naphthalene 1,2-oxide [22], [7-^{3}H]styrene oxide [23], octene 1,2-oxide [24] and 3-MC 11,12-oxide [25] were prepared as previously described. Substrates were purified until homogeneous as judged by NMR spectroscopy and thin-layer chromatography. All other materials were obtained from commercial sources. Male Sprague–Dawley and Long Evans rats, 60–80 g in weight, were used in these studies. The livers from the animals were removed, homogenized in cold 0.25 M sucrose (1:5, w/v), and the homogenate was centrifuged at 9,000 *g* for 15 min at 5°. The supernatant was further centrifuged at 105,000 *g* for 1 hr at 5° and the soluble fraction was discarded. The microsomes were suspended in 0.15 M KCl to a final concentration of 20–30 mg protein/ml. Protein concentration of the microsomal preparation was determined by the method of Lowry *et al.* [26] with bovine serum albumin as the reference standard.

Each tube contained 1.0 to 3.0 mg of microsomal protein, 0.1 M sodium phosphate buffer, pH 8.0, to a final volume of 0.4 ml, and 0.21 μmole 3-MC 11,12-oxide in 25 μl dimethylsulfoxide (DMSO). The formation of *trans*-dihydrodiol from 3-MC 11,12-oxide was measured by gas chromatography as previously described [27, 28]. DMSO at the concentration employed in the assay (6%) was without effect on the system. A preparation containing no microsomal protein or microsomes that had been boiled for 30 min served as controls for non-enzymatic diol formation. The activity in the latter was never more than 5 per cent of that seen with control microsomes.

For the assay of glycol formation from [^{3}H]-styrene oxide, the method of Oesch *et al.* [23] was employed. The data were corrected for a zero time control which was identical to the boiled enzyme control described above, but are not corrected for recovery (approximately 86 per cent).

The hydration of [^{3}H]-naphthalene oxide was measured at 30° for 10 min as described by Dansette *et al.* [22]. Results were corrected for boiled enzyme values and for recovery of *trans*-1,2-dihydroxy-1,2-dihydronaphthalene (77 ± 2 per cent) through the isolation procedure.

The hydration of octene 1,-2-oxide was assessed exactly as described by Oesch *et al.* [24]. Results were corrected for boiled enzyme values and recovery (83 ± 2 per cent).

The effects of phenobarbital or 3-MC administration upon the ability of Sprague–Dawley liver microsomes to catalyze the hydration of a series of oxides are presented in Table 1. Phenobarbital administration to Sprague–Dawley rats resulted in increases in the specific activity of the hydrase(s) toward styrene oxide (134 per cent), naphthalene oxide (171 per cent), octene oxide (152 per cent) and 3-MC 11,12-oxide (133 per cent). At 20 mg/kg, 3-MC moderately affected the hydration of octene oxide by increasing enzyme activity after 48 hr by 29 per cent At 40 mg/kg of 3-MC, the hydration of octene was elevated by 40 per cent at 72 hr. No elevations in hydrase activity were noted with styrene oxide, naphthalene oxide, or 3-MC 11,12-oxide as substrates after 3-MC administration under the conditions of the latter experiments. However, in separate experiments, induction with 40 mg/kg of 3-MC given daily for 3 days resulted in 40–60 per cent induction activity toward octene oxide and 3-MC 11,12-oxide.

The ability of liver epoxide hydrase activity to respond to the administration of either PB or 3-MC with 3-MC 11,12-oxide as substrate was also tested in the Long Evans rat (data not shown). Although 3-MC administration at 25 mg/kg was ineffective in elevating the hydration of 3-MC 11,12-oxide, phenobarbital did increase enzyme activity by approximately 80 per cent at 72 hr after PB administration.

In a recent review on mammalian epoxide hydrases [29], Oesch stated that the latter are inducible. This conclusion was largely based upon the choice of an assay system in which styrene oxide is employed as substrate. However, the data of the present report would suggest that this statement is only partially accurate. With phenobarbital as the administered agent, the hydrations of all substrates were clearly elevated: styrene oxide (134 per cent), naphthalene oxide (171 per cent), octene oxide (152 per cent) and 3-MC

Table 1. Effect of 3-MC or PB administration on epoxide hydrase in Sprague–Dawley rats*

Treatment	Diol (nmoles/5 min/mg protein) Styrene oxide	Naphthalene oxide	Octene oxide	3-MC 11, 12-oxide
Saline	12.7 ± 1.0	6.2 ± 1.3	50.5 ± 5.2	6.7 ± 0.7
PB (72 hr)	29.7 ± 1.3	16.8 ± 1.3	127.2 ± 4.4	15.6 ± 1.5
Corn oil	12.2 ± 0.9	4.9 ± 0.4	57.0 ± 1.1	7.1 ± 0.3
3-MC, 20 mg/kg (24 hr)	8.9 ± 0.1	3.5 ± 0.3	54.7 ± 2.6	7.2 ± 0.4
3-MC, 20 mg/kg (48 hr)	12.5 ± 0.4	5.0 ± 0.6	73.5 ± 6.1	8.0 ± 0.4
Corn oil			59.2 ± 3.2	7.7 ± 0.3
3-MC, 40 mg/kg (72 hr)			83.0 ± 2.8	7.9 ± 0.4

* Sprague–Dawley rats were injected with PB (75 mg/kg body wt) in saline, 3-MC (at doses indicated) in corn oil, i.p., or with the vehicles alone every 24 hr. The rats were sacrificed 24 hr after the last injection and hydrase activity was determined as described in the text. The data are presented for three to six rats as mean ± standard error.

11,12-oxide (133 per cent). After 3-MC administration, the hydration of octene oxide was elevated. At 20 mg/kg, the increase was 29 per cent after 48 hr and at 40 mg/kg, the elevation was 40 per cent at 72 hr. In contrast the hydrations of styrene oxide, naphthalene oxide and 3-MC 11,12-oxide (Table 1) were unaffected by pretreatment with 3-MC (20 mg/kg, 48 hr). However, in other experiments, when 3-MC was given at a higher concentration (40 mg/kg), approximately 50 per cent elevations of epoxide hydrase activity toward octene oxide or 3-MC 11,12-oxide were noted. The extent of induction was variable between sets of animals and was occasionally not significantly above control. In more than ten groups of animals (four to six animals/group) examined over a period of 1 year, no basis for this variability in induction could be established. The data presented in this report do indicate, however, that prior exposure of animals to certain drugs, e.g. phenobarbital, may result in an increase in the ability to form potentially pharmacologically active *trans*-dihydrodiol derivatives of polycyclic hydrocarbons and, therefore, regulation of the activity of epoxide hydrase may be an important determinant in the carcinogenic process.

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