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QUALITATIVE AND QUANTITATIVE DIFFERENCES IN THE INDUCTION AND INHIBITION OF HEPATIC BENZO[*a*]PYRENE METABOLISM IN THE RAT AND HAMSTER*

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Abstract—The present study compared the induction and inhibition of the metabolism of the prototype polycyclic aromatic hydrocarbon, benzo[*a*]pyrene (BaP), in rat and hamster liver microsomes. The production of total polar metabolites was quantitated by separating ^{3}H-metabolites from [^{3}H]-BaP using reverse-phase thin-layer chromatography. The rate of hepatic microsomal BaP metabolism was similar in the rat and hamster (0.81 vs 0.72 nmol/min/nmol cytochrome P-450 respectively). In the rat, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; 5 μg/kg, i.p.) and 3-methylcholanthrene (3-MC; 50 mg/kg, i.p., × 3 days) pretreatments doubled the rate of BaP metabolism, whereas phenobarbital pretreatment (PB; 80 mg/kg, i.p., × 3 days) had no effect. In contrast, hamster hepatic microsomal BaP metabolism was elevated 2.3-fold by PB pretreatment, whereas TCDD and 3-MC pretreatments had no effect. Isosafrole pretreatment (ISO; 150 mg/kg, i.p., × 3 days) elevated the rate by almost 2-fold in each species. Another cytochrome P-448-mediated activity, 7-ethoxyresorufin *O*-deethylase (EROD), was induced by the same compounds that induced BaP metabolism in the rat. In hamster liver microsomes, in contrast to BaP metabolism, EROD was induced by TCDD and 3-MC but not PB or ISO pretreatments. The results suggest differences in the substrate specificity of the cytochromes P-448–450 induced by TCDD, 3-MC and PB in these species. This was supported by the different selectivity of the *in vitro* inhibitors, metyrapone and 7,8-benzoflavone, towards BaP metabolism and EROD in hepatic microsomes from TCDD- or PB-pretreated rats and hamsters. Reverse-phase HPLC analysis indicated that, while 3-hydroxy-BaP was the major metabolite formed by the untreated rat, untreated hamster liver microsomes formed predominantly BaP-4,5-diol. Microsomes from TCDD-treated rats generated elevated levels of all BaP-diols, diones and 3-hydroxy-BaP, with the major metabolites being BaP-9,10- and BaP-7,8-diols. In contrast, the metabolite profile from TCDD-pretreated hamsters was unchanged from the control. PB-treated hamster microsomes produced elevated levels of BaP-diones and 3-hydroxy-BaP. However, the major hepatic metabolite formed by PB-pretreated hamsters was BaP-4,5-diol, while BaP-9,10- and BaP-7,8-diols were not detected. The results of the study indicate differences in the induced cytochrome P-450s and the generation of toxic BaP metabolites in the liver of the rat and hamster.

Polycyclic aromatic hydrocarbons (PAH‖) are widely distributed environmental contaminants. An important and very extensively studied member of this class of compounds is benzo[*a*]pyrene (BaP), which has been shown to cause carcinogenic, mutagenic and cytotoxic effects in various species and tissues [1]. BaP requires metabolic activation in order to exert many of its biological effects. BaP has been shown to be converted to phenols, dihydrodiols, quinones, epoxides, diol-epoxides and water-soluble conjugates through the action of the cytochrome P-450-dependent monooxygenase system coupled with epoxide hydrolases and phase II enzyme systems present in mammalian tissues [2–5].

Studies examining the effects of chemical-inducing agents on the cytochrome P-450-dependent metabolism of BaP have established that in rat liver the major PAH-inducible cytochrome P-450 (P-450$_c$) is important in the metabolism of BaP [6–8]. This enzyme has also been determined to be the major form of cytochrome P-450 capable of converting BaP-7,8-dihydrodiol to the ultimate carcinogenic metabolite, BaP-7,8-diol 9,10-epoxide [9–11]. While the role of different hepatic cytochrome P-450 forms in the metabolism of BaP has been well characterized in the rat, little information is available in this regard in the hamster. Several reports have indicated that aryl hydrocarbon hydroxylase (AHH, BaP metabolism) activity in hamster liver microsomes is not stimulated by pretreatment of animals with PAHs

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‖ Abbreviations: PAH, polycyclic aromatic hydrocarbons; BaP, benzo[*a*]pyrene; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; PB, phenobarbital; 3-MC, 3-methylcholanthrene; ISO, isosafrole; ANF, 7,8-benzoflavone; DMSO, dimethyl sulfoxide; RP-TLC, reverse-phase thin-layer chromatography; BND, benzphetamine *N*-demethylase; and EROD, 7-ethoxyresorufin *O*-deethylase.

such as 3-methylcholanthrene or β-napthoflavone [8, 12–14]. However, these studies only quantitated fluorescent products and did not identify BaP metabolites formed under these conditions. Few investigations have dealt with the identity of BaP metabolites formed by the hamster. As in rat microsomes, uninduced hamster liver microsomes metabolize BaP mainly to dihydrodiols, quinones, and phenolic derivatives [15]. Using thin-layer chromatographic analysis, BaP metabolites formed by liver microsomes from hamsters pretreated with BaP were shown to be the same as those formed in untreated hamsters [16]. Contradictory reports also exist on the activation of BaP to mutagenic intermediates in hamster liver microsomes [7, 14]. Since the hamster has been shown to be more efficient than other animal species in activating certain chemical carcinogens and is becoming a more frequently used animal model in toxicity studies [8, 17, 18], it is important to provide a more detailed characterization of xenobiotic-metabolizing enzyme systems in this species. The present study compares the hepatic microsomal metabolism of BaP in the hamster and rat through the use of selective cytochrome P-450 inducing agents and *in vitro* inhibitors, and qualitatively evaluates the profile of BaP metabolites formed under various conditions.

MATERIALS AND METHODS

Drugs and chemicals. [G-^{3}H]-Benzo[*a*]pyrene (88 Ci/mmol) was purchased from Amersham International (Amersham, UK). Unlabeled benzo[*a*]pyrene, 3-methylcholanthrene (3-MC), and 7,8-benzoflavone (alpha-napthoflavone, ANF) were purchased from the Sigma Chemical Co. (St. Louis, MO). Unlabeled 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was a gift from the Dow Chemical Co. (Midland, MI). Isosafrole was purchased from the Eastman Kodak Co. (Rochester, NY). D-Benzphetamine-HCl was obtained as a gift from the Upjohn Co. (Kalamazoo, MI), and 7-ethoxyresorufin was purchased from the Pierce Chemical Co. (Rockford, IL). Metyrapone was obtained as a gift from CIBA-Pharmaceuticals (Summit, NJ). BaP metabolite standards were obtained from the Chemical and Physical Carcinogenesis Branch of the National Cancer Institute.

Pretreatment of animals. Male Sprague–Dawley rats (175–220 g) and male Golden Syrian hamsters (75–105 g) were purchased from Harlan Sprague–Dawley Inc. (Indianapolis, IN). Liver microsomes were prepared from animals 24 hr following treatment with either sodium phenobarbital (PB) in 0.9% saline (80 mg/kg, i.p. × 3 days), 3-methylcholanthrene (3-MC) in olive oil (50 mg/kg, i.p. × 3 days), or isosafrole in olive oil (150 mg/kg, i.p. × 3 days). Liver microsomes were also prepared 72 hr following a single treatment of animals with TCDD in olive oil (5 μg/kg, i.p.).

Preparation of microsomes. Livers from rats and hamsters were excised immediately after being cleared of blood via the portal vein using 0·9% saline. All procedures were then carried out at 4°. Livers were weighed and homogenized in 4 vol. (w/v) of ice-cold 10 mM Tris–HCl (pH 7.4) containing 0.25 M sucrose. Liver homogenates were then centrifuged for 30 min at 10,900 *g*. The supernatant fraction from this spin was centrifuged at 100,000 *g* for 60 min to obtain the microsomal pellet. The pellet was then resuspended in 10 mM Tris–HCl (pH 7.4) containing 0.25 M sucrose, 20% (v/v) glycerol, and 1 mM EDTA and used either immediately or frozen at −80°.

Assay of benzo[a]*pyrene metabolism.* The metabolism of [^{3}H]benzo[*a*]pyrene was measured by separating polar metabolites from parent compound by reverse-phase thin-layer chromatography (RP–TLC). Prior to use in these studies [^{3}H]-BaP was purified by the method of DePierre *et al.* [19]. The microsomal assay was performed in 50 mM Tris–HCl (pH 7.4 at 37°), containing 5 mM $MgCl_2$, an NADPH-generating system (1.4 mM $NADP^+$, 10 mM glucose-6-phosphate, 1 unit/ml glucose-6-phosphate dehydrogenase), 2 mM NADPH, 0.5 mg microsomal protein and 80 μM [^{3}H]-BaP (3.33 mCi/mmol) in a total volume of 1.0 ml. The reaction was initiated by the addition of [^{3}H]-BaP substrate in acetone (0.5% of total volume). Conditions were chosen to give linear kinetics with respect to time and protein concentration. Depending on the treatment, the extent of substrate conversion over 10 min was between 10 and 50%. The reaction was terminated at 10 min by the addition of 0.5 ml acetone. Precipitated protein was pelleted by centrifugation at 1000 *g* for 10 min. The supernatant fraction was analyzed by RP–TLC (Whatman $LKC_{18}D$). A 150 μl sample of reaction supernatant was applied to the TLC plates which were then developed in 85% methanol/15% water. Plates were then scraped into sections separating metabolites from parent compound. The silica gel from these sections was added to scintillation vials containing 2 ml of 1,4-dioxane. Liquiscint (National Diagnostics) (10 ml) was added to the vials, and radioactivity was measured by liquid scintillation counting (LSC). Incubation carried out for 10 min without a generating system and NADPH served as controls for the reaction.

The RP–TLC system cleanly separated nine BaP

Table 1. Separation of benzo[*a*]pyrene metabolite standards from benzo[*a*]pyrene by reverse-phase thin-layer chromatography*

	R_f value
Benzo[*a*]pyrene 3,6-dione	0.33
Benzo[*a*]pyrene 1,6-dione	0.34
Benzo[*a*]pyrene 6,12-dione	0.32
Benzo[*a*]pyrene 4,5-epoxide	0.37
Benzo[*a*]pyrene 4,5-diol	0.58
Benzo[*a*]pyrene 9,10-diol	0.67
Benzo[*a*]pyrene 7,8-diol	0.51
3-Hydroxy-benzo[*a*]pyrene	0.35
9-Hydroxy-benzo[*a*]pyrene	0.37
Benzo[*a*]pyrene	0.24

* Whatman $LKC_{18}D$ thin-layer chromatography plates were developed in 85% methanol/15% water. Standards in acetone were spotted onto the plates as described in Methods and detected by UV absorbance.

metabolite standards consisting of phenols, diols and quinones, from BaP as detected by UV fluorescence. R_f values for BaP and the metabolite standards are given in Table 1. BaP had an R_f of 0.24, whereas the metabolites had R_f values ranging from 0.32 to 0.67.

Analysis of BaP *metabolites by high performance liquid chromatography (HPLC).* [^{3}H]-BaP metabolites formed by microsomal incubations were analyzed by reverse-phase HPLC. The metabolites were identified by co-elution with BaP metabolite standards chromatographed under the same conditions. Metabolite standards were detected by absorbance at 254 nm. The reaction was carried out as described above except that the reaction was scaled down to 0.1 ml and stopped with 0.05 ml acetone. The specific activity of [^{3}H]-BaP used in these studies was 20 mCi/mmol. After pelleting the protein in a tabletop centrifuge as above, 0.1 ml of reaction supernatant was analyzed directly by RP-HPLC. An Altex Ultrasphere ODS column (4.6 mm × 25 cm) was eluted with 60% methanol/40% water for 10 min followed by a 50-min gradient to 100% methanol using a flow rate of 1 ml/min at room temperature [20]. Column effluent was collected as 0.5-min fractions into scintillation vials. Radioactivity was measured by LSC.

Inhibition of BaP metabolism. Assays were performed in a volume of 1 ml as described above and in the presence of either metyrapone or ANF. Inhibitors were added immediately prior to addition of [^{3}H]-BaP at final concentrations of 1, 10, 50, 100, and 300 μM. The inhibitors were added in dimethyl sulfoxide (DMSO) which accounted for <0.5% of the total volume. This concentration of solvent did not affect the rate of metabolism of BaP under any of the conditions studied.

Inhibition of EROD. 7-Ethoxyresorufin *O*-deethylase (EROD) activity was determined by the fluorometric method of Prough *et al.* [21]. Assays were performed in the presence or absence of metyrapone or ANF. Inhibitors were added immediately prior to addition of 7-ethoxyresorufin (0.5 μM), at final concentrations of 1, 10, 100 and 1000 nM. The inhibitors were added in DMSO which accounted for 0.25% of the assay volume. This concentration of DMSO did not affect EROD activity under the conditions examined.

Cytochrome P-450 content and other monooxygenase activities. Cytochrome P-450 content was determined by the method of Omura and Sato [22] in a 0.1 M phosphate buffer, pH 7.4, containing 20% (v/v) glycerol, 1 mM EDTA, 1 mM dithiothreitol, and 0.2% (w/v) Emulgen [23]. The demethylation of benzphetamine was measured by following the formation of formaldehyde [24], essentially as described by Lake *et al.* [25] using 1 mM benzphetamine and 0.5 mg microsomal protein in a total volume of 1.5 ml. Microsomal protein was determined by the biuret procedure [26] with bovine serum albumin as the standard.

Statistical analysis. Results were statistically evaluated using one-way analysis of variance. Duncan's multiple range test was used for multiple comparisons between group means. A significance level of $P < 0.05$ was chosen for acceptance or rejection of the null hypothesis.

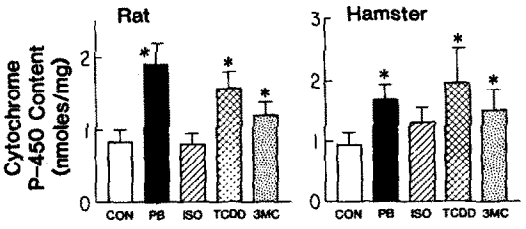

Fig. 1. Hepatic microsomal cytochrome P-450 content in untreated (CON), phenobarbital (PB), isosafrole (ISO), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 3-methylcholanthrene (3-MC) pretreated rats and hamsters. Pretreatment schedules were as described in Methods. Values are the mean ± SD of four to seven animals per treatment. Key: (*) significantly different from the respective control group, $P < 0.05$.

RESULTS

The effects of the inducing agents on rat and hamster hepatic microsomal cytochrome P-450 content are shown in Fig. 1. In the context of this paper, cytochrome P-450 is used as a general term which includes the measurement of the major carbon monoxide binding pigments absorbing from 448 to 450 nm. Cytochrome P-450 levels were very similar in control rat and hamster hepatic microsomes. In the rat, TCDD and 3-MC elevated the concentration of P-450 by 1.8- and 1.4-fold respectively. PB pretreatment elevated P-450 by 2.3-fold, while isosafrole had no effect. In hamster microsomes, PB, ISO, TCDD and 3-MC pretreatments elevated P-450 content by 1.8-, 1.4-, 2.1- and 1.6-fold respectively.

The effects of the inducers on hepatic microsomal benzphetamine *N*-demethylase (BND) activity are shown in Fig. 2. When expressed as turnover number (nmol HCHO/min/nmol P-450), 3-MC and TCDD depressed BND activity by 40–60% in the rat and hamster. Isosafrole pretreatment had no effect on BND in either species. PB increased BND by 1.3-fold in the rat and doubled the activity in hamster microsomes. Thus, the effects of these inducers on cytochrome P-450 content and BND activity are very similar in the two species.

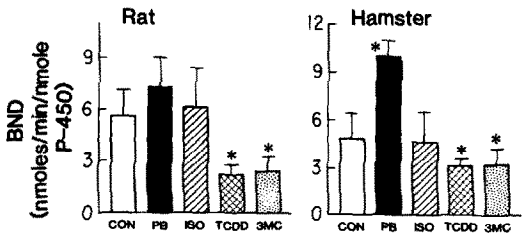

Fig. 2. Hepatic benzphetamine *N*-demethylase (BND) activity, expressed as turnover number, in microsomes from untreated (CON), phenobarbital (PB), isosafrole (ISO), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 3-methylcholanthrene (3-MC) pretreated rats and hamsters. Pretreatment schedules were as described in Methods. Values are the mean ± SD of four to six animals per treatment. Key: (*) significantly different from the respective control group, $P < 0.05$.

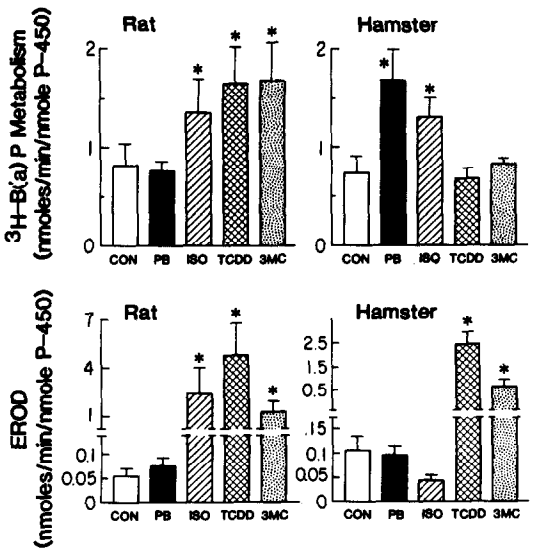

Fig. 3. Hepatic microsomal [^{3}H]-benzo[*a*]pyrene (BaP) metabolism and 7-ethoxyresorufin *O*-deethylase (EROD) activity in rats and hamsters, expressed as turnover number. Data are from untreated (CON), phenobarbital (PB), isosafrole (ISO), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 3-methylcholanthrene (3-MC) pretreated animals. Pretreatments were as described in Methods. Values are the mean ± SD of three to seven animals per treatment. Key: (*) significantly different from the respective control, $P < 0.05$.

The effects of the inducers on hepatic microsomal [^{3}H]BaP metabolism and EROD activity (expressed per nmol P-450) are shown in Fig. 3. In the rat, PB pretreatment did not affect either activity. In contrast to the rat, PB pretreatment in the hamster produced a 2.3-fold increase in BaP metabolism but no change in EROD activity. TCDD and 3-MC pretreatments doubled BaP metabolism in rat microsomes and increased EROD activity by 94- and 23-fold respectively. However, in the hamster, TCDD and 3-MC had no effect on BaP metabolism but increased EROD activity by 24- and 6-fold respectively. Isosafrole pretreatment increased rat microsomal BaP metabolism by 1.6-fold and increased EROD activity 42-fold. In the hamster, isosafrole elevated BaP metabolism by 1.8-fold but decreased EROD activity to less than 50% of the control activity.

Figure 4 illustrates the concentration-related inhibition of hepatic microsomal BaP metabolism by metyrapone and ANF. Studies were performed in microsomes from TCDD- and PB-pretreated rats and hamsters. As expected, in TCDD-treated rat microsomes (Fig. 4A), metyrapone had no effect on the rate of BaP metabolism at concentrations up to 300 μM. However, ANF produced a concentration-dependent inhibition, with 50% inhibition occurring at about 50 μM. In contrast, in microsomes from PB-treated rats, ANF did not affect BaP metabolism at concentrations up to 300 μM (Fig. 4B). However, under these conditions metyrapone inhibited BaP

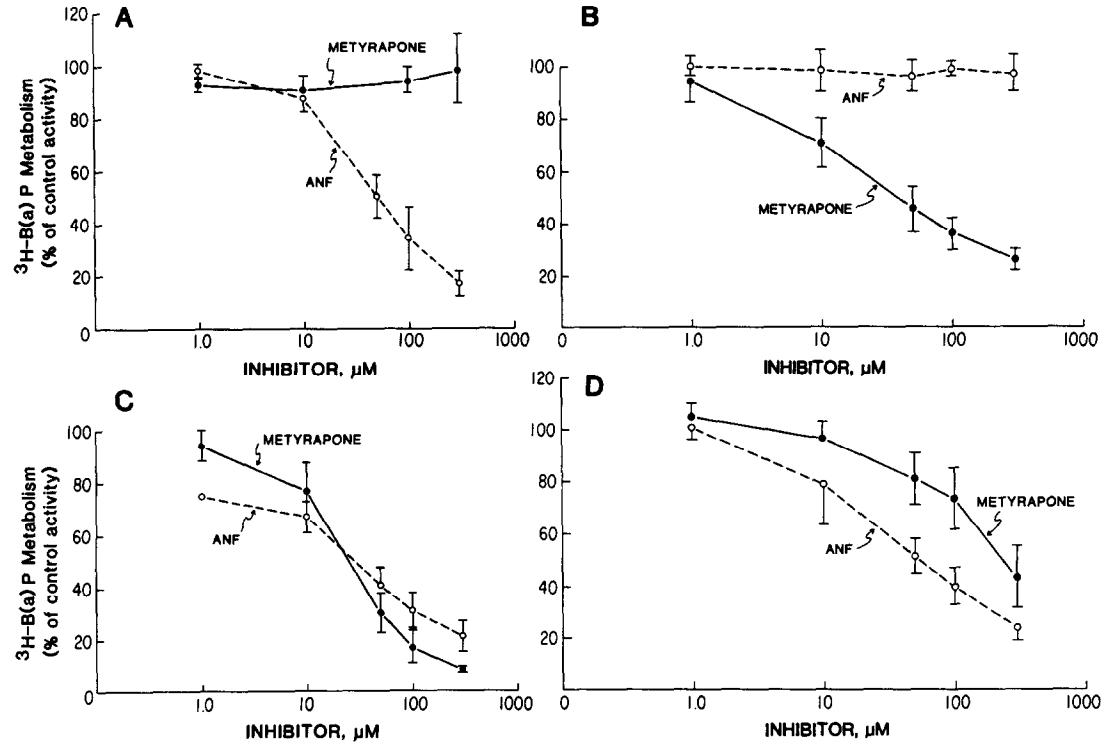

Fig. 4. Inhibition of [^{3}H]-benzo[*a*]pyrene (BaP) metabolism by metyrapone and 7,8-benzoflavone (ANF) in hepatic microsomes from TCDD-pretreated rats (A), PB-pretreated rats (B), TCDD-pretreated hamsters(C), and PB-pretreated hamsters (D). Data are expressed as percent of activity in the absence of inhibitor. Values are the mean ± SD of measurements made in three animals. Activities (nmol/min/nmole P-450, mean ± SD) in the absence of inhibitors were: TCDD-pretreated rats (1.66 ± 0.35); TCDD-pretreated hamsters (0.67 ± 0.10); PB-pretreated rats (0.76 ± 0.10); and PB-pretreated hamsters (1.68 ± 0.34).

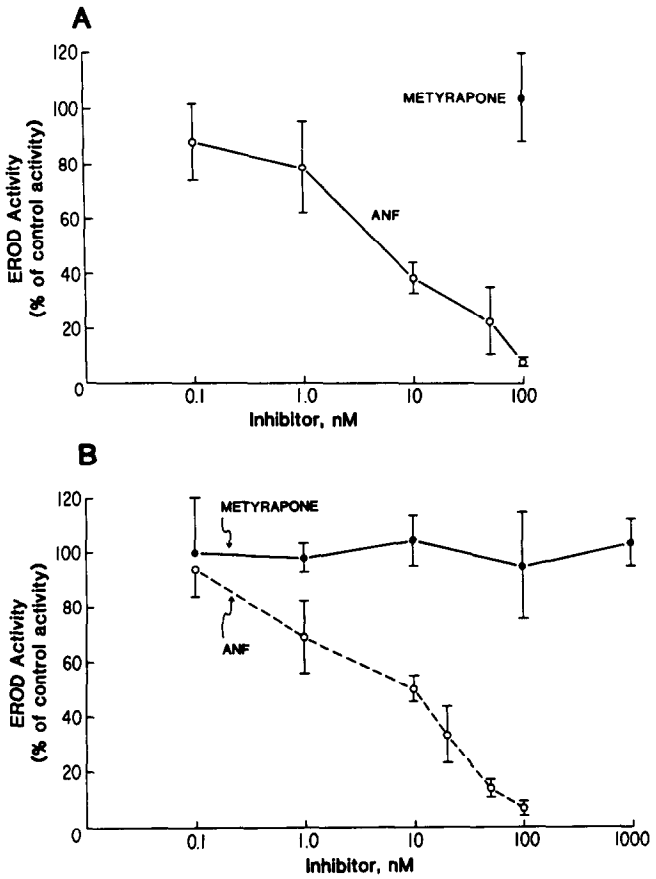

Fig. 5. Inhibition of 7-ethoxyresorufin *O*-deethylase (EROD) activity by metyrapone and 7,8-benzoflavone (ANF) in hepatic microsomes from TCDD-pretreated rats (A) and hamsters (B). Data are expressed as percent of activity in the absence of inhibitors. Values are the mean ± SD of measurements made in three animals. Activities in the absence of inhibitors (nmol/min/nmole P-450, mean ± SD): TCDD-pretreated rat (4.69 ± 2.04); and TCDD-pretreated hamster (2.49 ± 0.49).

metabolism in a concentration-related manner with 50% inhibition occurring at about 30 μM. In microsomes from TCDD-pretreated hamsters, both ANF and metyrapone produced a concentration-related inhibition of BaP metabolism (Fig. 4C), with 50% inhibition observed at 40–50 μM with each compound. However, in microsomes from PB-treated hamsters, ANF was more potent than metyrapone as an inhibitor of BaP metabolism (Fig. 4D). ANF produced 50% inhibition at about 50 μM, whereas inhibition by metyrapone was observed at concentrations ranging from 100 to 300 μM.

Figure 5 illustrates the inhibition of hepatic microsomal EROD activity by metyrapone and ANF in microsomes from TCDD-pretreated rats and hamsters. In the rat (Fig. 5A), the effectiveness of ANF and metyrapone as inhibitors of EROD was similar to that observed for inhibition of BaP metabolism (Fig. 4A), although the concentrations of ANF necessary for inhibition were 3 orders of magnitude less in the case of EROD inhibition. ANF produced 50% inhibition of EROD at a concentration of about 8 nM, whereas metyrapone was not effective at concentrations as high as 100 nM. Unlike in the studies with BaP metabolism (Fig. 4C), metyrapone and ANF exhibited differential effects on EROD activity in microsomes from TCDD-treated hamsters (Fig. 5B). ANF produced a 50% inhibition of EROD at a concentration of about 10 nM, whereas metyrapone was not effective at concentrations as high as 1 μM.

Reverse phase-HPLC analyses of [^{3}H]BaP metabolites formed by hepatic microsomes are shown in Fig. 6. The retention times of BaP metabolite standards, detected by absorbance at 254 nm, are shown by arrows on the figures. Recovery of BaP-derived ^{3}H in the supernatant fraction ranged from 51 to 66% under the various conditions, indicating a similar degree of binding of radioactivity to the microsomal membranes from the different preparations. The recovery of radioactivity applied to the column was greater than 90% in all cases. This indicates that the chromatographs provide a quantitative as well as a qualitative comparison of hepatic BaP metabolism in the rat and hamster. The earliest radioactive peak (4 min) in all chromatographs represents an unidentified polar metabolite, possibly tritiated water. Hepatic microsomes from control rats form metabolites having retention characteristics similar to several dione and diol metabolites, with 3-hydroxy-BaP representing the major radioactive product (Fig. 6A). However, in control hamster microsomes (Fig. 6D), the major radioactive peak corresponded to

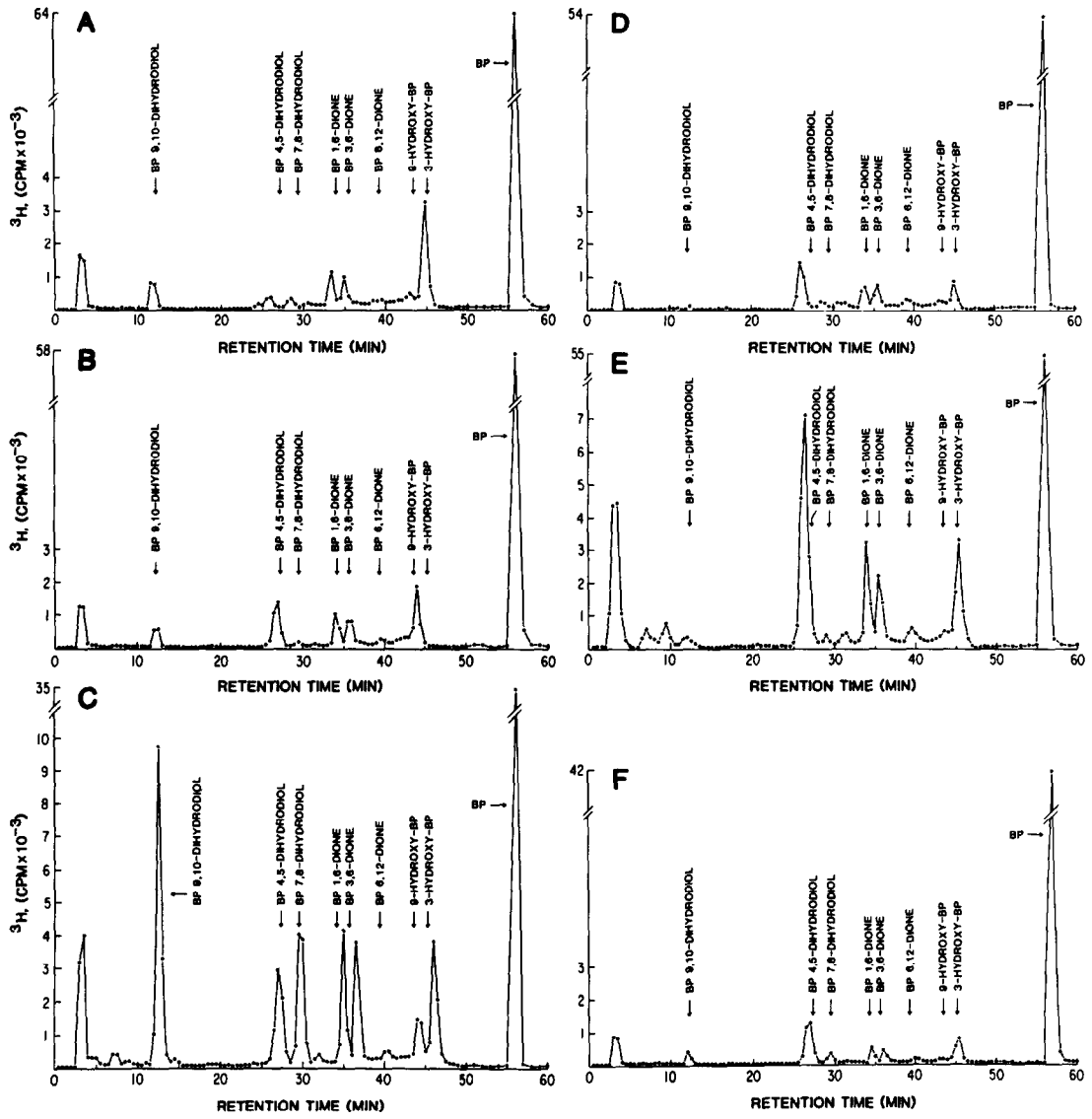

Fig. 6. Reverse-phase HPLC analysis of metabolites formed after incubation of [^{3}H]-benzo[*a*]pyrene with hepatic microsomes from untreated rats (A), PB-pretreated rats (B), TCDD-pretreated rats (C), untreated hamsters (D), PB-pretreated hamsters (E) and TCDD-pretreated hamsters (F). Arrows indicate the retention time of benzo[*a*]pyrene metabolite standards chromatographed under identical conditions and detected by absorbance at 254 nm.

BaP-4,5-diol, with diones and phenolic metabolites formed to a lesser degree. In microsomes from PB-pretreated rats (Fig. 6B), a small increase was observed in the formation of BaP-4,5-diol along with a small decrease in formation of 3-hydroxy-BaP relative to the control profile. PB pretreatment of hamsters resulted in a dramatic increase in the quantity of radioactive products having the retention characteristics of diones, 3-hydroxy-BaP, BaP-4,5-diol and the polar metabolite peak (Fig. 6E). TCDD treatment of rats resulted in a dramatic increase in the quantity of all metabolites formed compared to the control (Fig. 6C), with the largest increases observed with BaP-7,8-diol and BaP-9,10-diol. Pretreatment of hamsters with TCDD did not increase the quantity of any BaP metabolites compared to the control profile (Fig. 6F).

DISCUSSION

In the present study, a reverse-phase thin-layer chromatogrphy (RP–TLC) method was used to assess the hepatic microsomal metabolism of [^{3}H]BaP in the rat and hamster. Other laboratories have used thin-layer chromatographic methods to separate BaP metabolites from parent compound [16, 27], but few investigators have employed this technique for use in routine assays. We believe that this method has several advantages over the commonly employed fluorescent assay (aryl-hydrocarbon hydroxylase, AHH) and radiometric methods [19, 28, 29]. The RP–TLC method involves the direct application of the reaction sample to the TLC plate and thus provides a quantitative measure of total polar BaP metabolites. Since extraction procedures

are not employed in our assay method, routine analysis of multiple samples is simple and less time consuming. Therefore, assessing extraction efficiency using microsomes from different species under different treatment conditions is not necessary. Since the AHH assay measures mainly phenolic metabolites, one must assume that these metabolites are the major products, representative of all other metabolites formed in the species under investigation. Analysis of BaP metabolites in the reaction mixture (no extraction) by HPLC clearly indicates that this is not the case in the control or treated hamster or treated rat (>80% non-phenolic; see Fig. 6). The analysis of [^{3}H]BaP metabolism by reverse-phase TLC thus provides a rapid method for accurately quantitating the formation of polar BaP metabolites without the need for liquid–liquid extractions.

Previous studies have indicated that marked qualitative and quantitative differences exist in both the constitutive and induced cytochrome P-450 enzymes in the rat and hamster. Enzymes immunologically related to rat liver cytochrome P-450$_c$ and P-450$_d$ have been shown to be present in hepatic microsomes from 3-methylcholanthrene-pretreated hamsters, although induced levels are lower than in the rat [30, 31]. Comparison of polypeptide patterns and peptide maps of rat and hamster cytochrome P-450s has indicated that several polypeptides present in pregnenolone-16 α-carbonitrile and phenobarbital-treated rat liver are not present in hamster liver microsomes [32]. The results from the present study indicate clear differences in the substrate specificity of the induced cytochrome P-450 isozymes that metabolize BaP in the rat and hamster liver. In the rat, the major polycyclic aromatic hydrocarbon (PAH) inducible cytochrome P-450 (P-450$_c$) catalyzes both BaP metabolism and EROD activity, while the major phenobarbital inducible cytochrome P-450 is unimportant in the metabolism of these substrates [6, 33]. The induction and inhibition data in microsomes from PAH- and PB-pretreated rats in this study support these findings. However, in the hamster liver, BaP metabolism and EROD are differentially induced. TCDD and 3-MC pretreatment increased microsomal EROD but not BaP metabolism, whereas PB had the opposite effect. This suggests that, in the hamster, EROD is metabolized by an enzyme homologous to rat cytochrome P-450$_c$ which appears to have poor catalytic activity towards BaP. Similarly, the use of monoclonal antibodies to rat cytochrome P-450$_c$ to inhibit aryl hydrocarbon hydroxylase and ethoxycoumarin *O*-deethylase has indicated that this enzyme is important in both reactions in the rat, whereas it contributes only to the former reaction in hamster liver [34]. In contrast to the rat, hepatic microsomes from PB-induced hamsters efficiently metabolize BaP. The induction of hepatic BaP metabolism by PB in the hamster has not been reported previously [31, 34–37]. The findings in the present study suggest that the active site on hamster PB inducible cytochrome P-450 may be quite different from that in the rat. The difference between our results and that of earlier studies may be related to our use of the RP–TLC method, which quantitates total polar metabolite production. The AHH assay was used in the other studies [31, 34, 37] but seems inappropriate for study of the hamster, since HPLC analysis indicates that phenolic metabolites are relatively minor products of BaP metabolism in this species. In the rat, isosafrole induces a novel cytochrome P-450 (P-450$_d$) along with elevating the levels of both P-450$_b$ and P-450$_c$ [36, 37]. Since purified cytochrome P-450$_d$ has been shown to have little catalytic activity towards either BaP or 7-ethoxyresorufin [36, 37], the increase in these activities by isosafrole in the rat microsomes is probably due to concomitant induction of P-450$_c$. In hamster, isosafrole behaves like PB in increasing BaP metabolism but not EROD. From this result, it appears that the PAH-inducible cytochrome P450 is not increased by isosafrole in this species. The increase in BaP metabolism as a result of isosafrole pretreatment may be explained by induction of the major PB cytochrome P-450 or to the induction of a novel hamster cytochrome P-450 which can metabolize BaP.

As has been well documented in the literature, the *in vitro* inhibitors ANF and metyrapone demonstrate selectivity towards PAH- and PB-induced rat liver microsomes respectively [38, 39]. Our results with TCDD- and PB-induced rat liver microsomes are in agreement with these findings (Figs. 4 and 5). However, both compounds were equally effective inhibitors of BaP metabolism in microsomes from both TCDD- and PB-induced hamsters. This finding further indicates the differing substrate specificity of hepatic cytochrome P-450s in these species. The enzyme(s)-catalyzing EROD activity and BaP metabolism in TCDD-pretreated hamster liver microsomes appears distinct since the inhibitors demonstrate differential effects on the former but not the latter activity. The ability of ANF to inhibit BaP metabolism in microsomes from PB-induced hamsters and of metyrapone to inhibit BaP metabolism in microsomes from TCDD-pretreated hamsters are unusual findings. The data suggest that the induced hamster enzymes possess an active site or an alternative binding site which can accommodate the structures of both inhibitors as well as BaP.

Data based on the use of microsomal systems, which contain the entire complement of cytochrome P-450 isozymes, must be interpreted cautiously due to the overlapping substrate specificity of cytochrome P-450s [40]. However, microsomes from pretreated rats have been shown to accurately reflect the activity and stereoselectivity of a reconstituted enzyme system containing purified cytochrome P-450 and epoxide hydrolase towards the metabolism of BaP [41–43]. Since a single isozyme of cytochrome P-450 is capable of oxidizing BaP at multiple sites [41–43], the profiles of metabolites formed probably reflect the activity of the major enzyme present under the given conditions. Therefore, the similarity in the BaP metabolites formed by microsomes from control and TCDD-treated hamsters (Fig. 6D and F) suggests that the same enzyme is responsible for metabolism in both situations. However, BaP metabolism in the untreated hamster was stimulated (approximately 20%) by concentrations of ANF that were inhibitory in TCDD-treated microsomes (data not shown). This finding is similar to that observed in studies with rat

and rabbit liver microsomes [38, 44, 45] and suggests the presence of a different enzyme in the liver of untreated and TCDD-treated hamsters.

HPLC analysis indicated major differences in the BaP metabolites formed by hepatic microsomes from control and TCDD- or PB-pretreated rats and hamsters (Fig. 6). In control rats, the major metabolite formed was 3-hydroxy-BaP, whereas BaP-4,5-dihydrodiol was the main metabolite formed by control hamster microsomes. This is significant in that 3-hydroxy-BaP but not BaP-4,5-dihydrodiol is converted by S9 fractions of rat and hamster liver to products mutagenic in *Salmonella typhimurium* strain TA 100 [46]. 3-Hydroxy-BaP has also been shown to be converted to products that covalently bind to DNA [47]. Although liver microsomes from PB-pretreated hamsters showed increased rates of BaP metabolism, the major metabolite observed in this situation was BaP-4,5-dihydrodiol with only minor quantities of BaP-9,10-dihydrodiol and BaP-7,8-dihydrodiol detected under these conditions. Pretreatment of rats with TCDD increased the quantity of all BaP metabolites formed and greatly enhanced the production of the bay-region dihydrodiol, BaP-9,10-dihydrodiol, and BaP-7,8-dihydrodiol. In contrast to the rat, the formation of these dihydrodiols were not increased in microsomes from TCDD-treated hamsters, with the HPLC profile remaining essentially the same as that observed with control hamster microsomes. BaP dihydrodiols are very biologically active and can act as substrates for P-450 enzymes to yield diol-epoxides. In particular, BaP-7,8-dihydrodiol can act as a substrate for cytochrome P-450 enzymes to yield BaP-7,8-diol 9,10-epoxide. This diol epoxide has been shown to be the major reactive metabolite responsible for binding to DNA [48] and is an ultimate carcinogen derived from BaP [9–11, 49]. Neither BaP-9,10-dihydrodiol nor BaP-4,5-dihydrodiol is metabolized to products as mutagenic as those formed from BaP-7,8-dihydrodiol [5, 48, 50]. In rat and rabbit, the major PAH-inducible cytochrome P-450 is mainly responsible for conversion of BaP-7,8-dihydrodiol to BaP-7,8-diol 9,10-epoxide [5, 10, 50]. The differences in the regional selectivity of hepatic cytrochrome P-450s observed in the rat and hamster suggest possible differences in the *in vivo* generation of metabolites that can be activated in target organs distal to initial sites of oxidation and conjugation [51].

The respiratory tract is a highly sensitive target organ for BaP carcinogenicity in humans and laboratory animals [52]. In particular, the hamster has been shown to be very sensitive to PAH-induced respiratory tract tumors [18, 53, 54]. The sensitivity of the hamster to PAH-induced respiratory tumors may be explained, in part, by the lack of induction of hepatic BaP metabolism as has been reported after PAH treatment in earlier studies [8–10] and TCDD treatment in this study. As a result of a low level of hepatic metabolism in the hamster, more parent compound would appear to be available for pulmonary biotransformation and activation. In this regard, tracheal, bronchial and lung cultures from humans, rats and hamsters have been shown to form 3-hydroxy-BaP, BaP-9,10,dihydrodiol, and BaP-7,8-dihydrodiol [55, 56].

In conclusion, significant differences were observed in the substrate specificity of the hepatic cytochrome P-450-dependent enzyme systems in the rat and hamster. These differences appear to be related to major differences in the abilities of these species to generate the ultimate carcinogenic metabolite of BaP. These results indicate that further examination of species differences in the substrate specificity of cytochrome P-450s is critical for the proper interpretation of toxicity and carcinogenicity studies involving different species or PAHs.

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