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SOME CHARACTERISTICS OF HAMSTER LIVER AND LUNG MICROSOMAL ARYL HYDROCARBON (BIPHENYL AND BENZO(a)PYRENE) HYDROXYLATION REACTIONS

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Abstract—Aryl hydrocarbon hydroxylation (AHH) reactions were compared using liver and lung microsomes of corn oil- and 3-methylcholanthrene (3-MC)-treated hamsters, employing benzo(a)pyrene (BAP) and biphenyl as substrates. The predominant AHH activity of liver and lung microsomes from corn oil- or 3-MC-treated hamsters was biphenyl 4-hydroxylase. Biphenyl 2-hydroxylase and BAP-hydroxylase activities were approximately 50 per cent as active as biphenyl 4-hydroxylase in liver and approximately 1–3 per cent as active as biphenyl 4-hydroxylase in lung microsomes. Biphenyl 4-hydroxylase activity was 70–80 per cent as active in lung as in liver microsomes. Treatment with 3-MC *in vivo* induced the biphenyl 4-hydroxylation reaction in liver but not in lung microsomes, the biphenyl 2-hydroxylation reaction both in lung and liver microsomes, and the BAP hydroxylation reaction in lung but not in liver microsomes. Biphenyl 2- and 4-hydroxylase activities of liver microsomes displayed similar sensitivities to inhibition by a number of chemical inhibitors *in vitro*. Inhibition of biphenyl hydroxylation reactions by metyrapone or carbon monoxide did not distinguish between lung or liver microsomal mono-oxygenases of corn oil- or 3-MC-treated hamsters. While small differences were expressed by inhibition with ethylmorphine, large differences became apparent through inhibition studies with BAP or α-naphthoflavone. It is concluded that the major aromatic hydroxylase activity of lung microsomes from corn oil- or 3-MC-treated hamsters resembles the constitutive (uninduced) AHH of the liver microsomes and that the minor aromatic hydroxylase activity of lung microsomes from corn oil- or 3-MC-treated hamsters resembles the induced AHH of the liver microsomes.

Benzo(a)pyrene (BAP) and a number of polycyclic aromatic hydrocarbons (PAH) promote cancer in many tissues of man and laboratory animals, and the respiratory tract is a highly sensitive target organ [1]. While some evidence suggests that the respiratory tract is inherently more susceptible than other tissues to PAH-induced cancer [2], it is possible that a preponderance of lung tumors occurs only after intra-tracheal administration of PAH to animals [3–5] or their inhalation by humans [1, 6].

Polycyclic aromatic hydrocarbons are probably metabolized by their target tissues to the proximal carcinogens, which may be arene oxides [7–9], and the susceptibility of certain tissues to PAH-induced cancer correlates directly with their ability to metabolize PAH [10–12]. However, PAH may also be metabolized to compounds which are less- or non-carcinogenic [8, 11, 13].

BAP and other polycyclic aromatic hydrocarbons such as biphenyl are metabolized mainly by an NADPH- and oxygen-dependent mono-oxygenase enzyme system active in the microsomes of many tissues, including the liver and the lung [14–16]. The metabolites of BAP are many and various and have been shown to include several phenols, dihydrodiols, quinones, epoxides and diol-epoxides [17–23]. The relative abundances of these various metabolites may be altered by pretreatment of animals with PAH or phenobarbital [19, 20, 22], and the effects of PAH pretreatment *in vivo* on the pattern of microsomal BAP metabolites are different in the lung and the liver [24]. There is a wide species-variability in the ratio for the mono-oxygenase activity of lung microsomes relative to liver microsomes, and there are both similarities and differences between the lung and the liver for the metabolism of BAP and other compounds, including non-carcinogenic substrates [15, 19, 25–32].

In this study we have undertaken to compare some characteristics of the hydroxylation of aromatic hydrocarbons by lung and liver microsomes by contrasting the hydroxylation of biphenyl and benzo(a)pyrene, using the hamster because it is highly susceptible to PAH-induced respiratory tract cancer [3–5]. Benzo(a)pyrene hydroxylase activity can be measured by three methods: high pressure liquid or thin-layer chromatography techniques which measure various classes of metabolites [15, 19–22], fluorescence measurements which measure only phenols [20, 22, 33–35], and radiometric assays which measure only total metabolites [36, 37]. Because all three methods have some limitations, we have used biphenyl as a second substrate for the aryl hydrocarbon hydroxylase activity since it has two easily measured metabolites, 2- and 4-hydroxybiphenyl, that comprise at least 95 per cent of the total metabolites produced by rat or hamster liver microsomes [38, 39].

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Biphenyl hydroxylase activity has been shown to exist with appreciable specific activity in rodent lung microsomes [25, 28, 29].

MATERIALS AND METHODS

Chemicals. Chemicals were purchased in the highest purity available and were used as supplied. Biphenyl and its hydroxybiphenyl derivatives were recrystallized from petroleum ether. Authentic 3-hydroxybenzo(a)pyrene was provided by Dr. Harry V. Gelboin, Chemistry Branch, National Cancer Institute.

Animals. Male Syrian Golden hamsters, 14–18 weeks old, were allowed food (laboratory rat chow pellets) and water *ad lib.* They received either corn oil (0.3 ml), 3-MC (20 mg/kg of body weight as a 2% solution in corn oil), or phenobarbital (80 mg/kg of body weight as a 4% aqueous solution of the sodium salt) i.p. once daily for 4 days, with the final injection being given 24 hr before sacrifice. The animals were not starved before sacrifice.

Microsome preparation and enzyme assays. Hamsters were killed by cervical dislocation and suspensions of their liver and lung microsomes prepared as previously described [29, 38]. The concentration of protein in the microsomal suspensions was determined by the method of Lowry *et al.* [40] and the contents of their cytochromes P-450 and b_5 were assayed as described previously [29]. NADPH- and NADH-cytochrome *c* reductase activities were measured as detailed by Masters *et al.* [41].

Benzphetamine and ethylmorphine demethylation reactions were assayed as reported in Ref. 42, employing 1 mM benzphetamine or 8 mM ethylmorphine. BAP hydroxylation activity was determined as products showing fluorescence equal to 1 nmole of authentic 3-hydroxy BAP by a modification of the method of Dehnen *et al.* [35]. α-Naphthoflavone (ANF) or biphenyl was added to the incubations dissolved in the acetone solutions of BAP itself, while ethylmorphine was dissolved in the reaction buffer. The hepatic microsomal reactions were monitored every 30 sec and were linear for no longer than 3 min; the lung microsomal reactions were monitored every 2 min and were linear for 15 min.

Biphenyl 2- and 4-hydroxylase activities were measured by an adaptation [38, 39] of the method of Creaven *et al.* [43]; the reaction included either liver microsomes or lung microsomes (1 mg protein/ml of reaction) and was started with NADPH (0.25 mM). Biphenyl was present at a 1 mM concentration. A constant volume (10 μl) of various concentrations of ANF in methanol or BAP in acetone was added to the reaction mixtures, and metyrapone or ethylmorphine was dissolved at various concentrations in the reaction buffer. None of these additions affected the fluorescence of standard 2- or 4-hydroxybiphenyl solutions at pH 5.5, and the effects of the solvents themselves on the reaction are discussed in Results (Inhibition of biphenyl hydroxylation by ethylmorphine). The liver microsomal biphenyl hydroxylation reactions were linear for 7.5 min, and 5-min incubations were routinely used, while the lung microsomal reactions were linear for 20 min, and 15-min incubations were used. For determination of carbon monoxide inhibition, the reaction tubes were continuously gassed with gas mixtures of either N_2–O_2 (80–20 per cent) or CO–O_2 (80–20 per cent). The spectrally apparent interaction of biphenyl with lung microsomes was investigated by the technique of Schenkman *et al.* [44].

RESULTS

Liver and lung microsomal activities. Of the aryl hydrocarbon hydroxylation (AHH) activities measured in liver microsomes of corn oil-treated hamsters, biphenyl 4-hydroxylation was the most active, biphenyl 2-hydroxylation was the least active, and BAP hydroxylation was intermediate in activity (Table 1). Benzphetamine and ethylmorphine demethylase activities were as large as the biphenyl 4-hydroxylase activity but were not induced by animal pretreatment with 3-methylcholanthrene (3-MC). 3-MC treatment of hamsters induced hepatic biphenyl 2- and 4-hydroxylase activities 3.4- and 2.3-fold, respectively, but did not greatly induce BAP hydroxylase activity. The microsomal cytochrome P-450, measured as carbon monoxy-ferrous cytochrome P-450, had a spectral maximum at 450 nm in liver or lung microsomes from corn oil-treated hamsters but had a spectral maximum at 448 nm and an increased concentration per mg of protein in liver or lung microsomes from 3-MC-treated hamsters (Table 1). The lack of induction of liver microsomal BAP hydroxylase activity by 3-MC treatment is in contrast to the 3 to 12-fold induction of activity in rat liver microsomes after 3-MC administration [19, 29]. Other reports have shown that BAP hydroxylation is minimally induced in hamster liver microsomes by PAH [24, 30].

In lung microsomal fractions, only biphenyl 2- and BAP-hydroxylase activities were induced by 3-MC pretreatment of hamsters, while all other activities measured were not affected or slightly diminished (Table 1). However, the lung microsomal specific activities for biphenyl 4-hydroxylation, benzphetamine demethylation, and NADPH-cytochrome *c* reduction are nearly as large as those of liver in both corn oil- or 3-MC-treated hamsters but the activities of biphenyl 2- or BAP-hydroxylation and ethylmorphine demethylation were only a few per cent of those of liver (Table 1 and 2).

The specific activities of BAP- and biphenyl 2-hydroxylation with liver or lung microsomes of corn oil- or 3-MC-treated hamsters showed a fairly direct correlation with the specific concentration (per mg of protein) of cytochrome P-450, whereas no such correlation was apparent for biphenyl 4-hydroxylation. Consequently, when the hydroxylation activities are expressed per nmole of cytochrome P450, the 'turnover numbers'* of lung microsomal BAP- or biphenyl 2-hydroxylation were 10–30 per cent of those of liver, while the lung microsomal biphenyl

* The term 'turnover number' will be based upon CO-reactive cytochrome P-450, which is the terminal oxidase in many o. .he mixed-function oxidase reactions, and will be expressed as the nmoles of product formed/min/nmole of cytochrome P-450.

Table 1. Activities and contents of microsomes of hamster lung and liver

Activity or content*	Lung corn oil	Lung 3-MC	Fold induction	Liver corn oil	Liver 3-MC	Fold induction
Biphenyl 4-hydroxylation†	1.7 ± 0.4	1.4 ± 0.5	0.8	2.2 ± 0.4	5.2 ± 1.2	2.4
Biphenyl 2-hydroxylation†	0.02 ± 0.01	0.05 ± 0.01	2.5	0.9 ± 0.3	3.10 ± 0.90	3.4
Benzo(a)pyrene hydroxylation†	0.006 ± 0.002	0.020 ± 0.005	3.3	1.3 ± 0.1	1.4 ± 0.3	1.1
Benzphetamine demethylation†	1.5	1.7	1.1	3.5	3.0	0.9
Ethylmorphine demethylation†	0.2	0.2	1.0	2.7	1.8	0.7
NADPH-cytochrome c reduction†	70	63	0.9	200	205	1.0
Cytochrome P-450 or P-448‡	0.06	0.21	3.5	0.95	1.61	1.7

* Values are given either as mean ± S. D. for five experiments for the three aromatic hydroxylation reactions or the mean for three experiments for the other activities. Each activity was measured on a different preparation of microsomes due to the low yield of lung microsomal protein; the demethylase and reductase activities are presented for comparison with other lung microsomal studies. Activities and contents were determined as described in Methods. Hamsters were pretreated with either corn oil or 3-MC as detailed in Methods. Liver and lung microsomes of corn oil-treated hamsters contained cytochrome P-450, while those of 3-MC-treated hamsters contained cytochrome P-448, as discussed in the text.

† Rate is nmoles of product formed/min/mg of microsomal protein.

‡ Expressed as nmoles/mg of protein.

4-hydroxylase turnover number was ten times larger than that of liver. Our results suggest that while biphenyl 2- and BAP-hydroxylase and ethylmorphine demethylase activities in lung were similar to those of liver, each molecule of lung cytochrome P-450 was either catalytically more active than its hepatic counterpart toward biphenyl 4-hydroxylation and benzphetamine *N*-demethylation or a large fraction of the hepatic cytochrome was catalytically inactive toward these reactions.

Inhibition of BAP hydroxylation by ANF, biphenyl and ethylmorphine. ANF at a concentration of 1×10^{-4} M potently inhibits BAP hydroxylation with liver microsomes from 3-MC-induced rats, whereas it slightly enhances the reaction with liver microsomes of corn oil-treated rats [27]. ANF strongly inhibited, by 85 per cent, BAP hydroxylation with the liver microsomes of 3-MC-treated hamsters and it also inhibited, but more weakly, by 31 per cent, the reaction with the liver microsomes of corn oil-treated hamsters (Fig. 1). The ANF was added in acetone, which had no effect on the reaction. Biphenyl (1×10^{-3} M) strongly inhibited BAP-hydroxylation by liver microsomes of corn oil- or 3-MC-treated hamsters, although the inhibition was greater with the microsomes of corn oil-treated animals (87 vs 68 per cent) (Fig. 1). BAP hydroxylation was also significantly inhibited by 10^{-1} M ethylmorphine with the liver microsomes of corn oil or 3-MC-treated hamsters (Fig. 1).

ANF and biphenyl at a concentration of 1×10^{-3} M strongly inhibit the lung microsomal benzo(a)pyrene hydroxylase activity of corn oil- or 3-MC-treated hamsters (Table 3). The activities with lung microsomes from hamsters were nearly identical with those reported for rat lung microsomes [27] and the amount of ANF resulting in a 50 per cent inhibition of control or induced benzo(a)pyrene activity was also nearly identical (1.0 to 1.3×10^{-5} M). The biphenyl inhibited equally well the control and

Table 2. Comparison of specific activities and turnover numbers of the aromatic hydroxylation reactions of hamster liver and lung

Activity*	Animal pretreatment	Sp. Act. lung / Sp. act. liver (%)	Turnover No.† Lung	Turnover No.† Liver
Biphenyl 4-hydroxylase	Corn oil	77	28.3	2.3
	3-MC	27	7.2	3.2
Biphenyl 2-hydroxylase	Corn oil	2.2	0.3	0.9
	3-MC	1.6	0.2	1.9
Benzo(a)pyrene hydroxylase	Corn oil	0.4	0.06	1.4
	3-MC	1.4	0.10	0.9

* Calculated from Table 1.

† Rate is nmoles product formed/min/nmole of cytochrome P-450.

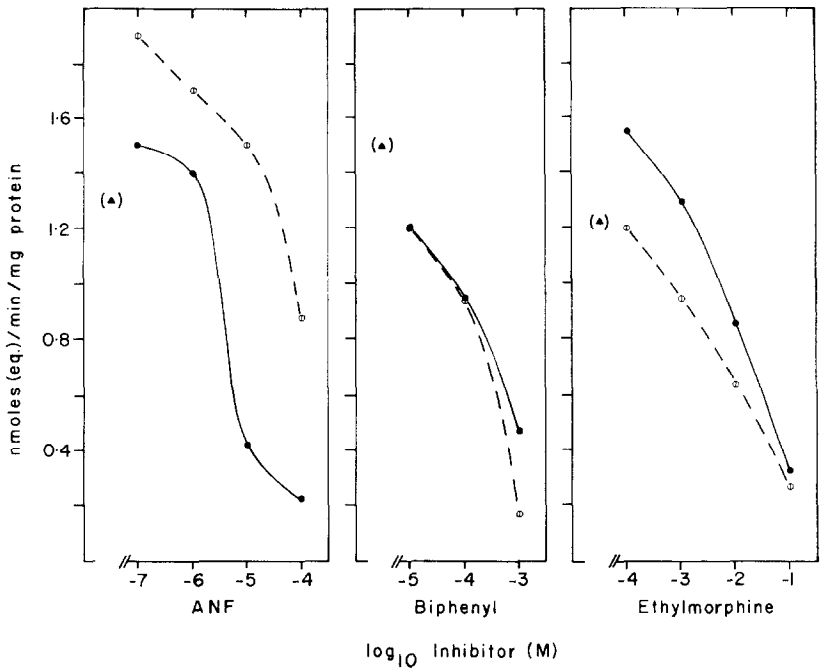

Fig. 1. Inhibition of liver microsomal benzo(a)pyrene hydroxylation. BAP was incubated, as described in Methods, with liver microsomes of either corn oil- (Φ) or 3-MC-treated (●) hamsters, in the presence of various concentrations of either α-naphthoflavone (ANF), biphenyl or ethylmorphine. BAP-hydroxylation activity is expressed as nmoles of metabolites exhibiting the fluorescence identical to 1 nmole of standard 3-hydroxy BAP. BAP-hydroxylase activities in the absence of added inhibitors were equal with the liver microsomes of either corn oil- or 3-MC-treated hamsters and are represented as (▲). The values given are the mean for experiments with microsomes from three separate sets of hamsters and the average deviation in all cases was less than 10 per cent.

induced BAP hydroxylase activity in hamster lung microsomes.

Inhibition of biphenyl hydroxylation by BAP and ANF. The biphenyl 2- and 4-hydroxylation reactions of liver microsomes from corn oil-treated hamsters were weakly inhibited to the same extent by BAP *in vitro* (Fig. 2). The induced liver microsomal 2- and 4-hydroxylation specific activities of 3-MC-treated hamsters were strongly inhibited by BAP *in vitro* to the same extent (Fig. 2). Benzo(a)pyrene inhibited the 3-MC-induced activities to approximately the activity for biphenyl 4-hydroxylation shown by the uninhibited liver microsomes of corn oil-treated hamsters. This result suggests that induced liver microsomes may contain both a constitutive and a 3-MC-induced mono-oxygenase which can catalyze biphenyl hydroxylation.

The biphenyl 4-hydroxylase activity of lung microsomes was not induced by 3-MC treatment *in vivo* (Table 1), and BAP *in vitro* did not inhibit the reaction with the lung microsomes of either corn oil- or 3-MC-treated hamsters (Fig. 3). The biphenyl 2-hydroxylase activity of lung microsomes from either corn oil- or 3-MC-treated hamsters was also not inhibited by BAP; instead 3-fold stimulations resulted in each case from the incorporation of 10^{-4} M BAP in the incubation mixture (Burke and Prough, unpublished results).

ANF weakly inhibited biphenyl 4-hydroxylation with liver microsomes from corn oil-treated hamsters but strongly inhibited the reaction in liver microsomes from 3-MC-treated hamsters (Fig. 4). Biphenyl 2-hydroxylation was inhibited identically as 4-hydroxylation in liver microsomes from corn oil- or 3-MC-treated hamsters (Burke and Prough, unpublished results). ANF did not inhibit the biphenyl 4-hydroxylation reaction of lung microsomes from either corn oil- or 3-MC-treated hamsters, while 10^{-4} M ANF enhanced the biphenyl 2-hydroxylation reaction 3- to 4-fold with lung microsomes of corn oil- or 3-MC-treated hamsters.

Acetone inhibited biphenyl 4-hydroxylation with the liver or lung microsomes of corn oil- or 3-MC-treated hamsters (10 and 40 μl of acetone/2 ml of incu-

Table 3. Effect of α-naphthoflavone and biphenyl on lung microsomal benzo(a)pyrene hydroxylase activity*

Animal pretreatment	Inhibitor	% Inhibition	I_{50}† (M)
Corn oil	Biphenyl	87	8×10^{-5}
3-MC	Biphenyl	90	1.2×10^{-5}
Corn oil	ANF	86	1×10^{-5}
3-MC	ANF	78	1.3×10^{-5}

* The activity of lung microsomal benzo(a)pyrene hydroxylase activity was approximately 7 and 25 pmoles of 3-hydroxybenzo(a)pyrene formed/min/mg of protein for control and 3-MC-pretreated hamsters respectively. The values are the mean of two experiments and the average deviation was 25 per cent.

† The I_{50} value is that molar concentration of inhibitor which causes a 50 per cent inhibition of activity.

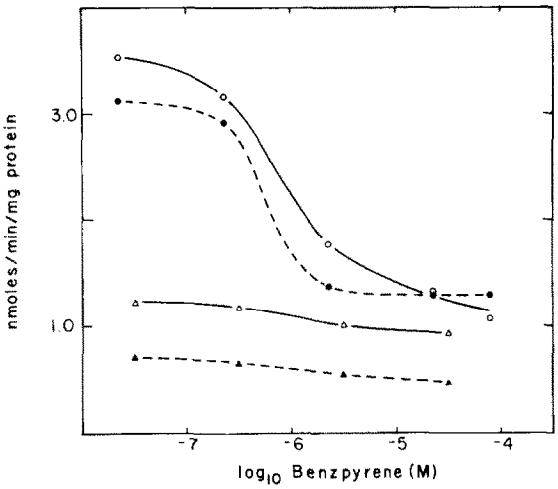

Fig. 2. BAP inhibition of liver microsomal biphenyl 2- and 4-hydroxylation. Biphenyl was incubated, as described in Methods, with liver microsomes of either corn oil- (△ or ▲) or 3-MC-treated (○ or ●) hamsters, in the presence of various concentrations of BAP. Hydroxylation activities are expressed as nmoles of either 2-hydroxybiphenyl (▲, ●) or 4-hydroxybiphenyl (△, ○) formed. The values given are the mean for microsomes from three separate sets of hamsters and the average deviation in all cases was less than 10 per cent.

bation mixture caused 28 and 50 per cent inhibition, respectively), but biphenyl 2-hydroxylation was not affected. The results in Fig. 3 are expressed as percentages of the activities in the presence of 10 μl acetone alone. Methanol (10 μl) added to 2 ml of incubation mixture inhibited biphenyl 4-hydroxylation by 15 per cent with the liver or lung microsomes of corn oil- or 3-MC-treated hamsters, but biphenyl 2-hydroxylation was not affected. Dimethylformamide (DMF) and dimethylsulfoxide (DMSO) were both as inhibitory (50 per cent maximally) to biphenyl 2-hydroxylation as to its 4-hydroxylation (Burke and Prough, unpublished results). Organic solvents are much less inhibitory to 3-MC-induced rat liver microsomal BAP-hydroxylase activity than to the constitutive activity [27, 45], and they have been shown to also inhibit biphenyl 4-hydroxylase activity of liver microsomes of untreated rabbits [46].

Inhibition of biphenyl hydroxylation by ethylmorphine. The ethylmorphine *N*-demethylation activity of the liver microsomes from corn oil-treated hamsters was approximately 3 nmoles/min/mg of protein, and this was not induced by pretreatment with 3-MC *in vivo*. In contrast, ethylmorphine was poorly *N*-demethylated to formaldehyde by lung microsomes of corn oil- or 3-MC-treated hamsters (≦ 0.2 nmole/min/mg), although alternative possible metabolites were not sought. High concentrations of ethylmorphine strongly inhibited to an approximately equal extent the biphenyl 4-hydroxylation activity of liver or lung microsomes from corn oil- or 3-MC-treated hamsters, although the lung activity was slightly more sensitive than that of the liver (Fig. 5). Ethylmorphine inhibited biphenyl 2-hydroxylation with liver microsomes from corn oil-treated hamsters more strongly than it did the activity of liver microsomes from 3-MC-treated hamsters, while the reaction of lung microsomes from 3-MC-treated hamsters was only weakly inhibited (Fig. 6). Biphenyl 2-hydroxylation with lung microsomes of corn oil-treated hamsters was also inhibited by ethylmorphine, but the inhibited activity under these conditions was below the level of accurate measurement.

Involvement of cytochrome P-450 in the binding and metabolism of biphenyl. The interaction of many substrates with the hepatic and lung microsomal monooxygenase systems is characterized by a shift in the spectral absorption of microsomes, which is attributed to the binding of substrate to cytochrome P-450 [29, 44]. Biphenyl evokes a type I binding difference spectrum with liver microsomes from corn oil- or 3-MC-treated hamsters [38]. A similar type I spectral change resulted from the addition of biphenyl to lung microsomes from corn oil- or 3-MC-treated hamsters. The binding difference spectrum was well defined at saturating substrate concentrations, with a peak at 395 nm and a trough at 425 nm, but the peak to trough magnitude (ΔE) was small in comparison with that obtained for the interaction of biphenyl with liver microsomes. This is consistent with the lower concentration of cytochrome P-450 in lung microsomes. The microsomes were titrated with increasing concentrations of biphenyl and the maximal effect at 2 mM biphenyl (ΔE_{max}) was 0.002 and 0.006 $E/2$ mg of protein (N = 3 experiments, average deviation 25 per cent) with lung microsomes from corn oil- and 3-MC-treated hamsters respectively. BAP also produces a weak, type I spectral change with lung microsomes of 3-MC-treated rats [47].

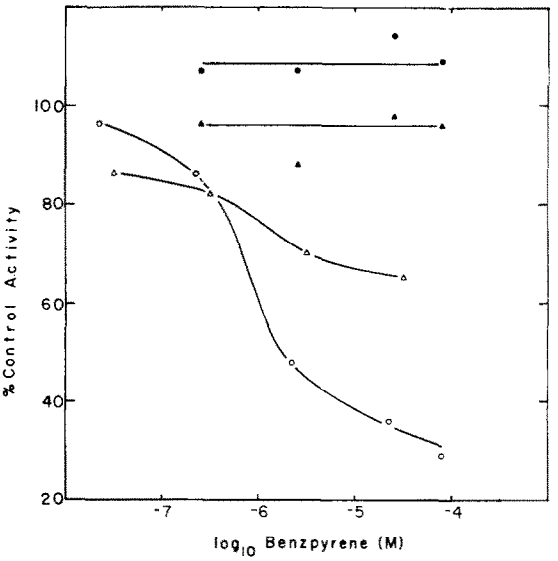

Fig. 3. BAP inhibition of lung and liver microsomal biphenyl 4-hydroxylation. Biphenyl was incubated, as described in Methods, with either lung (closed symbols) or liver (open symbols) microsomes of corn oil- (△ or ▲) or 3-MC-treated (○ or ●) hamsters, in the presence of various concentrations of BAP. Hydroxylation activities are expressed for each BAP concentration as a percentage of the corresponding specific activity (nmoles of 4-hydroxybiphenyl formed) in the absence of added BAP. The values given are the mean for microsomes from three separate sets of hamsters and the average deviation for liver microsomes was 10 per cent and for lung microsomes was 13 per cent.

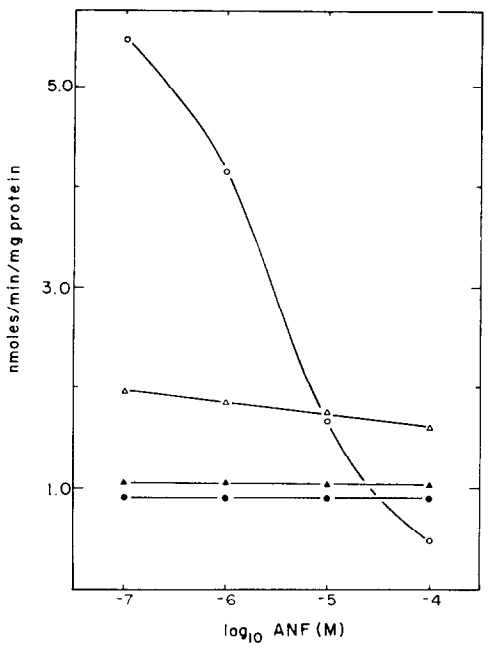

Fig. 4. ANF inhibition of lung and liver microsomal biphenyl 4-hydroxylation. Details are as described in Fig. 3, except that the added inhibitor was α-naphthoflavone and the actual specific activities of 4-hydroxylation are plotted. The specific activities in the absence of ANF were as follows: control lung, 1.1 nmoles/min/mg; 3-MC lung, 0.9 nmole/min/mg; control liver, 2.2 nmoles/min/mg; and 3-MC liver, 4.4 nmoles/min/mg.

Carbon monoxide has been shown to inhibit cytochrome P-450-mediated microsomal reactions [48]. The cytochrome P-450-mediated microsomal biphenyl 2- and 4-hydroxylation reactions of liver and lung microsomes from corn oil- or 3-MC-treated hamsters were equally sensitive to inhibition by carbon monoxide (Table 4). An atmosphere of 80% CO–20% O_2 above the incubation mixture inhibited the hydroxylation reactions by 75–80 per cent relative to the activities with an atmosphere of 80% N_2–20% O_2 (mean result for eight experiments). Since only small differences exist between the two activities regardless of organ and animal pretreatment, the 2- and 4-hydroxylase activities appear to be very similar in their sensitivity to CO inhibition.

Metyrapone is a potent inhibitor of liver microsomal drug metabolism and probably acts by interacting directly with the heme iron of cytochrome P-450 [49, 50]; on the other hand, many substrates appear to bind to cytochrome P-450 without interacting with the iron as an axial ligand [44]. Constitutive BAP hydroxylation by liver microsomes of (untreated) mice is much more sensitive to metyrapone than is the 3-MC-induced reaction [45, 46]. However, 1 mM metyrapone strongly inhibited to an equal extent the biphenyl 4-hydroxylation activities of liver or lung microsomes from corn oil-, phenobarbital- or 3-MC-treated hamsters (Figs. 7 and 8). A comparison of these two figures illustrates how an impression of an apparent difference in the sensitivities of reactions with different microsomes can mistakenly arise from plotting the specific activities directly instead of

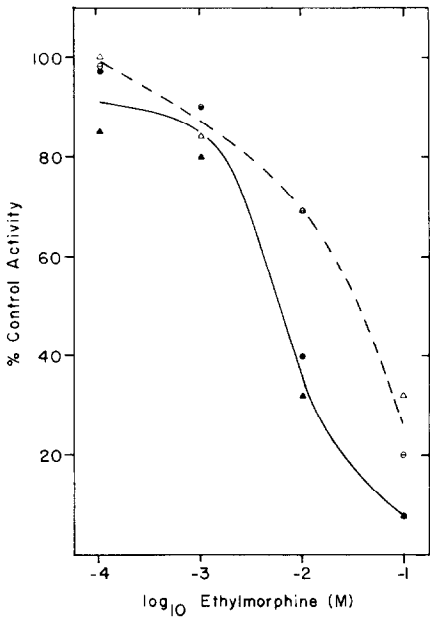

Fig. 5. Ethylmorphine inhibition of lung and liver microsomal biphenyl 4-hydroxylation. Details are as described in Fig. 3, except that the added inhibitor was ethylmorphine.

Table 4. Carbon monoxide inhibition of the biphenyl hydroxylase activities of hamster liver and lung

Organ/pretreatment	Types of biphenyl hydroxylase activity	Activity*		
		N_2:O_2	CO:O_2	% Inhibition
Control liver	4	2.30	0.37	84
	2	1.10	0.30	73
3-MC liver	4	5.26	1.34	79
	2	2.20	0.74	75
Control lung	4	1.10	0.10	95
	2	<0.03	<0.01†	>66†
3-MC lung	4	0.90	0.03	95
	2	0.05	0.01	80

* Rate is nmoles product formed/min/mg of protein.
† Lower level of assay reliability.

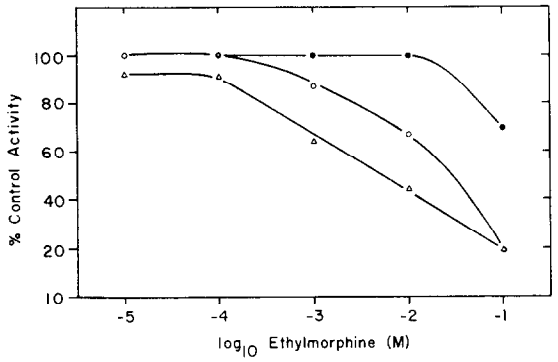

Fig. 6. Ethylmorphine inhibition of lung and liver microsomal biphenyl 2-hydroxylation. Details are as described in Fig. 3, except that ethylmorphine was the added inhibitor and the results are for nmoles of 2-hydroxybiphenyl formed. No curve is included for lung microsomes of untreated hamsters (see text).

as percentages of the control activity (i.e. in the absence of inhibitor). The inhibition of biphenyl 2-hydroxylation by metyrapone in liver microsomes from corn oil-, phenobarbital- or 3-MC-treated hamsters or in lung microsomes from 3-MC-treated hamsters was identical with the inhibition of biphenyl 4-hydroxylation. The 2-hydroxylation activity of the lung microsomes from corn oil- or phenobarbital-treated hamsters was also inhibited by metyrapone, but to below the level of accurate measurement.

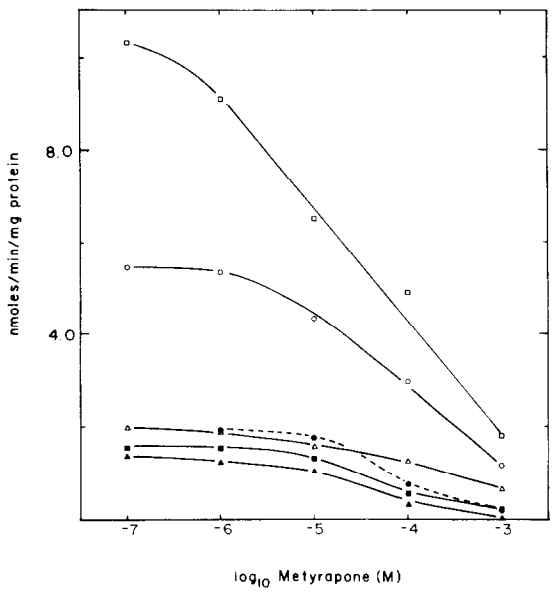

Fig. 7. Metyrapone inhibition of lung and liver microsomal biphenyl 4-hydroxylation. Biphenyl was incubated, as described in Methods, with either lung (closed symbols) or liver (open symbols) microsomes of either corn oil- (▲ or △), 3-MC-treated (● or ○) or phenobarbital-treated (■ or □) hamsters, in the presence of various concentrations of metyrapone. The hydroxylation activities are expressed for each metyrapone concentration as nmoles of 4-hydroxybiphenyl formed. The values given are the mean of experiments performed with microsomes from three separate sets of hamsters and the average deviation was 15 and 10 per cent for lung and liver microsomes respectively.

DISCUSSION

Biphenyl is a toxicologically important xenobiotic in its own right, since it is used as a fungistat on fruit and forms the nucleus of the polychlorobiphenyl plasticizers. Several experimental observations suggest that the hamster liver microsomal hydroxylation reactions of biphenyl and BAP are catalyzed by similar AHH systems. Biphenyl and BAP both interact with liver microsomal cytochrome P-450 to yield a type I binding difference spectrum [38, 46], and the induction of rat liver microsomal biphenyl and BAP hydroxylase activities by either phenobarbital or 3-methylcholanthrene is quite similar [29, 38]. Both hydroxylation reactions are inhibited in a parallel fashion by an antibody against NADPH-cytochrome *c* (P-450) reductase [39]. However, no biphenyl-epoxide or -dihydrodiol metabolites have been observed corresponding to those formed during the microsomal metabolism of BAP.

Hamster lung microsomes catalyzed BAP hydroxylation and biphenyl 2- and 4-hydroxylation reactions, but the major AHH activity was biphenyl 4-hydroxylation, with a specific activity (nmoles product formed/min/mg of protein) comparable to that of liver microsomes and over 25-fold higher than the specific activities for BAP- or biphenyl 2-hydroxylations of lung microsomes. Because the concentrations of CO-reactive cytochromes P-450 or P-448 in hamster lung microsomes were much less than their concentrations in liver microsomes, the implication is that either each molecule of the lung cytochromes was catalytically more active than its hepatic counterpart toward biphenyl 4-hydroxylation or that a large portion of the hepatic cytochrome was catalytically inactive toward this reaction. On the other hand, there was a close similarity between the lung and liver

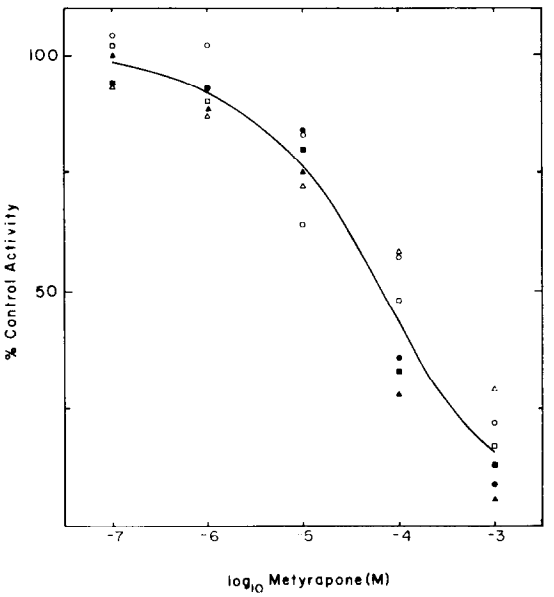

Fig. 8. Metyrapone inhibition of lung and liver microsomal biphenyl 4-hydroxylation. Details are as for Fig. 7, except that the hydroxylase activities for each metyrapone concentration are expressed as percentages of the corresponding specific activity in the absence of added inhibitor.

cytochromes P-450 or P-448 with respect to activity toward biphenyl 2- and BAP-hydroxylation.

Goujon *et al.* [45] and Wiebel *et al.* [27] have used chemical inhibitors to differentiate between constitutive and PAH-induced benzo(a)pyrene hydroxylase (AHH) activities. Both reported that the liver microsomal BAP hydroxylase activity of untreated mice or rats was refractory to inhibition by ANF but that this activity of 3-MC-pretreated animals was extremely sensitive to ANF as an inhibitor. In agreement with Wiebel *et al.*, we have noted that lung microsomal BAP hydroxylase activities of control or 3-MC-treated rats or hamsters were nearly equally sensitive to ANF.

Using a number of chemical inhibitors, we have contrasted biphenyl and benzo(a)pyrene as substrates of the hamster liver and lung mono-oxygenase system which hydroxylates aromatic hydrocarbons. Biphenyl inhibited BAP hydroxylation reactions; the biphenyl concentration causing 50 per cent inhibition of BAP hydroxylase activity was approximately equal to the apparent K_m for biphenyl 2-hydroxylase (2 to 4×10^{-4} M, Ref. 38). Conversely, BAP inhibited 3-MC-induced liver microsomal biphenyl 2- and 4-hydroxylase activities. The liver microsomal biphenyl hydroxylases of control animals and the lung microsomal biphenyl hydroxylases were slightly or not at all inhibited by BAP respectively. ANF behaved much like BAP as an inhibitor of biphenyl hydroxylase activity in that only the activity of 3-MC-induced hamster liver microsomes was sensitive to ANF.

The biphenyl 2- and 4-hydroxylase activities with liver microsomes of corn oil-treated hamsters were much less sensitive to ANF inhibition than was the control BAP-hydroxylase, while the 3-MC-induced biphenyl- and BAP-hydroxylase activities were highly sensitive to ANF inhibition. These results suggest that a closer similarity exists between the 3-MC-induced biphenyl- and BAP-hydroxylases than between the constitutive activities.

The liver microsomal biphenyl 2-hydroxylase of corn oil- or 3-MC-pretreated hamsters showed the same sensitivity as biphenyl 4-hydroxylase toward inhibition by ANF, BAP, metyrapone, ethylmorphine or carbon monoxide. This result supports the previous suggestions that these biphenyl hydroxylases are generally identical although subtly different [38, 39]. At the same time, constitutive and 3-MC-inducible biphenyl hydroxylases can be differentiated by their sensitivities to ANF or BAP. These observations suggest that the constitutive biphenyl hydroxylase of hamster liver microsomes catalyzes both the 2- and the 4-hydroxylation reactions, in contrast to rat liver microsomes wherein only a 3-MC-inducible hydroxylase catalyzes biphenyl 2-hydroxylation reactions [51].

Experiments using specific inhibitory antibodies have indicated that NADPH-cytochrome *c* (P-450) reductase plays an essentially identical, important role as the flavoprotein reductase of cytochrome P-450 in the biphenyl hydroxylases of both liver and lung microsomes [39]. Our observation of the type I binding of biphenyl with lung microsomes and metyrapone- and carbon monoxide-inhibitions in lung and liver biphenyl hydroxylase activities suggests that cytochrome P-450 assumes the important role of a terminal oxidase for the lung hydroxylase activities, as it does for the liver mono-oxygenases [52, 53].

There were some large differences between the lung and liver microsomal AHH. The major AHH activity of hamster lung microsomes, biphenyl-4-hydroxylation, was not inducible in lung microsomes upon 3-MC pretreatment and displayed the ANF- and BAP-inhibition characteristics *in vitro* of the constitutive liver microsomal activity. A minor AHH activity of lung microsomes, biphenyl 2-hydroxylation, was induced by 3-MC in both liver and lung microsomes, while another minor lung microsomal AHH activity, BAP hydroxylation, was induced by 3-MC in hamster lung but not liver microsomes. Wang *et al.* [24] have reported that BAP treatment of female hamsters *in vivo* also induces BAP-hydroxylase activity with lung but not liver microsomes [19, 28, 29], and biphenyl 4-hydroxylase activity is reportedly induced in lung and liver microsomes by 3-MC treatment *in vivo* of Wistar rats [29]. ANF and BAP enhanced hamster lung microsomal biphenyl 2-hydroxylation *in vitro* but inhibited the reaction with liver microsomes. The enhancement may have been caused by metabolites of ANF or BAP generated during the relatively prolonged lung microsomal reactions, whereas the shorter duration liver microsomal reactions may have preempted the formation of sufficient concentrations of such metabolites. A similar enhancement *in vitro* of liver microsomal biphenyl-2-hydroxylation by metabolites of various PAH carcinogens has been reported [54].

As similarly proposed for rat or mouse liver benzo(a)pyrene hydroxylase [27, 45], there appear to be two types of biphenyl hydroxylase activities in hamster liver microsomes capable of catalyzing both the 2- and 4-hydroxylation reactions: a constitutive enzyme complex and a 3-MC-inducible enzyme system. In hamster lung, there appear to be both a constitutive and a 3-MC-inducible AHH enzyme system. The constitutive system is the major lung AHH activity and catalyzes primarily biphenyl 4-hydroxylation at a higher turnover than liver microsomes (10–30 nmoles product/min/nmole of cytochrome P-450). The 3-MC-inducible enzyme is a minor lung AHH activity and appears to catalyze only BAP- and biphenyl 2-hydroxylation reactions at a turnover similar to liver microsomes from control animals (0.5 nmole product/min/nmole of cytochrome P-450). The existence of two types of cytochrome P-450-dependent mono-oxygenases in rodent lung is similar to the situation in liver in which several types or classes of cytochrome P-450 exist. Further, it may be relevant to BAP lung tumorigenesis that only one type of lung mono-oxygenase which is inducible by PAH appears to be very specific for BAP metabolism.

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