MOLECULAR PHARMACOLOGY, 10, 484–493

Genetic Expression of Aryl Hydrocarbon Hydroxylase Induction

Presence or Absence of Association with Zoxazolamine, Diphenylhydantoin, and Hexobarbital Metabolism

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(Received November 27, 1973)

SUMMARY

ROBINSON, JOSEPH R., AND NEBERT, DANIEL W.: Genetic expression of aryl hydrocarbon hydroxylase induction. Presence or absence of association with zoxazolamine, diphenylhydantoin, and hexobarbital metabolism. *Mol. Pharmacol.* **10**, 484–493 (1974).

An increase in zoxazolamine (2-amino-5-chlorobenzoxazole) metabolism, as determined either by paralysis time or by an enzyme assay *in vitro*, is associated with aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase induction among inbred strains of aromatic hydrocarbon-treated, genetically "responsive" mice, does not occur in inbred, genetically "nonresponsive," aromatic hydrocarbon-treated mice, and segregates ($p < 0.01$) with the *Ah* locus in the appropriate genetic crosses between "responsive" and "nonresponsive" mice. Hexobarbital metabolism, as determined by sleeping time, is not associated with the *Ah* locus in either inbred or hybrid mice. The inbred strains used for zoxazolamine and hexobarbital studies were C57BL/6N, BALB/cAnN, C3H/HeN, DBA/2N, and AKR/N. A rise in 5,5-diphenylhydantoin metabolism, as determined by the increased formation of *p*-hydroxy and 3,4-dihydrodiol derivatives *in vitro*, is associated with the presence of aryl hydrocarbon hydroxylase induction among aromatic hydrocarbon-treated inbred C57BL/6N, does not occur in aromatic hydrocarbon-treated inbred DBA/2N, but does not segregate ($p > 0.10$) with the *Ah* locus among offspring of the appropriate crosses between "responsive" and "nonresponsive" mice. Induction of both diphenylhydantoin hydroxylase and aryl hydrocarbon hydroxylase occurs in the liver of 3-methylcholanthrene-treated Osborne-Mendel rats. Therefore monooxygenation (i.e., ring hydroxylation) of benzo[*a*]pyrene and zoxazolamine probably involves the same newly synthesized cytochrome P_1-450 regulated by the *Ah* locus; diphenylhydantoin hydroxylation may be involved with a different P-450 species regulated by an "aromatic hydrocarbon responsiveness" genetic locus other than the *Ah* locus.

INTRODUCTION

In previous papers of this series (1–7), genetic differences in the expression of aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase[1] by aromatic hydrocarbons administered to mice have been examined in detail. The im-

These results were presented in part at the Meeting of the American Society of Biological Chemists, Atlantic City, April 1974.

[1] The aryl hydrocarbon hydroxylase assay measures the rate of formation of hydroxylated benzo[*a*]pyrene; such phenols may be formed either by direct hydroxylation or by a two-step process (see ref. 8 for discussion). In a two-step process in which an epoxide is formed, dihydrodiols and other metabolites may be formed and

portance of aryl hydroxylations of drugs, polycyclic hydrocarbons, and insecticides mediated by monooxygenases (such as this aryl hydrocarbon hydroxylase) to pharmacology, chemical carcinogenesis, and toxicology has recently been reviewed (9). The genetic locus for aromatic hydrocarbon responsiveness, designated the *Ah* locus (3, 10, 11), is closely associated with new formation of the CO-binding hemoprotein[2] cytochrome P_1-450 (2–7, 10, 11), and with the increase in several monooxygenase activities (6). Aromatic hydrocarbon "responsiveness" appears to represent a threshold effect, because exposure of liver cell cultures derived from genetically "nonresponsive" mice to aromatic hydrocarbons dissolved in the culture medium and treatment of genetically "nonresponsive" mice with certain halogenated dioxins and furans *in vivo* produce rises in the hydroxylase activity that are as high as those found in so-called genetically "responsive" mice (1, 18).

Zoxazolamine (2-amino-5-chlorobenzoxazole) was introduced in 1957 as a muscle relaxant in the treatment of various spastic disorders. Because of potent uricosuric properties (19, 20), the drug was also indicated in the treatment of gout. However, studies implicating zoxazolamine as the etiological agent in several instances of fatal hepatic necrosis in man (21) caused removal of the drug from the pharmaceutical market in 1963.

Since the introduction of 5,5-diphenylhydantoin in 1938 (22), it has remained one of the most widely used anticonvulsants. Few subjectively disturbing side effects, relative to good seizure control at moderate dosages, have ensured the popular use of this drug. In certain rodent species DPH[3] causes such teratogenic anomalies as cleft lip and palate (23, 24). In man an apparent increase in birth defects such as cleft lip and palate (25–28) and a lowering of the intelligence quotient (29) has been noted in the offspring of epileptic mothers receiving anticonvulsant drugs such as hydantoin derivatives.

The popularity of hexobarbital as a model substrate (30) for the liver monooxygenase system probably stems from the rapid absorption of the drug, and its distribution, metabolism, and hypnotic activity in laboratory animals; also, the duration of sleep reflects primarily metabolism rather than tissue redistribution, in contrast, for example, with thiopental.

The metabolism of zoxazolamine (31), DPH (32–34), and hexobarbital (30) involves monooxygenation (9, 35, 36) (Fig. 1) via liver microsomal cytochrome(s) P-450. The major metabolite for zoxazolamine in man is the 6-hydroxy derivative, and very small amounts of chlorzoxazone—formed by replacement of the amino group of zoxazolamine with a hydroxyl group—are found (31, 37). The major metabolites of DPH in rats, rhesus monkeys, and man are 5-(*p*-hydroxyphenyl)-5-phenylhydantoin (32) and 5-(3,4-dihydroxy-1,5-cyclohexadien-1-yl)-5-phenylhydantoin (33). The major product of hexobarbital metabolism in dog, rabbit, mouse, rat, and man is the 3′-hydroxy derivative, with subsequent 3′-keto formation, *N*-demethylation of the 3′-keto product, and glucuronide conjugation of 3′-hydroxyhexobarbital (30). Because the *Ah* locus is associated in genetically responsive mice with increases in certain monooxygenase activities, and because major metabolic pathways for zoxazolamine, DPH, and hexobarbital involve the liver monooxygenase system, we therefore examined the metabolism of these drugs among strains of genetically responsive and nonresponsive mice and among hybrids resulting from certain genetic crosses.

presumably would not be associated with the hydroxylase activity; the relative importance of these other pathways for the substrate benzo[*a*]pyrene has not been determined.

[2] Cytochrome P_1-450 (12)—also known as cytochrome P-448 (13) or P-446 (14)—is that species of CO-binding hemoprotein which increases in concentration in response to aromatic hydrocarbon treatment either *in vivo* (12, 13) or in cell culture (14). It is becoming increasingly evident (15–17) that numerous species of cytochrome(s) P-450 exist.

[3] The abbreviations used are: DPH, 5,5-diphenylhydantoin (Dilantin); MC, 3-methylcholanthrene; B6, the inbred C57BL/6N mouse strain; D2, the inbred DBA/2N mouse strain.

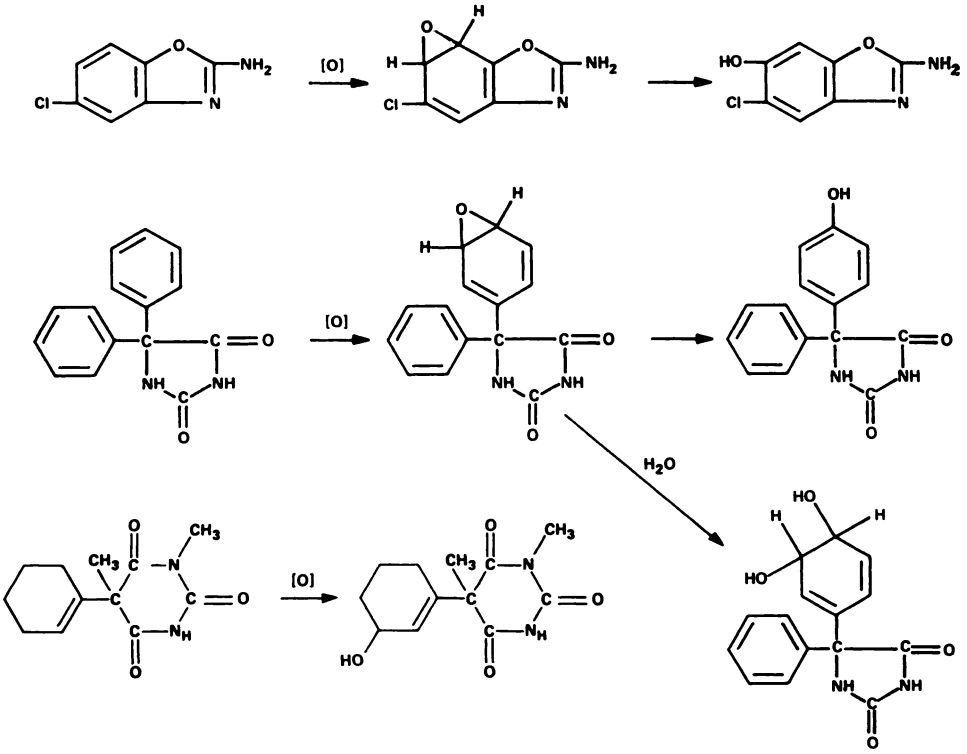

FIG. 1. *Proposed reaction schemes for the three drugs under study in this report: zoxazolamine (upper left), DPH (middle left), and hexobarbital (lower left)*

It is postulated that zoxazolamine and DPH undergo hydroxylation via arene oxide intermediates, whereas the hydroxylation of hexobarbital in position 3′ involves a simple aliphatic hydroxylation. The hypothetical arene oxide intermediates, 1,6-epoxyzoxazolamine and 5-(3,4-epoxy-1,5-cyclohexadien-1-yl)-5-phenylhydantoin, shown here have not been isolated or otherwise identified. It is entirely possible that the hydroxy product of zoxazolamine is formed only by a direct hydroxylation and not by a two-step process involving the arene oxide. Shown at right are the hydroxy product of zoxazolamine, the monohydroxy and dihydrodiol derivatives of DPH, and the hydroxy product of hexobarbital.

MATERIALS AND METHODS

Zoxazolamine was a generous gift of Dr. Alan P. Poland (University of Rochester Medical School), who had received it as a gift from McNeil Laboratories, Inc., Fort Washington, Pa. Unlabeled DPH was generously donated by Parke Davis & Company, and sodium phenobarbital and hexobarbital were purchased from Merck & Company. [4-^{14}C]DPH (4.65 mCi/mmole) was purchased from New England Nuclear; MC, from J. T. Baker Company; α-naphthoflavone, from Aldrich Chemical Company; and benzo[*a*]pyrene, NADPH, and NADH, from Sigma. National Institutes of Health Animal Supply provided sexually immature male or female (age, 3–6 weeks) inbred mice and 100-g male Osborne-Mendel rats. The environment in the animal room, feeding of the animals, the intraperitoneal administration of MC or phenobarbital (80 mg/kg of body weight), and the preparation of liver microsomes were exactly as described previously (2, 5).

Zoxazolamine and hexobarbital sleeping times. Twenty-four hours after a single dose of either MC in corn oil or corn oil alone, the mice received zoxazolamine (225 mg/kg of body weight) intraperitoneally in corn oil. Within 1 min the animals were asleep; each mouse was then placed on its back. The sleeping time was recorded as that period of time until the animal had regained enough consciousness to right itself repeatedly.

Hexobarbital sleeping times, with a dose of 100 mg/kg of body weight, were similarly measured.

Hydroxylase assay. The hydroxylase activity and protein determinations were determined essentially as described before (2, 10). One unit of aryl hydrocarbon hydroxylase activity is defined as that amount of enzyme catalyzing per minute at 37° the formation of hydroxylated product causing fluorescence equivalent to that of 1 pmole of the 3-hydroxybenzo[*a*]pyrene recrystallized standard.

Zoxazolamine metabolism in vitro. The originally described method (31) was modified considerably. The 1.00-ml reaction volume included 50 μmoles of potassium phosphate buffer (pH 7.4), 0.36 μmole of NADH, 0.10 ml of liver microsomes (containing 200–400 μg of protein), and 60 or 120 nmoles of the substrate zoxazolamine. The substrate was first dissolved in a minimal amount of 1 N HCl and then diluted with water to a final concentration of 500 μg/ml. The reaction mixture was incubated in a shaker at 37° in air for 20 min, and the reaction was stopped by adding 1.0 ml of 0.5 N NaOH and placing the reaction flasks in ice. This 2-ml mixture was extracted with 2 ml of cold ethylene dichloride and centrifuged briefly. The ethylene dichloride extract was shaken with 1.0 ml of 0.3 M sodium borate, and the aqueous fraction was removed. The zoxazolamine was then extracted from ethylene dichloride into 2.0 ml of 1 N HCl and measured spectrophotometrically at 278 nm. The percentage of substrate remaining, relative to appropriate blanks and standards, allowed calculation of nanomoles of zoxazolamine metabolized per minute per milligram of microsomal protein.

DPH metabolism in vitro. The procedure (34) for measuring DPH metabolism *in vitro* was followed exactly, except that a 1.00-ml reaction volume, as described above, was used. Thus potassium phosphate buffer, NADPH, NADH, liver microsomes, 50 nmoles of [4-^{14}C]DPN, and 150 nmoles of unlabeled DPH were incubated at 37° in air for 15 min, unless otherwise indicated. The reaction was stopped with 3.0 ml of cold ethyl acetate; this mixture was shaken at room temperature for 5 min and briefly centrifuged. The ethyl acetate layer was then removed. This extraction with ethyl acetate was repeated four more times, and the ethyl acetate phases were combined and then evaporated to dryness. To the dried extracts were added 25 μl of methanol, and 20 μl of this methanol residue mixture were chromatographed on F-254 silica gel thin-layer plates (Brinkmann) with a benzene–methanol–acetic acid (45:8:4) solvent system. The dried plates were placed in contact with Eastman Kodak BB-54 medical X-ray film and stored for 2–14 days. The radioactive spots on the plates were also scraped into vials for radioactivity determinations by liquid scintillation counting.

RESULTS

Zoxazolamine and hexobarbital sleeping times. Table 1 shows zoxazolamine and hexobarbital sleeping times for control and MC-treated inbred mice of five different strains. C57BL/6N, BALB/cAnN, and C3H/HeN are three strains that are genetically responsive to aromatic hydrocarbons such as MC with respect to the induction of aryl hydrocarbon hydroxylase (2) and other monooxygenase (6) activities; DBA/2N and AKR/N are two strains which demonstrate little or no genetically mediated response (2, 6). Prior treatment with MC caused a 2- to more than 10-fold shortening of the zoxazolamine paralysis time in the three responsive strains but not in the two nonresponsive strains. That benzo[*a*]pyrene is a more potent inducer of zoxazolamine hydroxylase activity than phenobarbital in rat liver has been reported (31). The AKR/N mouse, which invariably has a higher basal hydroxylase activity in the liver than the other four strains studied (6), had a slightly shorter paralysis time among the control animals receiving zoxazolamine. The dose of zoxazolamine used was more lethal in the D2 control mouse than in any of the other four strains. With lesser doses of 100–200 mg of zoxazolamine per kilogram of body weight, some of the MC-treated, genetically responsive mice did not sleep at all.

No significant differences in hexobarbital sleeping times between control and MC-treated mice of these five strains were found.

TABLE 1

Effect of prior treatment with MC on zoxazolamine and hexobarbital sleeping times for five strains of inbred mice

The range of sleeping times is given for each group, and the number of animals in each group is shown in parentheses. A maximal sleeping time of 180 min was arbitrarily assigned, because most animals that slept longer than 180 min usually remained near death for another 3 hr or more and eventually died.

Strain	Sleeping time			
	Zoxazolamine		Hexobarbital	
	Control	MC	Control	MC
	min	*min*	*min*	*min*
C57BL/6N	120–180 (10)	20–50 (10)	60–90 (10)	50–90 (10)
BALB/cAnN	130–180 (8)	18–45 (8)	40–70 (8)	50–80 (8)
C3H/HeN	120–180 (8)	25–50 (8)	60–90 (8)	50–90 (8)
DBA/2N	160–180 (10)	120–180 (10)	50–110 (10)	60–80 (10)
AKR/N	100–130 (8)	105–160 (8)	60–100 (8)	70–90 (8)

Doses of DPH ranging from 20 to 150 mg/kg of body weight were similarly tried with control and MC-treated mice. However, the mice developed ataxia and tremors—which often lasted for several days before death—and no well-defined genetic differences in sleeping times with DPH could be determined. We also found that intraperitoneal administration of zoxazolamine, hexobarbital, or DPH did not cause aryl hydrocarbon hydroxylase induction.

Figure 2 shows that there is a good correlation between MC-induced hydroxylase activity and shortened zoxazolamine sleeping time in the F_2 generation from B6 and D2 mice. We have similarly found[4] this association without exception in more than 60 offspring from various back-crosses and intercrosses; e.g., B6D2 × D2, (C3H/HeN) (DBA/2N) × C3H/HeN, and (B6) (AKR/N) × B6. For each zoxazolamine sleeping time experiment, we always included several MC-treated B6 and D2 inbred controls, in order to determine the range of sleeping times found on any given day. As expected, phenobarbital administration *in vivo* produced shortened zoxazolamine sleeping times in both genetically responsive and nonresponsive mice (data not shown). Induction of the monooxygenase activities by phenobarbital has been demonstrated (2, 10, 11) not to be associated closely with the *Ah* locus. We also found a good correlation between the MC-induced hydroxylase activity and the assay for zoxazolamine

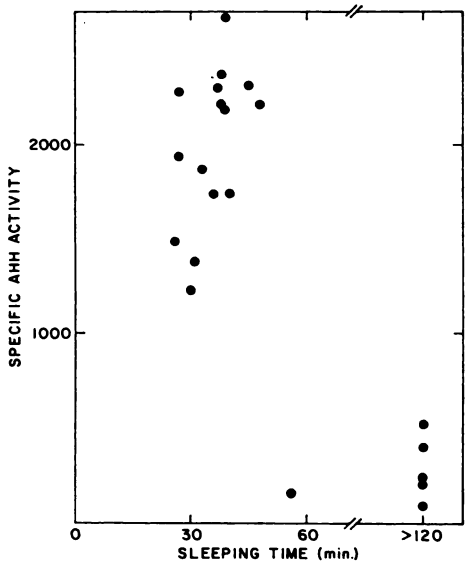

FIG. 2. *Relationship between liver microsomal aryl hydrocarbon hydroxylase (AHH) activity and zoxazolamine sleeping time in MC-treated (B6D2)F_2 mice*

After each sleeping time had been recorded, the specific hydroxylase activity, in units per milligram of microsomal protein, was determined for each mouse. The correlation coefficient, r is -0.79 ($p < 0.001$) for these 21 samples.

[4] J. R. Robinson, J. W. Daly, and D. W. Nebert, unpublished data.

metabolism *in vitro:* a range of 45–70 nmoles of zoxazolamine metabolized per minute per milligram of microsomal protein was observed in MC-treated, genetically responsive mice, as compared with a range of 10–26 nmoles/min/mg of protein in MC-treated, genetically nonresponsive mice. These studies included B6, BALB/cAnN, D2, and AKR/N inbred mice plus progeny of several crosses involving these strains.

Since α-naphthoflavone is a good inhibitor of MC-inducible aryl hydrocarbon hydroxylase activity *in vitro* (4), we wished to determine whether this compound would affect the zoxazolamine paralysis time *in vivo*. However, doses of 50–200 mg/kg of body weight given intraperitoneally 0.5–2 hr prior to the zoxazolamine test dose did not significantly alter the zoxazolamine paralysis time of MC-treated B6 mice.

DPH metabolism. Thin-layer chromatographic separations of DPH and its metabolites are shown in Fig. 3. We found that the dihydrodiol and monohydroxy derivatives of DPH had approximate R_F values of 0.19 and 0.35, respectively (34), that another product, of lesser quantity, had an R_F value of about 0.38, and that formation of these products required the presence of microsomes and was NADPH-dependent. Under these conditions less than 2% of the substrate was metabolized to the above derivatives. Figure 3A and Table 2 illustrate that DPH hydroxylation was stimulated in rat liver by intraperitoneal MC but not by phenobarbital. This effect was more pronounced with microsomes than with the postmitochondrial supernatant fraction; perhaps the presence of conjugating enzymes in the cytosol and not in the microsomal pellet would explain these differences, since conjugations of epoxidated intermediates readily occur (9).

Figure 3B shows that the monohydroxy derivative is increased in MC-treated B6 mice but not in MC-treated D2 mice. The product can be seen after 2 min of incubation of the labeled substate with liver microsomes, is most intense after 15 min, and actually is less after 30 min. Further metabolism of the monohydroxy product would explain these findings. Figure 4 illustrates that increased monohydroxy-DPH formation does not occur in every genetically responsive B6D2 × D2 or (B6D2)F_2 mouse. Thus, although induction of both aryl hydrocarbon hydroxylase and "DPH hydroxylase" by MC was absent or very small in the inbred D2 mouse and present in the inbred B6 mouse and (B6D2)F_1 hybrid, either or both MC-inducible enzyme activities were sometimes expressed in animals from the B6D2 × D2 back-cross or the B6D2 × B6D2 intercross.

DISCUSSION

From the results of this study we conclude that "zoxazolamine hydroxylase" is similar to or identical with the other monooxygenase activities (6) which appear to be closely associated with the *Ah* locus: aryl hydrocarbon hydroxylase, 7-ethoxycoumarin *O*-deethylase, *p*-nitroanisole *O*-demethylase, and 3-methyl-4-methylaminoazobenzene *N*-demethylase. This means that the increased hydroxylation of zoxazolamine in aromatic hydrocarbon-treated, genetically responsive animals is correlated with newly synthesized cytochrome P_1-450, which can be identified by spectral (2, 3, 5–7, 10, 11), paramagnetic (5, 38), and preferential inhibitor (4) differences.

The zoxazolamine sleeping time affords a quick, convenient, and reliable laboratory test for determining the phenotype, with respect to the *Ah* locus, without sacrificing the mouse or performing a liver biopsy. We still cannot distinguish the genotype between genetically responsive homozygous (Ah^b/Ah^b) and heterozygous (Ah^b/Ah^d) mice (3, 39–41), since the hydroxylase activity in both genotypes is fully inducible by aromatic hydrocarbon administration (2, 3, 10, 11). In certain studies involving genetic differences in susceptibility to polycyclic hydrocarbon-initiated carcinogenesis (39–43) or in susceptibility to the toxic effects of numerous xenobiotics,[5] it may be advantageous to know the phenotype without killing the animal. Likewise, for breeding animals having the highest or lowest basal hydroxylase activity or having the highest inducible hydroxylase activity, the zoxa-

[5] J. R. Robinson, J. S. Felton, and D. W. Nebert, manuscript in preparation.

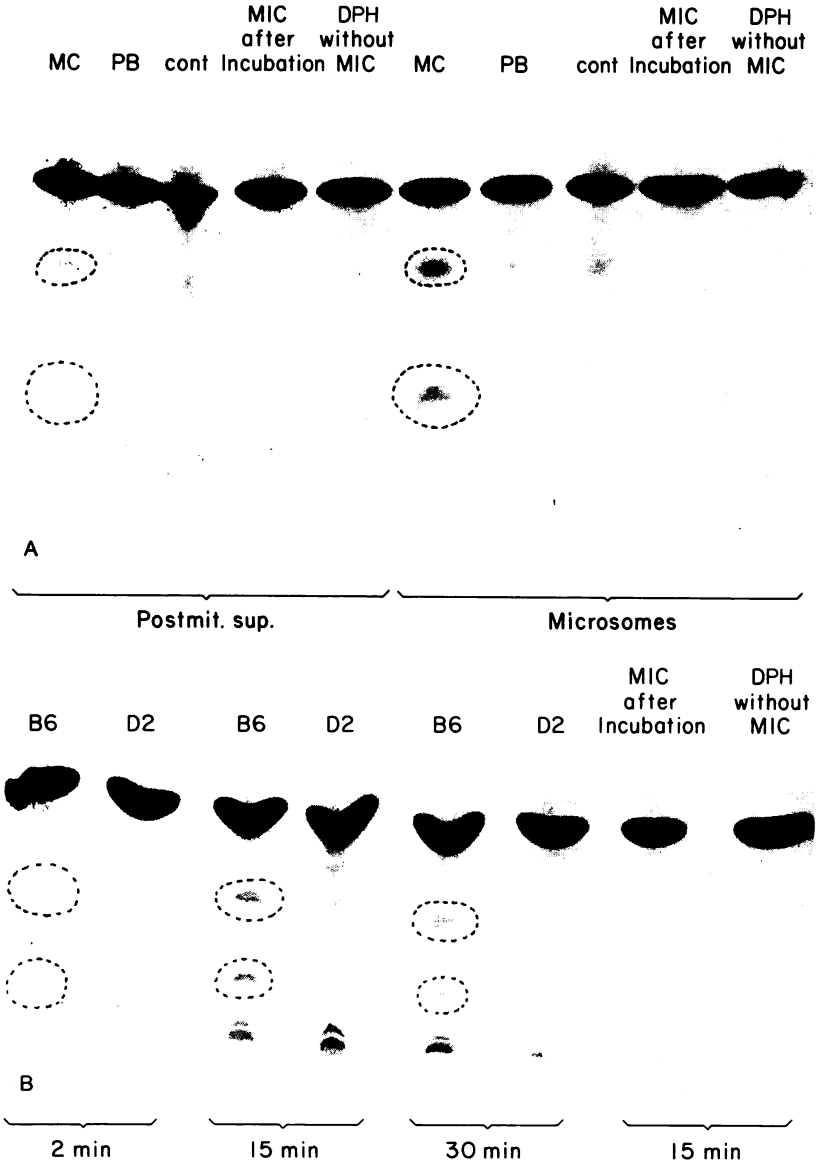

FIG. 3. *X-ray films of thin-layer chromatographs from hepatic postmitochondrial supernatant or microsomal fractions incubated with [4-^{14}C]DPH*

The origin is at the bottom, and the most prominent spot, with an R_F value of about 0.45, is the substrate [4-^{14}C]DPH. The monohydroxy and dihydrodiol derivatives, with R_F values of approximately 0.35 and 0.19, respectively, are encircled with a dashed line wherever the spots appear most prominent. A. Effect of prior treatment with MC or phenobarbital in the rat. B. Kinetics of product formation in MC-treated B6 and D2 mice. The postmitochondrial supernatant fraction (Postmit. sup.) in Fig. 3A represents that fraction poured off after centrifugation of liver homogenate for 15 min at 15,000 × *g*. Microsomal aryl hydrocarbon hydroxylase specific activities in the MC-treated, phenobarbital-treated, and control rats (Fig. 3A) were 790, 130, and 140, respectively, and in the MC-treated B6 and D2 mice (Fig. 3B) were 2140 and 380, respectively. Control chromatographs include the addition of microsomes (MIC) after the 15-min incubation and the presence of labeled substrate without microsomes (DPH without MIC). The amount of microsomal protein in each reaction flask (about 0.5 mg) was about the same in all experiments.

TABLE 2

Effect of prior treatment with MC or phenobarbital on DPH hydroxylation in rats

Under the conditions employed, 1000 dpm/chromatographic spot correspond to approximately 1.0 nmole of labeled plus unlabeled product formed per milligram of microsomal protein during the 15-min incubation at 37°. Conditions are described in MATERIALS AND METHODS and in Fig. 3.

Fraction used *in vitro*	*in vivo* Treatment	Dihydrodiol product	Monohydroxy product	Parent compound
			dpm/sample	
Postmitochondrial supernant	MC	710	530	329,000
	Phenobarbital	380	210	303,000
	Control	400	290	294,000
	Microsomes after incubation	70	98	303,000
	DPH without microsomes	17	20	328,000
Microsomal	MC	270	940	258,000
	Phenobarbital	130	110	279,000
	Control	160	190	282,000
	Microsomes after incubation	32	98	288,000
	DPH without microsomes	2	14	294,000

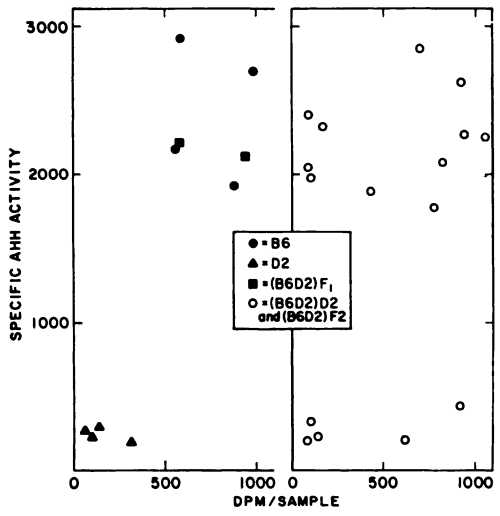

FIG. 4. *Relationship between hepatic microsomal aryl hydrocarbon hydroxylase (AHH) activity and monohydroxy-DPH formation in MC-treated B6 and D2 inbred mice, (B6D2)F1 hybrids, and offspring from the B6D2 × D2 back-cross or B6D2 × B6D2 intercross*

Conditions were the same as those described in MATERIALS AND METHODS, Fig. 3, and Table 2. The correlation coefficient, r, is +0.84 ($p < 0.01$) for the graph at left and 0.28 ($p > 0.10$) for the 16 values depicted at right.

zolamine sleeping time provides a useful genetic probe. An alternative test which does not consume the animal has been described (44): genetically responsive strains develop skin ulcers 5–15 days after a single topical application of 7,12-dimethylbenz[*a*]anthracene, whereas nonresponsive strains show little or no reaction. This response has recently been shown (45) to be related to MC-inducible aryl hydrocarbon hydroxylase activity. However, the grading of an inflammatory response on the skin of mice over a 15-day period is technically more difficult and more subject to error than the zoxazolamine sleeping time.

Clearly hexobarbital hydroxylation is not at all associated with the *Ah* locus. This finding is in agreement with studies[6] involving hexobarbital hydroxylation *in vitro* among three genetically responsive and three nonresponsive inbred strains.

At first glance, DPH hydroxylase induction appears to be correlated with aryl hydrocarbon hydroxylase induction by aromatic hydrocarbon treatment in the inbred B6 and D2 mice and (B6D2)F_1 hybrid. Thus

[6] W. Levin, A. Y. H. Lu, and D. W. Nebert, unpublished data.

DPH hydroxylation is stimulated in the MC-treated, genetically responsive B6 inbred mouse but not in the MC-treated, nonresponsive D2 inbred mouse, and the trait is expressed dominantly in the F_1 hybrid. However, the value of examining further appropriate genetic crosses is aptly demonstrated in this case. Thus, from the B6D2 × D2 and B6D2 × B6D2 crosses, MC-treated, genetically responsive Ah^b/Ah^b or Ah^b/Ah^d offspring may have high or low DPH hydroxylase activities, and MC-treated, genetically nonresponsive Ah^d/Ah^d progeny also may have high or low DPH hydroxylase activities (Fig. 4). The number of animals tested is too small to determine whether one or more loci in addition to the *Ah* locus are involved. We did not examine DPH metabolism in detail among other inbred strains of mice or their offspring. These data indicate, however, that DPH hydroxylation may be involved with a different cytochrome P-450 species, regulated by an aromatic hydrocarbon responsiveness locus other than the *Ah* locus. Several other monooxygenase activities seem to fit in this category,[4] and studies are under way to see whether each of these MC-inducible enzymes, which are not closely associated with the *Ah* locus, is instead closely linked to the MC-inducible DPH hydroxylase activity in the mouse. From genetic crosses involving more than 12 other strains (46), our results indicate that genetic expression of MC-inducible aryl hydrocarbon hydroxylase activity is regulated by at least two, if not three, nonlinked loci.

In man there are genetic differences in the hydroxylase inducible by aromatic hydrocarbons. Reproducible, significant differences in the magnitude of hydroxylase induction by MC were determined in cultured lymphocytes from a sample population of several hundred patients and their close relatives, and genetic expression of aryl hydrocarbon hydroxylase induction appears to segregate as two alleles at a single locus (47). Moreover, susceptibility to bronchogenic carcinoma in man appears to be associated with the higher levels of inducible hydroxylase activity as determined in this cultured lymphocyte system (48). Dissimilar responses to other inducers of drug-metabolizing enzymes may explain some of the reported pharmacogenetic disorders (49, 50) in man. DPH intoxication, a syndrome which includes nystagmus, ataxia, and drowsiness, has been described in one family and is apparently caused by genetic differences in the hydroxylation rate of DPH (51). The degree of susceptibility of different individuals to the teratogenic effect of certain drugs might be explained on the basis of genetically controlled differences in the metabolism of those drugs (52). Either the slow degradation of a reactive parent compound to a nonreactive metabolite or the rapid conversion of an inert parent compound to a reactive metabolite might therefore be important for the pharmacological, toxicological, carcinogenic, or teratogenic effect of a drug or other xenobiotic.

ACKNOWLEDGMENT

We thank Noreen Considine for her valuable technical assistance.

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