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Effect of Enzyme Induction on the Metabolism and Tissue Distribution of Benzo(α)pyrene[1]

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SUMMARY

Pretreatment of rats with benzo(α)pyrene, 3-methylcholanthrene, or 7,12-dimethylbenz(α)anthracene p.o. once daily for two days prior to the i.v. administration of tritiated benzo(α)pyrene (BP-^{3}H) on the third day stimulates the disappearance of BP-^{3}H from blood and decreases the concentration of BP-^{3}H in various tissues. Pretreatment of rats with pyrene, anthracene, or phenobarbital does not stimulate the *in vivo* disappearance of BP-^{3}H. The concentration of BP-^{3}H in the fat of rats is lower after seven daily p.o. doses of BP-^{3}H than after a single dose of BP-^{3}H, which suggests that this hydrocarbon stimulates its own metabolism during chronic administration.

INTRODUCTION

Treatment of rats with BP,[5] MC, or certain other polycyclic aromatic hydrocarbons enhances severalfold the hydroxylation of BP and various drugs by enzymes in liver and nonhepatic tissues (2, 4, 6, 7, 9, 21, 26, 30). Although much of the human population is chronically exposed to low levels of BP and other carcinogenic hydrocarbons in the environment, little is known about the effects of enzyme induction on the *in vivo* metabolism of carcinogenic hydrocarbons. Such considerations have prompted our laboratory to determine whether the induction of BP-hydroxylating enzymes by polycyclic hydrocarbons or phenobarbital influences the *in vivo* metabolism and tissue distribution of BP. This report indicates that pretreatment of rats with MC, BP, or DMBA stimulates the metabolism of BP *in vivo*, whereas phenobarbital does not exert this effect.

MATERIALS AND METHODS

Adult female Sprague-Dawley rats obtained from Blue Spruce Farms, Altamont, N. Y., were fed Wayne Lab-Blox meal (Allied Mills, Inc., Peoria, Ill.) and water *ad libitum*. Tritiated BP (generally labeled) was obtained from the Amersham/Searle Corporation, Arlington Heights, Ill., and nonlabeled BP was obtained from the Sigma Chemical Company, St. Louis, Mo., or the Aldrich Chemical Company, Inc., Milwaukee, Wis. Rats were pretreated with polycyclic hydrocarbons in 0.5 ml of corn oil p.o. once daily for 2 days prior to the i.v. administration of BP-^{3}H on the 3rd day. In some experiments, rats were treated with 37 mg/kg of sodium phenobarbital in 0.9% NaCl solution i.p. twice daily for 4 days, and BP-^{3}H was injected the following day. Control rats received injections of the appropriate vehicle.

Tritiated BP was prepared for i.v. injection by dissolving 100 μg of this hydrocarbon in approximately 50 μl of acetone. Enough rat plasma was added to obtain a final concentration of 10 μg of BP-^{3}H (50 μCi)/0.5 ml of plasma. This mixture was shaken gently at room temperature for 45 min prior to an i.v. injection of 0.5 ml of this solution into the rat's tail vein. In some experiments (Tables 2 and 3), tritiated BP (1 to 10 mg, 115 to 250 μCi) was dissolved in 0.5 ml of dimethyl sulfoxide, which was administered by stomach tube. After administration of BP-^{3}H, the rats were killed by decapitation, and blood was collected in tubes containing a small volume of heparin. Liver, fat, lung, adrenal gland, and brain were rapidly removed and chilled. The tissues were weighed rapidly and homogenized in 3 volumes of ice-cold water with a Potter-Elvehjem homogenizer. Two volumes of acetone and 6 volumes of hexane were added to the blood or tissue homogenates, and the mixtures were shaken for 20 min. Following centrifugation, a portion of the organic solvent was evaporated to dryness under a stream of nitrogen. The residue was dissolved in a small volume of acetone, and an aliquot was chromatographed on thin-layer chromatography paper (type SA, Gelman Instrument Company, Ann Arbor, Mich.) with hexane:benzene (7:1). After the solvent was 15 cm from the origin, the paper was dried at room temperature for a few minutes and again developed with the same solvent mixture. Under these conditions, BP-^{3}H was found 13.5 cm from the origin. For some experiments, thin-layer chromatography was done on thick Silica Gel G plates with hexane:benzene (3:1). After development of the chromatograms, 0.5- to 1-cm sections of the silica gel or paper were placed in scintillation vials, and radioactivity was quantified in a Packard scintillation spectrometer with a scintillation mixture that contained 0.5% PPO and 0.01% POPOP in toluene or the scintillation mixture described by Bray (3). The concentration of BP-^{3}H in the various tissues was calculated from the amount of radioactivity with the mobility of BP. All values were corrected for the

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[5] The abbreviations used are: BP, benzo(α)pyrene; MC, 3-methylcholanthrene; DMBA, 7,12-dimethylbenz(α)anthracene.

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recovery of BP-^{3}H, which was 75 to 85% for the various tissues. The recovery of BP-^{3}H was determined by adding this hydrocarbon to homogenates of the various tissues, which were then extracted and chromatographed as described above. Appreciable amounts of metabolites of BP-^{3}H were found in the blood and liver of rats given injections of the tritiated hydrocarbon, whereas much smaller amounts of metabolites were found in the brain and fat. These metabolites were more polar than BP and were not studied further.

RESULTS

Effect of Pretreatment with Polycyclic Hydrocarbons on the Metabolism of i.v. Administered BP-^{3}H. Ten μg of BP-^{3}H were injected i.v. into rats, and the blood, liver, brain, and fat were analyzed for BP-^{3}H at various intervals after the injection. BP-^{3}H disappeared from the blood very rapidly for the first few minutes and then more slowly at later times (Chart 1). Pretreatment of the rats with nonradioactive BP for 2 days markedly enhanced the disappearance of BP-^{3}H from blood during the first 5 min after its i.v. injection. The concentration of BP-^{3}H in the blood 1 min after the i.v. injection of BP-^{3}H into control and pretreated rats was 193 ± 29 and 155 ± 34 ng of BP-^{3}H/ml, respectively; whereas the concentration of BP-^{3}H in the blood 5 min after the i.v. injection of BP-^{3}H into control and pretreated rats was 31 ± 1 and 4 ± 0.2 ng/ml, respectively (Chart 1). The concentration of BP-^{3}H was lower in blood obtained from pretreated animals than in blood from control animals for the 1st 4 hr after the injection of BP-^{3}H, but little or no difference was observed at 6 hr, and little or no difference in organic solvent-extractable hydrocarbon-^{3}H in blood was observed for the 2 groups of animals 24 hr after the injection of BP-^{3}H.

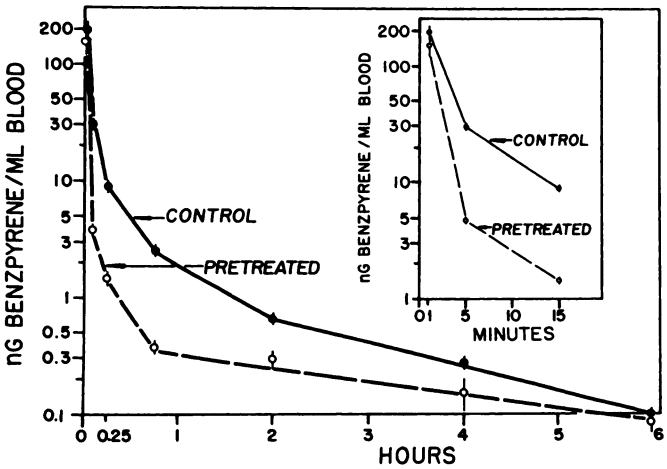

Chart 1. Effect of pretreatment of rats with nonradioactive BP on the disappearance of i.v. administered BP-^{3}H from blood. Female rats weighing 175 to 185 g were given corn oil or 20 mg of nonradioactive BP/kg of body weight in corn oil p.o. once daily for 2 days. The following day, 10 μg of BP-^{3}H was injected i.v. and the concentration of BP-^{3}H in the blood was determined as described in "Materials and Methods." The data represent the mean ± S.E. from 3 values (each value was obtained from 3 rats).

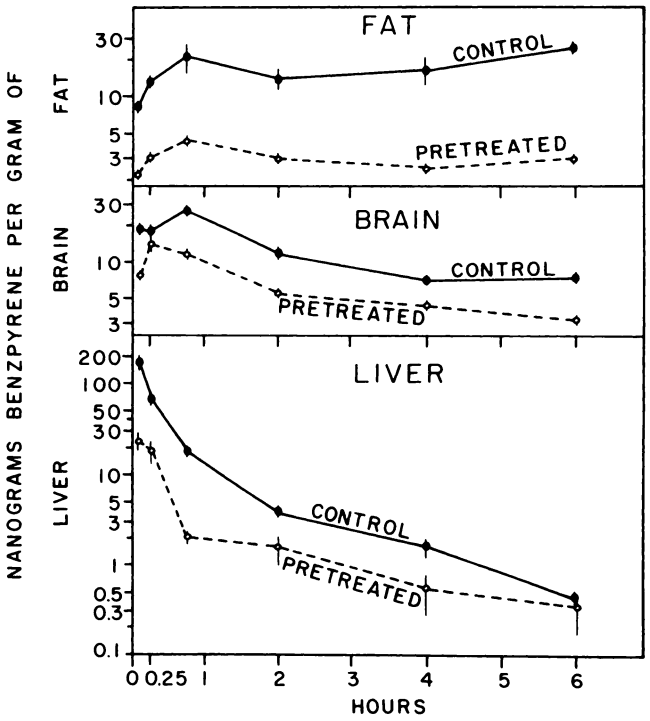

Chart 2. Effect of pretreatment of rats with nonradioactive BP on the appearance of BP-^{3}H in the tissues of animals given BP-^{3}H i.v. The concentration of BP-^{3}H in the dorsal fat, brain, and liver of the rats described in Chart 1 was determined as described in "Materials and Methods." The data represent the mean ± S.E. from 3 values (each value was obtained from 3 rats).

Table 1

Effect of pretreatment of rats with polycyclic hydrocarbons or phenobarbital on the metabolism of BP-^{3}H

Female rats weighing 175 to 185 g were treated p.o. with 20 mg/kg of BP once daily for 2 days. An equimolar amount of MC, DMBA, pyrene, or anthracene was administered in Experiment 2. In Experiment 3, rats were given i.p. injections of 37 mg/kg of sodium phenobarbital twice daily for 4 days. The day after the last dose of polycyclic hydrocarbon or phenobarbital, rats were given i.v. injections of 10 μg of BP-^{3}H, and the animals were killed 45 min later. The data represent the mean ± S.E. from 3 to 6 values (each value was obtained from 3 rats).

Experiment	Pretreatment	Concentration of BP-^{3}H in Blood (ng/ml)	Concentration of BP-^{3}H in Fat (ng/g)
1	Control	2.6 ± 0.2	21.6 ± 6.6
	BP	0.4 ± 0.1	4.1 ± 0.8
2	Control	2.9 ± 0.3	15.0 ± 3.5
	MC	0.5 ± 0.0	4.3 ± 0.7
	DMBA	0.7 ± 0.1	5.4 ± 0.6
	Pyrene	2.5 ± 0.1	18.9 ± 0.7
	Anthracene	2.3 ± 0.3	16.9 ± 2.5
3	Control	2.5 ± 0.2	14.4 ± 1.4
	Phenobarbital	2.3 ± 0.2	18.1 ± 1.8

The lower blood level of BP-^{3}H described above for BP-pretreated rats was paralleled by a lower concentration of BP-^{3}H in dorsal fat, brain, and liver (Chart 2). Similar results were obtained with the lung, but more variability was observed

Table 2

Effect of pretreatment of rats with nonradioactive BP on the tissue concentration of BP-^{3}H in rats given BP-^{3}H p.o.

Female rates weighing 150 g were treated i.p. with 33 mg/kg of nonradioactive BP once daily for 2 days. The rats were given 10 mg (115 μCi) of BP-^{3}H p.o. the following day and were killed 2 hr later. The data represent the mean ± S.E. from 4 values (each value was obtained from the pooled tissues of 7 rats).

Pretreatment	Concentration of BP-^{3}H in		
	Lung (μg/g)	Dorsal fat (μg/g)	Adrenals (μg/g)
Control	2.83 ± 0.28	5.86 ± 0.48	4.29 ± 0.62
BP	0.16 ± 0.02	1.38 ± 0.19	0.73 ± 0.06

Table 3

Decreased concentration of BP-^{3}H in the fat of rats treated chronically with BP-^{3}H

Female rats weighing 150 g were given 1 mg (250 μCi) of BP-^{3}H p.o. once daily for 1 or 7 days. The group that was given 1 dose of BP-^{3}H received an appropriate amount of vehicle prior to administration of tritiated hydrocarbon. All rats were killed 24 hr after the last dose of BP-^{3}H. The data represent the mean ± S.E. from 4 experiments (tissues from 2 rats were pooled for each experiment).

No. of doses of BP-^{3}H	Concentration of BP-^{3}H in:	
	Dorsal fat (ng/g)	Peritoneal fat (ng/g)
1	249 ± 9	228 ± 10
7	24 ± 5	34 ± 9

for this tissue. Two hr after the i.v. injection of 10 μg of BP-^{3}H, the concentration of BP-^{3}H in the lung was 53 ± 14 ng/g for control rats and 19 ± 9 ng/g for BP-pretreated rats. The disappearance of BP-^{3}H from liver paralleled the disappearance of this hydrocarbon from the blood that was described in Chart 1. These results suggest a rapid equilibrium for BP between blood and liver. The disappearance of BP-^{3}H from brain was slower than from blood or liver, and the concentration of BP-^{3}H in dorsal fat remained high for the duration of the experiment (Chart 2).

The effect of several compounds on the *in vivo* metabolism of BP-^{3}H was investigated (Table 1). MC, DMBA, and BP are potent stimulators of hepatic microsomal hydroxylase activity (2, 6, 7, 16), and treatment of rats with these compounds enhanced the metabolism of i.v. administered BP-^{3}H and decreased the fat concentration of this hydrocarbon (Table 1). However, pretreatment of rats with pyrene or anthracene had little or no stimulatory effect on hepatic microsomal hydroxylase activity (2, 6, 9, 16) or on the *in vivo* metabolism of BP-^{3}H. Pretreatment of rats with phenobarbital, a compound with a small stimulatory effect on hepatic BP hydroxylase activity (1, 5), did not stimulate the *in vivo* metabolism of BP-^{3}H (Table 1).

Effect of Pretreatment with BP on the Tissue Concentration of p.o. Administered BP-^{3}H. The p.o. administration of 10 mg of BP-^{3}H to rats resulted in a high concentration of this hydrocarbon in lung, fat, and adrenal glands 2 hr later. The concentration of tritiated hydrocarbon in these tissues was markedly decreased when the rats were pretreated with 33 mg/kg of nonradioactive BP i.p. once daily for 2 days prior to the p.o. administration of BP-^{3}H (Table 2).

Because we were concerned that nonradioactive BP might influence the distribution or binding of subsequently administered radioactive BP, we measured the tissue concentration of BP-^{3}H after a single dose of *tritiated* BP or after 7 daily doses of *tritiated* BP. Although the concentration of BP-^{3}H in dorsal and peritoneal fat averaged 239 ng/g 24 hr after a single p.o. dose of 1 mg of BP-^{3}H, the concentration of this polycyclic hydrocarbon in fat decreased to 29ng/g 24 hr after the last of 7 daily doses of 1 mg of BP-^{3}H (Table 3). These results suggest that BP stimulates its own metabolism during chronic administration in rats.

DISCUSSION

The stimulatory effect of BP, MC, and DMBA on BP hydroxylase activity in rats (7, 9) is paralleled by enhanced metabolism of BP *in vivo*. Pretreatment of rats with nonradioactive BP for 2 days enhanced the disappearance of BP-^{3}H from blood during the 1st few minutes after its i.v. administration. Pretreatment of rats with nonradioactive BP may have accelerated the disappearance of BP-^{3}H from blood for only a few minutes after its i.v. injection because large amounts of BP-^{3}H were unbound and available for metabolism during the 1st few minutes after i.v. injection; whereas, at the very low concentrations of BP-^{3}H in the blood at later times, most of the BP-^{3}H may be bound to plasma protein and not readily available for metabolism. The rapid disappearance of BP-^{3}H from the blood of control and pretreated animals represents both metabolism and distribution into tissues. The increased rate of disappearance of BP-^{3}H found in pretreated rats represents increased metabolism, since examination of the brain, fat, liver, and lung revealed that less BP-^{3}H was present in the tissues of BP-pretreated animals than in the tissues of control animals, and studies presented in the following paper (24) indicate that pretreatment of rats with nonradioactive BP prior to an i.v. injection of BP-^{14}C enhances the excretion of metabolites of BP-^{14}C into the bile. The rapidity of the excretion of BP metabolites into bile (24) is consistent with the view that the rapid decrease in the blood level of BP found in the present study was due not only to distribution of BP into tissue but also to metabolism. The ability of BP to stimulate its own metabolism *in vivo* was also suggested from the studies described in Table 3. After a single p.o. dose of BP-^{3}H, a high concentration of this hydrocarbon occurred in the fat of rats, but when BP-^{3}H was given each day for 7 days, the concentration of this hydrocarbon in fat was reduced severalfold.

Although treatment of rats with MC, BP, or DMBA enhances the *in vitro* and *in vivo* metabolism of BP, treatment of rats with pyrene or anthracene has little or no effect on liver hydroxylase activity (2, 6, 9, 16), and these compounds do not stimulate the metabolism of BP *in vivo*. It is of considerable interest that, although phenobarbital administration stimulates the activity of hepatic enzymes that metabolize BP (5), this barbiturate does not stimulate the *in vivo* metabolism of BP. An examination of differences between the

enzyme-inducing properties of polycyclic hydrocarbons and phenobarbital revealed that treatment of rats with MC increases the V_{max} and decreases the K_m for the enzymatic hydroxylation of BP by liver; whereas phenobarbital increases the V_{max} to a smaller extent than polycyclic hydrocarbons and has no effect on the K_m (1). The results presented here indicate that the effect of phenobarbital administration on BP hydroxylase activity in the rat is not sufficient to enhance the hydroxylation of BP *in vivo*.

Treatment of rats with stimulators of hepatic DMBA metabolism stimulates the metabolism of DMBA *in vivo*. Pretreatment of rats with MC prior to DMBA-^{3}H administration resulted in a decreased concentration of tritiated hydrocarbon in the adrenal gland, mammary gland, and fat (16), and this effect provides an explanation for the inhibitory effect of MC on the production of adrenal necrosis and mammary cancer by DMBA (12, 14, 33).

Although microsomal enzyme inducers stimulate the *in vitro* formation of noncarcinogenic metabolites (6–8, 27, 31) and inhibit the carcinogenicity of several structurally unrelated chemicals in animals (13, 15, 18–20, 23, 25, 28, 29, 33), further work is needed to evaluate the effects of enzyme induction on chemical carcinogenesis under various circumstances. This is particularly important because enzyme induction can enhance metabolic pathways leading to metabolites that are more carcinogenic than the parent compound (17). In addition, the induction of microsomal enzymes that metabolize BP results in enhanced formation of BP metabolites that bind to DNA *in vitro* (10), and recent studies have suggested that hydroxylated metabolites of BP are more cytotoxic than BP in tissue culture (11). Attempts to examine the levels of carcinogen-metabolizing enzymes in the human population revealed that cigarette smoking markedly stimulates the hydroxylation of BP and the *N*-demethylation of 3-methyl-4-monomethylaminoazobenzene by enzymes in the placenta, a readily obtainable human tissue (22, 32). It was of considerable interest that BP hydroxylase activity in human placenta varied widely in individuals that smoked the same number of cigarettes, and a knowledge of whether the marked variability in the induction of BP hydroxylase activity in cigarette smokers can explain why some people develop cancers when exposed to cigarette smoke whereas other individuals do not would be important.

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