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THE EFFECT OF CARCINOGENIC AND NONCARCINOGENIC COMPOUNDS ON THE *O*-DEALKYLATING ACTIVITY OF THE HEPATIC MICROSOMAL ENZYME SYSTEM OF THE RAT

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Abstract—Pretreatment of young rats with phenobarbital, dichlorodiphenyltrichloroethane (DDT), gammexane, and carcinogenic polycyclic hydrocarbons, stimulates the *O*-dealkylation of 2- and 4-alkoxybiphenyls by rat liver microsomes. Phenobarbital, gammexane, and DDT stimulate the activity 80–178 per cent, the degree of stimulation being independent of the length of the alkyl side chain of the substrate. Imipramine, meprobamate, and butylatedhydr oxytoluene (BHT) have little or no effect at the dose level used. The degree of stimulation by carcinogenic hydrocarbons increases with increase in substrate side-chain length from 0 to 70 per cent for CH_3O– to over 300 per cent for C_4H_9O–. Noncarcinogenic hydrocarbons do not show this inductive pattern and are mostly without significant effect.

PRETREATMENT of animals with a variety of compounds increases the activity of the hepatic microsomal enzyme systems responsible for the metabolism of foreign compounds.[1] The long-acting barbiturates, chlorinated hydrocarbon insecticides, and polycyclic aromatic hydrocarbons are among the most potent of these agents. Previous reports have indicated that the mechanism of induction of polycyclic hydrocarbons differs from that by chlordane and phenobarbital,[2, 3] and later evidence has been presented that chlordane may have effects on the microsomal system slightly different from those of phenobarbital.[4] It has also been shown that carcinogenic and noncarcinogenic polycyclic hydrocarbons can be distinguished by the difference in the pattern of induction of hydroxylating activity which they produce.[5]

In the present work the effect of several drugs, chlorinated hydrocarbon insecticides, and carcinogenic and noncarcinogenic polycyclic hydrocarbons on the ability of liver microsomal preparations to dealkylate 2- and 4-methoxy, 2- and 4-ethoxy, 4-propoxy and 4-butoxy biphenyl has been examined. It was found that, at the dose level used, carcinogenic polycyclic hydrocarbons had a characteristic pattern of induction which differed from that obtained with noncarcinogenic hydrocarbons, drugs, and insecticides.

MATERIALS AND METHODS

Preparation of substrates. 2- and 4-hydroxybiphenyls and 2- and 4-methoxybiphenyls were prepared and purified as previously described.[6] 2-Ethoxybiphenyl

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(m.p. 35°), 4-ethoxybiphenyl (m.p. 74·5°), 4-propoxybiphenyl (m.p. 76·5°), and 4-butoxybiphenyl (m.p. 75°) were prepared from the corresponding phenols by the Williamson synthesis and recrystallized to constant melting point from aqueous ethanol and low-boiling petrol. They were shown to be without free phenols by chromatography in three solvent systems, as previously described.[7]

Pretreatment of animals. Male Wistar albino rats (Porton strain), 100 g each, were pretreated for 3 consecutive days with the test compound at a dose level of 10 mg/kg in 0·2 ml arachis oil, i.p. Control animals received only arachis oil.

Assay of dealkylating activity. The animals were killed 24 hr after the last injection and the 10,000 *g* supernatant of liver homogenate was prepared as previously described.[8]

Incubation mixture. The incubation mixture consisted of 1 ml of the 10,000 *g* supernatant (250 mg liver), 0·25 μmole NADP in 0·1 ml of 1·15% KCl, 1 ml of 0·05 M Tris buffer, pH 8, 6 μmole substrate in 0·05 ml acetone, and 1·15% KCl solution to make the volume up to 3 ml. The mixture was incubated at 37° for 15 min. These conditions were found to be optimal for the system. The reaction was stopped and extraction and fluorimetric estimation of the hydroxybiphenyls produced were carried out by the method previously described.[7] The rate of production of the appropriate hydroxybiphenyl was used as a measure of the dealkylating activity.

Fluorescence measurements. The fluorescence of the alkoxybiphenyls was examined in an Aminco–Bowman spectrophotofluorimeter with 1 cm^2 quartz cuvettes and a 150 W Osram Xenon arc lamp as light source, at a concentration of 0·1 μg/ml in 5% aqueous ethanol. Measurements were made at maximum sensitivity with 1/16th, 1/16th, and 3/16th in. slits in positions 3, 5, and 7 respectively, and an uncalibrated photomultiplier I.P. 28. The data obtained are given in Table 1.

TABLE 1. FLUORESCENCE CHARACTERISTICS OF SOME ALKOXYBIPHENYLS*

Compound	λ_{exc} (mμ)	λ_{fl} (mμ)	Fluorescence intensity
4-Methoxybiphenyl	275	338	100
4-Ethoxybiphenyl	275	338	100
4-Propoxybiphenyl	275	338	113
4-Butoxybiphenyl	275	338	106
2-Methoxybiphenyl	255	348	24
	288		39
2-Ethoxybiphenyl	255	348	24
	288		39

* Compounds were examined at a concentration of 0·1 μg/ml in 5% aqueous ethanol. The wavelengths quoted are uncorrected instrumental values.

† Relative intensities, 4-methoxybiphenyl = 100.

The substrates, though highly fluorescent, do not interfere with the estimation procedure, since there is a large difference between the wavelengths of the alkoxybiphenyls and the anionic form of the corresponding hydroxybiphenyls (2-hydroxybiphenyl λ_{exc} 270, 320 λ_{fl} 415; 4-hydroxybiphenyl λ_{exc} 311 λ_{fl} 401).[6]

RESULTS

The induction of dealkylating activity by nonhydrocarbons is shown in Table 2. Marked induction was found with phenobarbital, but not with imipramine, meprobamate or butylated hydroxytoluene (BHT) at the dose level used in this study. With phenobarbital, stimulation of dealkylating activity toward 2- and 4-methoxy- and 2- and 4-ethoxybiphenyl is at the same level and somewhat more marked than stimulation of activity toward 4-propoxy- and 4-butoxybiphenyl. Pretreatment with dichlorodiphenyltrichloroethane (DDT) and gammexane produces a similar pattern. At this dose level both compounds are potent inducers of the activity toward all six substrates and the degree of stimulation is of the same order for each substrate.

The polycyclic hydrocarbons examined consisted of six compounds known to have carcinogenic activity and eight which are reputedly innocent.[9] The results obtained from the latter group are shown in Table 3. Six of the compounds were without significant activity and two showed moderate stimulation with no consistent pattern. The carcinogens tested (Table 4) were all powerful stimulators and the pattern of stimulation is the same in each case. Demethylation of 2- and 4-methoxybiphenyl is comparatively little affected, whereas marked stimulation of the activities toward the other substrates occurs, the degree of stimulation increasing with substrate chain length.

DISCUSSION

Although the compounds that cause induction of microsomal oxidative metabolism are a diverse group, it is recognized that at least two types of stimulation occur, that by polycyclic hydrocarbons and that by other agents.[2, 3] For the former to induce, they must lie between certain limits of molecular size.[10] In previous work it has been shown that, if the pattern of induction of biphenyl hydroxylation expressed as the ratio of 4-hydroxylation:2-hydroxylation is studied, a distinction can be made not only between polycyclic hydrocarbons and other agents, but also between carcinogenic and noncarcinogenic polycyclic hydrocarbons.[5] In the present work we have again shown that the three groups of compounds—carcinogenic polycyclic hydrocarbons, noncarcinogenic polycyclic hydrocarbons, and nonhydrocarbon inducers—can be differentiated on the basis of their stimulation of microsomal oxidative metabolism. This differentiation is made on the basis of the pattern of alteration of dealkylating activities. This is best seen in the histogram (Fig. 1) from which it is clear that the differentiation could be overlooked if a single activity only were measured. Thus, measurement of demethylation of 2-methoxybiphenyl will not differentiate between noncarcinogenic and carcinogenic hydrocarbons, although the lack of stimulation of this activity is part of the characteristic pattern that distinguishes the latter group from drugs and insecticides. Large and highly significant quantitative differences between the effects of carcinogenic and noncarcinogenic hydrocarbons are found on some activities: e.g. mean increase in dealkylation of 4-butoxybiphenyl after noncarcinogens, 8 per cent (0–68 per cent, 8 compounds); after carcinogens, 488 per cent (328–635 per cent, 6 compounds). These differences are not, however, the main basis of the differentiation proposed here between these two groups. All the carcinogens tested gave a characteristic inductive pattern, the increase in activity toward substrates being in the order 4-C_4H_9O– > 4-C_3H_7O– > 4-C_2H_5O– > 4-CH_3O– and 2-C_2H_5O– > 2-CH_3O–. No noncarcinogenic hydrocarbon or nonhydrocarbon showed this pattern,

TABLE 2. THE EFFECT OF PRETREATMENT WITH NONHYDROCARBONS ON THE DEALKYLATION OF ALKOXYBIPHENYLS BY RAT LIVER*

Pretreatment	Rate of dealkylation of substrate (μmole/g liver/hr)						Percentage stimulation of the rate of dealkylation of substrate					
	I	II	III	IV	V	VI	I	II	III	IV	V	VI
Control†	1·19 ± 0·2	1·24 ± 0·17	0·65 ± 0·04	0·25 ± 0·04	0·93 ± 0·1	1·17 ± 0·21	—	—	—	—	—	—
Phenobarbital	3·12 ± 0·2	3·45 ± 0·17	1·17 ± 0·08	0·47 ± 0·08	2·20 ± 0·1	2·72 ± 0·2	162	178	80	88	136	132
Imipramine‡	1·53 ± 0·19	1·56 ± 0·18	0·74 ± 0·05	0·37 ± 0·06	1·14 ± 0·0	1·14 ± 0·1	28	26	14	48	23	0
Meprobamate‡	1·39 ± 0·2	1·53 ± 0·15	0·66 ± 0·04	0·31 ± 0·04	1·06 ± 0·06	0·95 ± 0·15	17	23	0	24	14	0
DDT	2·56 ± 0·07	2·56 ± 0·09	1·25 ± 0·08	0·55 ± 0·06	2·30 ± 0·1	2·76 ± 0·16	115	106	92	120	147	136
Gammexane	2·62 ± 0·19	2·45 ± 0·18	1·23 ± 0·09	0·64 ± 0·07	1·75 ± 0·08	2·20 ± 0·15	120	98	89	156	88	88
BHT	1·19 ± 0·05	1·34 ± 0·08	0·62 ± 0·01	0·28 ± 0·01	1·00 ± 0·05	1·05 ± 0·01	0	8	0	12	7·5	0

* Groups of six 100-g male rats were pretreated with 10 mg/kg of compound i.p. in 0·2 ml arachis oil daily for 3 days and killed on the fourth day. Mean values are given ± the S.E.M. Substrates: I = 4-methoxybiphenyl, II = 4-ethoxybiphenyl, III = 4-propoxybiphenyl, IV = 4-butoxybiphenyl, V = 2-methoxybiphenyl, VI = 2-ethoxybiphenyl.

† This group contained 40 animals.

‡ These compounds were given in 0·2 ml water.

TABLE 3. THE EFFECT OF PRETREATMENT WITH NONCARCINOGENIC HYDROCARBONS ON THE DEALKYLATION OF ALKOXYBIPHENYLS BY RAT LIVER*

Pretreatment	Rate of dealkylation of substrate (μmole/g liver/hr)†						Percentage stimulation of the rate of dealkylation of substrate†					
	I	II	III	IV	V	VI	I	II	III	IV	V	VI
Control	1·19 ± 0·2	1·24 ± 0·17	0·65 ± 0·04	0·25 ± 0·04	0·93 ± 0·1	1·17 ± 0·21	—	—	—	—	—	—
Phenanthrene	1·23 ± 0·09	1·32 ± 0·08	0·72 ± 0·07	0·42 ± 0·02	1·04 ± 0·02	1·00 ± 0·06	3	6	10	68	12	0
Pentacene	1·19 ± 0·1	1·11 ± 0·04	0·49 ± 0·06	0·23 ± 0·02	1·03 ± 0·1	1·12 ± 0·12	0	0	0	0	11	0
1,2,8,9-Dibenz-pentacene	1·03 ± 0·4	1·16 ± 0·01	0·52 ± 0·01	0·24 ± 0·01	1·08 ± 0·01	1·18 ± 0·12	0	0	0	0	16	0
3,4,5,6-Dibenz-phenanthrene	1·17 ± 0·01	1·30 ± 0·01	0·65 ± 0·01	0·23 ± 0·01	0·99 ± 0·01	1·14 ± 0·01	0	5	0	0	6	0
Anthracene	1·46 ± 0·13	1·62 ± 0·14	1·06 ± 0·07	0·32 ± 0·02	0·98 ± 0·12	1·72 ± 0·06	23	31	63	28	5	47
2-Methylanthracene	0·81 ± 0·15	0·85 ± 0·14	0·45 ± 0·08	0·25 ± 0·05	0·76 ± 0·18	0·83 ± 0·16	0	0	0	32	0	0
9-Methylanthracene	1·03 ± 0·2	1·01 ± 0·16	0·55 ± 0·07	0·33 ± 0·04	0·99 ± 0·17	1·35 ± 0·22	0	0	0	0	6	15
Naphthalene	0·80 ± 0·25	0·88 ± 0·03	0·47 ± 0·1	0·26 ± 0·04	0·80 ± 0·1	1·04 ± 0·14	0	0	0	0	0	0

* Groups of six 100-g male rats were pretreated with 10 mg/kg of compound i.p. in 0·2 ml arachis oil daily for 3 days and killed on the fourth day. Mean values are given ± the S.E.M.

† Substrates are identified by Roman numerals as in Table 2.

TABLE 4. THE EFFECT OF PRETREATMENT WITH CARCINOGENIC HYDROCARBONS ON THE DEALKYLATION OF ALKOXYBIPHENYLS BY RAT LIVER*

Pretreatment	Rate of dealkylation of substrate (μmole/g liver/hr)†						Percentage stimulation of the rate of dealkylation of substrate†					
	I	II	III	IV	V	VI	I	II	III	IV	V	VI
Control	1·19 ± 0·2	1·24 ± 0·17	0·65 ± 0·04	0·25 ± 0·04	0·93 ± 0·1	1·17 ± 0·21	—	—	—	—	—	—
1,2-Benzopyrene	1·80 ± 0·0	3·91 ± 0·25	2·18 ± 0·2	1·07 ± 0·18	0·95 ± 0·06	3·05 ± 0·19	51	215	235	328	2	161
20-Methylcholanthrene	1·68 ± 0·1	3·93 ± 0·4	3·44 ± 0·24	1·84 ± 0·15	1·01 ± 0·09	3·20 ± 0·29	41	217	429	635	9	174
22-Methylcholanthrene	1·35 ± 0·2	2·47 ± 0·19	2·54 ± 0·23	1·22 ± 0·1	1·20 ± 0·14	3·32 ± 0·17	13	99	291	388	29	184
1,2-Benzanthracene	1·80 ± 0·12	3·30 ± 0·29	3·09 ± 0·2	1·58 ± 0·23	1·03 ± 0·05	2·70 ± 0·17	51	166	375	532	11	131
1,2,3,4-Di-benzanthracene	1·77 ± 0·03	3·52 ± 0·13	3·40 ± 0·09	1·60 ± 0·17	1·05 ± 0·05	3·28 ± 0·32	49	184	423	540	13	180
1,2,5,6-Di-benzanthracene	1·99 ± 0·15	3·60 ± 0·4	3·33 ± 0·22	1·52 ± 0·1	1·15 ± 0·06	2·25 ± 0·11	67	190	412	508	24	92

* Groups of six 100-g male rats were pretreated with 10 mg/kg of compound i.p. in 0·2 ml arachis oil daily for 3 days and killed on the fourth day. Mean values are given ± the S.E.M.

† Substrates are identified by Roman numerals as in Table 2.

The difference found between different groups of compounds is not, therefore, merely one of greater or less activity but is an absolute qualitative difference in effect (Fig. 1).

The present work thus provides further evidence that induction of microsomal oxidative metabolism by carcinogens differs from that by noncarcinogens and that, with polycyclic hydrocarbons, carcinogenicity and inductive activity are correlated.

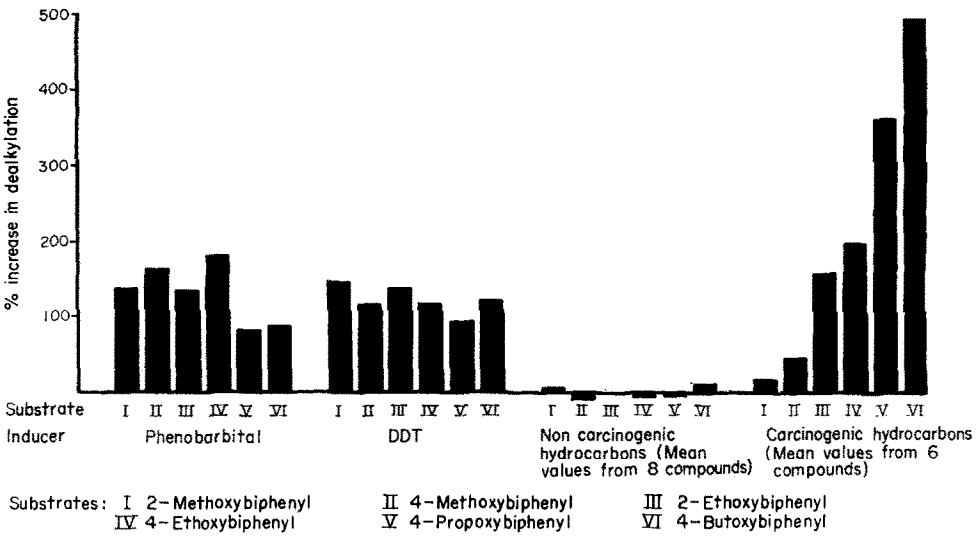

FIG. 1. Comparison of the inductive effect of carcinogenic and noncarcinogenic hydrocarbons and nonhydrocarbon inducers.

However, further work with a larger number of compounds and at a range of dose levels will be necessary to substantiate this hypothesis.

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