Biochemical Pharmacology, Vol. 17, pp. 1377–1383. Pergamon Press. 1968. Printed in Great Britain

EFFECTS OF VARIOUS CHEMICAL AGENTS ON DRUG METABOLISM AND CHOLESTEROL BIOSYNTHESIS*

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(*Received* 16 *November* 1967; *accepted* 29 *January* 1968)

Abstract—The effects of representative inhibitors of drug metabolism, hypocholesterolemic agents and insecticide synergists on microsome-catalyzed drug metabolism and cholesterol biosynthesis were examined in the rat. In the experiments *in vitro*, both biochemical reactions were inhibited to varying degrees by all of the test compounds. Cholesterol biosynthesis appeared to be more sensitive to these compounds than was the drug metabolism system. However, in an experiment *in vivo*, only microsome inhibitors prolonged the sleeping time of mice induced by hexobarbital. The administration of AY-9944 and Compound A at doses sufficient to lower the plasma cholesterol level had no effect on the microsomal enzyme activity.

Two effects on cholesterol metabolism were observed with microsome inducers. Administration of 3,4-benzpyrene resulted in an elevated plasma cholesterol level in the rat. However, the incorporation of ^{14}C-labeled acetate or mevalonic acid into cholesterol by the liver of benzpyrene-treated rats was not higher than that by the liver of the control rats. Phenobarbital did not elevate the plasma cholesterol level, but the liver from phenobarbital-treated rats showed increased ^{14}C-acetate incorporation into cholesterol.

AFTER the early observation that diethylaminoethyl diphenylpropylacetic acid (SKF 525-A) inhibited the metabolism of drugs,[1, 2] a variety of compounds have been found to act as inhibitors of drug metabolism.[3] Similarly, there are many chemical agents that inhibit cholesterol biosynthesis and effectively lower the plasma cholesterol level.[4, 5] Since many steps in drug metabolism[6] and biosynthesis of cholesterol[7] are catalyzed by the hepatic microsomal system and have common requirements in atmospheric oxygen and reduced triphosphopyridine nucleotide (NADPH), some degree of overlap in the actions of these two types of inhibitors is expected.

In the present paper a systematic study was made of the actions of representative microsome inhibitors and hypocholesterolemic agents on drug metabolism and cholesterol biosynthesis. In addition, the effects of insecticide synergists and microsome inducers on these biochemical processes were also examined.

EXPERIMENTAL

Chemicals. The following experimental compounds were provided by the companies indicated in parentheses: AY-9944, *trans*-1,4-*bis*(2-chlorobenzylaminomethyl) cyclohexane dihydrochloride (Ayerst Laboratories, Inc.); benzmalacene, *N*-[1-methyl-2,3-*bis*(*p*-chlorophenyl)propyl]-maleamic acid, MK 135 (Merck Sharp & Dohme);

* This work was supported by Contract No. PH 43-65-1061 with the National Institutes of Health, Public Health Service, Department of Health, Education and Welfare.

chloramphenicol, Compound A, 2[*p*-(2-diethylaminoethoxy)phenyl]benzimidazole hydrochloride and Compound B, 1′-[2-(1-naphthylamino)ethyl]-1,4′-bipiperidine dihydrochloride (Parke, Davis); iproniazid phosphate (Hoffmann-LaRoche); Lilly 18947, 2,4-dichloro-6-phenylphenoxyethyl-*N*,*N*-diethylamine hydrobromide (Eli Lilly); Lilly 32391 (DPEA), the primary amine of Lilly 18947; MGK 264, *N*-octyl bicycloheptene dicarboximide (McLaughlin Gormley King Co.); MGK 28344, an analog of piperonyl butoxide and piperonyl butoxide (McLaughlin Gormley King Co.); SKF 2314, diphenylpropylacetic acid and SKF 525-A, the diethylaminoethanol ester of SKF 2314 (Smith Kline & French).

Assay of drug metabolism in vitro. The method for preparation of the 10,000 *g* supernatant fraction, the incubation conditions and the analytical techniques used for hexobarbital, *o*-nitroanisole, aminopyrine and acetanilide have been described previously.[8] The enzymic hydroxylation of 3,4-benzpyrene was studied by using the method described by Conney *et al.*[9]

Assay of cholesterol biosynthesis in vitro. The incorporation of acetate-1-C^{14} or mevalonic acid-2-C^{14} into cholesterol was studied by using either the 10,000 *g* supernatant fraction of the rat liver homogenate or the partially purified soluble enzyme system fortified with microsomes.[10] Incubations were carried out according to the method of Dalidowicz and McDonald.[11] After the incubation, 5 mg of carrier cholesterol was added to the incubation mixture and cholesterol was isolated as the digitonide complex. The latter was cleaved with dimethyl sulfoxide[12] and cholesterol was purified and counted as described by Kabara *et al.*[13, 14]

Other assays. Protein concentration was determined by the method of Lowry *et al.*[15] The total plasma cholesterol level was assayed by a modified method of Bloor *et al.*[16]

RESULTS

Effects of inhibitors on drug metabolism. The inhibitory effects of three classes of compounds on the drug-metabolizing activities of liver microsomes are shown in Table 1. All compounds showed some degree of inhibition. However, at a concentration of 10^{-4} M the drug metabolism was more extensively inhibited by some of the microsome inhibitors than by the other two classes of compounds.

To assess the potency of some of these inhibitors *in vivo*, the ability of these compounds to prolong hexobarbital-induced sleep in the mouse was examined. As can be seen in Table 2, both of the microsome inhibitors, namely SKF 525-A and DPEA at 15 μmole/kg, prolonged the sleeping time in mice. Representative compounds from the other two classes, the hypocholesterolemic agents and insecticide synergists did not prolong the sleeping time even at doses of 100–200 μmole/kg.

In another experiment, the hypocholesterolemic agents AY-9944 and Compound A were administered orally to rats for 4 days so that a definite decrease in the plasma cholesterol level was observed. However, the drug-metabolizing activity of the liver from these rats was not adversely affected (Table 3). Estradiol benzoate, a compound known to lower the plasma cholesterol level and inhibit drug metabolism, was an effective inhibitor under similar experimental conditions.

Effects of inhibitors and inducers on cholesterol synthesis. The data presented in Table 4 show that the cholesterol synthetic system, the activity of which was measured by the extent of incorporation of mevalonic acid-2-C^{14} into cholesterol, was more sensitive to the actions of various inhibitors than was the drug-metabolizing system.

TABLE 1. EFFECT OF VARIOUS COMPOUNDS ON DRUG METABOLISM*

Inhibitors	Final concn (M)	Hexobarbital (μmoles)	(%)	*o*-Nitroanisole (μmoles)	(%)	Aminopyrine (μmoles)	(%)	Acetanilide (μmoles)	(%)
Microsome inhibitors									
	10^{-3}	0·03	97†	1·43	36†	0·11	80‡	0·64	46‡
SKF 525-A	10^{-4}	0·22	77	1·64	26	0·29	46	0·73	38
	10^{-5}	0·85	10	1·69	24	0·41	22	1·16	2
	10^{-3}	0·36	62†	2·23	0†	017·	62†	0·73	49†
KF 2314	10^{-4}	0·83	11	2·26	0	0·30	33	0·91	37
	10^{-5}	0·93	1	2·29	0	0·32	27	1·36	6
	10^{-3}	0·0	100‡	0·67	76‡	0·05	91‡	0·19	84‡
Lilly 18947	10^{-4}	0·10	91	1·31	53	0·06	90	0·54	54
	10^{-5}	0·16	86	1·99	28	0·21	61	0·79	33
	10^{-3}	0·0	100‡	0·29	90‡	0·02	96‡	0·04	97‡
Lilly 32391	10^{-4}	0·0	100	0·75	73	0·06	88	0·32	73
	10^{-5}	0·27	76	1·43	48	0·17	68	0·70	41
	10^{-3}	0·06	95‡	2·44	12‡	0·26	51‡	0·28	76‡
Iproniazid	10^{-4}	0·35	69	2·74	1	0·45	15	0·61	48
	10^{-5}	0·49	57	2·62	5	0·50	4	1·08	8
	10^{-3}	0·15	84†	2·01	10†	0·17	61†	1·12	23†
Chloramphenicol	10^{-4}	0·85	9	2·35	0	0·35	20	1·65	0
	10^{-5}	0·92	2	2·30	0	0·43	3	1·52	0
Hypocholesterolemic agents									
	10^{-3}	0·97	15‡	2·20	20‡	0·16	69‡	0·19	84‡
Compound A	10^{-4}	1·18	0	2·61	6	0·37	29	0·58	51
	10^{-5}	1·16	0	2·60	6	0·46	12	1·23	1
	10^{-3}	0·15	87‡	0·86	69‡	0·08	85‡	0·16	86‡
Compound B	10^{-4}	0·62	46	1·69	39	0·19	64	0·56	53
	10^{-5}	1·03	10	2·23	19	0·34	36	0·79	33
	10^{-3}	—	—	1·87	16†	0·13	72†	0·58	60†
AY-9944	10^{-4}	0·73	22†	2·14	4	0·29	34	1·24	14
	10^{-5}	0·94	0	2·23	0	0·42	5	1·46	0
	10^{-3}	0·03	97‡	2·85	0‡	0·26	50‡	0·77	35‡
Benzmalecene	10^{-4}	1·05	8	2·81	0	0·43	18	0·92	22
	10^{-5}	1·19	0	2·68	3	0·47	10	1·08	9
Insecticide synergists									
	10^{-3}	0·50	36‡	2·10	5†	0·22	50†	0·72	50†
Piperonyl butoxide	10^{-4}	0·71	21	2·23	0	0·34	23	0·77	47
	10^{-5}	0·96	0	2·46	0	0·35	20	1·73	0
	10^{-3}	0·69	39‡	2·17	21‡	0·37	29‡	0·72	39‡
MGK 264	10^{-4}	1·08	5	2·28	17	0·46	12	1·16	2
	10^{-5}	1·08	5	2·34	15	0·47	9	1·28	0
	10^{-3}	0·73	22†	2·08	6†	0·26	41†	0·88	39†
MGK 28344	10^{-4}	0·91	3	2·16	3	0·27	39	1·13	21
	10^{-5}	0·97	0	2·39	0	0·40	9	1·37	5

* A New Zealand white rabbit (approx. 2 kg) was treated twice daily with 38 mg/kg of phenobarbital for 3 days. On the day after the last phenobarbital treatment, the rabbit was killed and the liver was homogenized in 3 vol. isotonic KCl. The 10,000 *g* supernatant fraction was centrifuged for 30 min at 100,000 *g* to obtain the microsomes. Incubation was carried out with microsomes (16 mg protein) fortified with 0·51 ml of the 100,000 *g* supernatant. The concentrations of the substrates were hexobarbital, 5×10^{-4} M; *o*-nitroanisole, 2×10^{-3} M; aminopyrine, 3×10^{-3} M; and acetanilide, 3×10^{-3} M. The values are given as μmoles of substrate metabolized or product formed per beaker and the per cent inhibition.

† The control values for hexobarbital, *o*-nitroanisole, aminopyrine and acetanilide incubations were 0·94, 2·22, 0·44 and 1·44 μmole respectively.

‡ The control values for hexobarbital, *o*-nitroanisole, aminopyrine and acetanilide incubations were 1·14, 2·76, 0·52 and 1·18 μmole respectively.

TABLE 2. EFFECT OF VARIOUS COMPOUNDS ON SLEEPING TIME OF MICE

Compound*	Dose (μmole/kg)	No. of mice	Sleeping time (min)
Microsomal inhibitors			
None		5	47 ± 20
SKF 525-A	15	5	105 ± 51 $P < 0.05$
Lilly 32391 (DPEA)	15	5	125 ± 54 $P < 0.025$
Hypocholesterolemic agents			
None		4	39 ± 2
AY-9944	50	4	30 ± 9
	100	4	36 ± 24
None		5	36 ± 8
Compound A	50	7	32 ± 6
	100	6	33 ± 10
None		7	46 ± 25
Benzmalecene	50	7	46 ± 10
	100	6	56 ± 13 N.S.
Insecticide synergists			
None		7	39 ± 12
MGK 264	100	6	41 ± 18
	200	7	43 ± 10
None		7	43 ± 12
Piperonyl butoxide	100	7	35 ± 12
	200	7	53 ± 19 N.S.

* Test compounds were given i.p. 20 min before hexobartital (i.p.) injection. The results are the means ± S.D. N.S. = not significant.

TABLE 3. EFFECTS OF SOME COMPOUNDS ON THE PLASMA CHOLESTEROL LEVEL AND ON THE DRUG-METABOLIZING ACTIVITY OF THE LIVER*

Compound administered	Plasma cholesterol level (mg/100 ml)	Hexobarbital	*o*-Nitroanisole	Aminopyrine	Benzpyrene
None	54 ± 9 (11)†	26·2 ± 5·5 (11)	10·8 ± 3·3 (11)	1·6 ± 0·5 (11)	
AY-9944	37 ± 7 (10) $P < 0.001$	26·7 ± 7·8 (9)	9·7 ± 2·6 (10)	1·5 ± 0·7 (10)	
None	59 ± 7 (9)	17·2 ± 8·1 (8)	8·1 ± 1·2 (8)	1·5 ± 0·6 (8)	
Compound A	36 ± 7 (9) $P < 0.001$	13·7 ± 4·7 (10)	9·6 ± 2·7 (10)	1·5 ± 0·7 (10)	
None	59 ± 7 (9)	51·5 ± 7·9 (9)	13·5 ± 1·4 (9)	4·7 ± 1·1 (9)	1·1 ± 0·4 (9)
Estradiol benzoate‡	48 ± 8 (9) $P < 0.01$	24·6 ± 8·4 (9) $P < 0.001$	10·7 ± 1·1 (9) $P < 0.001$	2·1 ± 0·7 (9) $P < 0.001$	0·7 ± 0·4 (8) $P < 0.05$

* Long–Evans male rats weighing 110–120 g were used. AY-9944 (20 μmole or 9·28 mg/kg) or Compound A (20 μmole or 6·91 mg/kg) was given orally once a day for 4 days. Estradiol benzoate (6·6 μmole or 2·5 mg/kg) dispersed in Mazola oil was given i.p. once daily for 4 days. Control rats were given vehicle only. The rats were killed on day 5. The values are given as μmoles of substrate metabolized or product formed per gram of liver protein.

† Figures in parentheses refer to numbers of rats used in each assay.

‡ The extent of incorporation of mevalonic acid-2-C^{14} into cholesterol by the homogenates was 509 ± 166 cpm/mg cholesterol for the control rats and 301 ± 112 cpm/mg cholesterol for the estradiol-treated rats; the difference was significant at the 0·01 level. Homogenates were prepared with 3 ml of 0·1 M potassium phosphate, pH 7·4, instead of the usual 1·15% KCl, per gram of liver.

Even the insecticide synergists, which were relatively inactive against drug metabolism, showed substantial inhibition at 10^{-4} M against cholesterol synthesis. At 10^{-5}M, AY-9944 and Compound A showed greater inhibitory activities than did SKF 525-A.

Since the enzyme systems for cholesterol synthesis are localized in the microsomes, it was of interest to study the effects of microsome inducers on the plasma cholesterol

TABLE 4. EFFECT OF VARIOUS INHIBITORS ON CHOLESTEROL SYNTHESIS*

Experiment No.	Compounds	Concn (M)	Cholesterol-tomatine complex (cpm/mg)	Inhibition per cent
1	SKF 525-A	10^{-4}	75	88·5
	Compound A	10^{-4}	4	99·5
	Compound B	10^{-4}	6	99·0
	AY-9944	10^{-4}	14	98·0
2	SKF 525-A	10^{-5}	537	28·5
	Compound A	10^{-5}	15	98·0
	AY-9944	10^{-5}	20	97·5
3	Compound A	10^{-6}	155	65·0
	AY-9944	10^{-6}	161	63·0
	Benzmalecene	10^{-5}	247	43·0
4	Piperonyl butoxide	10^{-4}	41	88·5
	MGK 264	10^{-4}	24	93·4
	MGK 28344	10^{-4}	20	94·5

* The control values for cpm-mg cholesterol-tomatine complex for experiments 1 to 4 were 648, 750, 435 and 361, respectively, for each enzyme preparation. Incubations were performed in triplicate.

TABLE 5. EFFECTS OF MICROSOME INDUCERS ON THE PLASMA LEVEL OF CHOLESTEROL AND ON THE LIVER MICROSOMAL ENZYME SYSTEM*

Treatment	Plasma cholesterol (mg/100 ml)	*o*-Nitroanisole	Aminopyrine	Acetanilide	Benzpyrene
None	58 ± 8	14·1 ± 3·3	4·7 ± 2·5	14·5 ± 2·4	0·9 ± 0·1
Phenobarbital	53 ± 4	31·7 ± 1·8†	17·9 ± 2·3†	—	3·0 ± 0·2†
Aminopyrine	66 ± 5	20·0 ± 2·8†	—	26·3 ± 5·1†	2·2 ± 1·0†
None	55 ± 6	9·5 ± 2·3	3·9 ± 1·3	11·6 ± 1·5	1·3 ± 0·1
Benzpyrene	70 ± 5†	21·9 ± 3·2†	2·7 ± 0·4	49·6 ± 11·8†	5·8 ± 0·3†
3-Methyl-cholanthrene	56 ± 4	17·4 ± 3·4†	0·9 ± 0·4†	28·6 ± 10·3†	—

* In the first experiment, 4 Sprague–Dawley male rats weighing approximately 200 g were used in each group. Sodium phenobarbital (75 mg/kg) or aminopyrine (125 mg/kg) was given i.p. for 3 days in two equally divided doses. In the second experiment, 5 Sprague–Dawley rats were used in each group. Benzpyrene (20 mg/kg) or 3-methylcholanthrene (50 mg/kg) in Mazola oil was injected i.p. once a day for 3 days. The rats were killed the day after the last drug injection, and the liver was homogenized in 3 vol. of 0·1 M potassium phosphate buffer, pH 7·4, to prepare the postmitochondrial supernatant fraction. The values are given as μmoles of product formed per gram of liver protein.

† Significantly different from the control at $P < 0{\cdot}01$.

level. As can be seen in Table 5, all inducers increased the drug-metabolizing activities of the liver, with the exception of aminopyrine metabolism after 3-methylcholanthrene administration. Under these conditions, only 3,4-benzpyrene elevated the plasma cholesterol level to a significant extent.

Although phenobarbital treatment had no effect on the plasma cholesterol level in the rat, the liver from the rat was reported to show an increased rate of ^{14}C-labeled acetate incorporation into cholesterol.[17, 18] Although we confirmed this finding with phenobarbital, the liver preparation from 3,4-benzpyrene-treated rats did not stimulate the incorporation of either acetate or mevalonic acid into cholesterol (Table 6).

TABLE 6. INCORPORATION OF ^{14}C-ACETATE AND MEVALONIC ACID INTO CHOLESTEROL*

Animal group	Sp. act. of cholesterol (cpm/mg)	
	Acetate-1-C^{14}	Mevalonic acid-2-C^{14}
Control (5)	19 ± 4	6230 ± 1107
Phenobarbital (6)	219 ± 191†	6496 ± 1332
Control (6)	27 ± 10	2722 ± 451
3,4-Benzpyrene (6)	22 ± 6	1884 ± 540†

* Figures in parentheses refer to the number of rats in each group. Phenobarbital was given i.p. at 75 mg/kg/day in two equally divided doses for 3 days. 3,4-Benzpyrene was given i.p. at 20 mg/kg/day for 2 days. The plasma cholesterol level was 68 ± 13 mg/100 ml for the control and 90 ± 19 mg/100 ml for the benzpyrene group ($P < 0.05$). Two ml of the 10,000 *g* supernatant fraction was incubated with acetate-1-C^{14} (20 μc in 1 μmole) or mevalonic acid-2-C^{14} (1 μc in 0·53 μmole). Cholesterol isolated as tomatine complex was counted in a Nuclear-Chicago liquid scintillation system at a counting efficiency of 74·6 per cent.

† Significantly different from the control ($P < 0.05$).

DISCUSSION

The present study shows that both the drug-metabolizing and cholesterol synthetic systems are inhibited *in vitro* to varying extents by microsome inhibitors, hypocholesterolemic agents and insecticide synergists. Since the cholesterol synthetic system is more sensitive to inhibitors than is the drug-metabolizing system, it is expected that agents that inhibit the drug-metabolizing system might also inhibit cholesterol synthesis whereas the reverse may not be true.

In accord with this observation, a dosage of AY-9944 or Compound A sufficient to lower the plasma cholesterol level had no adverse effect on drug metabolism. Dosages of hypocholesterolemic agents considerably higher than those required to lower the plasma cholesterol level did not inhibit hexobarbital metabolism *in vivo*, as evidenced by the inability of these compounds to prolong hexobarbital-induced sleep in mice.

Benzmalacene[19] and AY-9944[20] have been reported to prolong or potentiate the actions of several drugs. These findings are in direct contrast to our present results. One possible explanation for this discrepancy is the more favorable environment in our laboratories for maintaining the body temperature of mice. AY-9944 was reported to lower the body temperature,[20] and as a result the rate of drug metabolism will be decreased.

In regard to the effect of microsome inhibitors on cholesterol biosynthesis, it has been reported that SKF 525-A lowered the plasma cholesterol level.[21] To our knowledge the hypocholesterolemic actions of other inhibitors such as Lilly 18947 and

DPEA have not been examined. Estradiol benzoate, administered to the rat at a high enough dosage (6·6 μmole/kg) to inhibit drug metabolism *in vitro*, lowered the plasma cholesterol level.

Insecticide synergists have been found to inhibit the metabolism of insecticides by insect homogenate preparations.[22, 23] They also inhibit the metabolism of insecticides by the mammalian microsomal system.[24, 25] However, the drug-metabolizing activity of rats and rabbits, in the few cases studied, appears to be considerably more resistant to the inhibitory action of insecticide synergists.[26] Our data are essentially in agreement with these observations.

In the experiments with microsome inducers, phenobarbital had no effect on the plasma cholesterol level in the rat, whereas 3,4-benzpyrene consistently raised the cholesterol level. Both of these compounds stimulated the microsomal drug-metabolizing activity under these experimental conditions. The results on incorporation studies of ^{14}C-labeled acetate or mevalonic acid into cholesterol indicated that phenobarbital stimulated acetate incorporation, but that 3,4-benzpyrene did not stimulate the incorporation either of acetate or of mevalonic acid. A possible explanation for these seemingly anomalous results is that phenobarbital probably stimulates the overall turnover of cholesterol, whereas 3,4-benzpyrene may inhibit the conversion of cholesterol to bile acids. Experiments are in progress to verify these points.

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