Inhibition of Drug Metabolism

IV. Induction of Drug Metabolism by 2-Diethylaminoethyl 2,2-Diphenylvalerate HCl (SKF 525-A) and 2,4-Dichloro-6-phenylphenoxyethyldiethylamine HBr (Lilly 18947) and the Effect of Induction on the Inhibitory Properties of SKF 525-A Type Compounds

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SUMMARY

The chronic administration (5 days) of SKF 525-A and Lilly 18947 to rats induced increased microsomal enzyme activity as measured by the N-dealkylation of these compounds themselves as well as that of ethylmorphine. In rats whose rates of hexobarbital metabolism had been increased as a result of the repeated administration of SKF 525-A, hexobarbital metabolism was still inhibited when a final dose of SKF 525-A was given. Similarly, in phenobarbital treated rats, SKF 525-A, its product of N-dealkylation, 2-ethylaminoethyl 2,2-diphenylvalerate HCl (SKF 8742-A), and its primary amine analog, 2-aminoethyl 2,2-diphenylvalerate HBr (AEDV), were all effective inhibitors of the enhanced rate of hexobarbital metabolism. These results are explained on the basis that the inductive process involves the synthesis of new enzymic protein that is as readily inhibited by SKF 525-A type compounds as that which was present before induction. Even though the rate of metabolism of SKF 525-A is increased as a result of induction it continues to exert its effects through its products of N-dealkylation, which are also excellent inhibitors.

INTRODUCTION

Several investigators (1–3) have observed that SKF 525-A exerts a biphasic effect on barbiturate sleeping time which is characterized by an initial prolonging effect followed by a decrease in sleeping time as the administration of the inhibitor is continued. Kato *et al.* (4) related these changes in sleeping time to changes in the rate of barbiturate metabolism. In an earlier publication these same authors (5) showed that SKF 525-A and other inhibitors had a stimulatory effect on drug metabolism both *in vitro* and *in vivo*. On the other hand, Rogers *et al.* (6) were unable to demonstrate any stimulatory effect of SKF 525-A on the rate of hexobarbital or codeine metabolism.

The stimulatory phase is thought to be due to an enhanced synthesis of the microsomal enzymes responsible for drug biotransformations. At first glance, this would seem to negate the repeated use of SKF 525-A type inhibitors for the prolongation of drug action when drugs are administered chronically. However, SKF 525-A should not be entirely ineffective as an inhibitor even though drug metabolizing activity has been increased because it should still inhibit the newly formed enzyme. In fact,

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Cook and co-workers (7), in their original study of the prolonging effect of SKF 525-A on hexobarbital sleeping time, showed that, when administered daily for 5 days, SKF 525-A was still an effective inhibitor although some "tolerance" to the compound had developed during that period. Even after 30 days of chronic administration, SKF 525-A had not lost its activity entirely. The relationship of prolonged sleeping time to the rate of hexobarbital metabolism was not studied.

SKF 525-A is oxidized by microsomal enzymes to form products of equal or greater inhibitory potency (8, 9). It would seem quite likely that during the stimulatory phase, SKF 525-A would produce not only an increased rate of metabolism of many drugs, but an increase in its own rate of metabolism as well, in which case its metabolic products would serve in turn to inhibit the metabolism of the drug in question.

The current study was designed (a) to determine whether SKF 525-A and Lilly 18947 were capable of inducing microsomal enzymes to increase their own metabolism, and (b) to determine whether an animal whose rate of hexobarbital metabolism had been increased as a result of repeated SKF 525-A or phenobarbital administration would still respond when SKF 525-A and its N-dealkylated metabolites were employed as inhibitors.

METHODS

In vitro studies. The preparation of the microsomal enzymes, the contents of the incubation mixtures, and the methods used for the determination of reaction rates have been described previously (8).

In vivo studies. Treatment of animals. Phenobarbital sodium (40 mg/kg), SKF 525-A,[2] (50 mg/kg), and Lilly 18947[3] (50 mg/kg) were dissolved in 0.9% sodium chloride solution and administered intraperitoneally once daily for 5 days to male Holtzman rats weighing 70–100 g. The concentrations of the solutions were adjusted so that 1 ml was administered per 100 g of body weight. Twenty-four hours after the last injection the livers were removed for the *in vitro* studies, or the rate of hexobarbital metabolism was studied in the intact rat.

Hexobarbital metabolism. Twenty-four hours after the last injection of phenobarbital or saline, the rats received SKF 525-A, 50 mg/kg (130 μmoles/kg), SKF 8742-A, 46 mg/kg (130 μmoles/kg) or AEDV 49 mg/kg (130 μmoles/kg). Forty-five minutes later 82.5 mg (320 μmoles/kg) of hexobarbital sodium was given. All compounds were dissolved in 0.9% sodium chloride solution and given intraperitoneally. The concentrations of the solutions were adjusted so that 1 ml was given per 100 g of body weight. At 30 and 90 min after the injections of hexobarbital, the rats were anesthetized with ether and 1.5 ml of blood was taken from each rat by aortic puncture using heparinized syringes. Hexobarbital blood levels were determined by the method of Cooper and Brodie (10).

Data processing and statistics. The statistical and kinetic analyses have been described previously (8).

RESULTS

Enzyme induction with SKF 525-A, Lilly 18947, and Phenobarbital

From the results presented in Table 1 it may be seen that SKF 525-A and Lilly 18947 induce an increase in their own microsomal N-dealkylation as well as that of ethylmorphine. As an inducing agent, phenobarbital is superior to either of these compounds.

Effect of SKF 525-A on the Rate of Hexobarbital Metabolism by Rats Treated Repeatedly with SKF 525-A

Groups of rats treated for 5 days with SKF 525-A were compared with saline treated rats for their ability to metabolize hexobarbital. As would be expected from the results obtained *in vitro*, SKF 525-A greatly accelerated the rate of disappearance of hexobarbital from the blood (Fig.

[2] Supplied by Smith Kline & French Laboratories, Philadelphia, Pennsylvania.

[3] Supplied by Eli Lilly and Co., Indianapolis, Indiana.

TABLE 1

Stimulatory effect of SKF 525-A, Lilly 18947, and phenobarbital on the N-dealkylation of SKF 525-A, Lilly 18947, and ethylmorphine

	Substrate					
	SKF 525-A[a]		Lilly 18947[a]		Ethylmorphine[b]	
Inducer	K_m(M × 10^5)	V_{max}[c]	K_m(M × 10^5)	V_{max}[c]	K_m(M × 10^4)	V_{max}[d]
None	3.64 ± 0.80 (5)[e]	3.73 ± 0.64	4.22 ± 1.15 (6)	2.37 ± 0.22	3.34 ± 0.54 (5)	5.62 ± 0.21
SKF 525-A	2.51 ± 0.74[ns] (5)	9.28 ± 0.69*	5.51 ± 1.25[ns] (5)	5.73 ± 0.67*	3.55 ± 0.00[ns] (4)	20.0 ± 1.46*
Lilly 18947	5.19 ± 1.97[ns] (4)	14.3 ± 1.63*	5.14 ± 0.69[ns] (6)	9.25 ± 0.64*	4.25 ± 0.68[ns] (4)	17.0 ± 0.62*
Phenobarbital	4.28 ± 0.68[ns] (4)	25.5 ± 3.75*	4.18 ± 0.61[ns] (3)	16.4 ± 2.34*	3.22 ± 0.33[ns] (3)	25.3 ± 0.58*

[a] S = 0.6 to 4 × 10^{-4} M.

[b] S = 0.8 to 2 × 10^{-3} M.

[c] Micromoles of acetaldehyde per gram of liver per hour.

[d] Micromoles of formaldehyde per gram of liver per hour.

[e] Values are given as mean ± standard error of the mean. Numbers in parentheses refer to number of determinations.

[ns] $P > 0.3$ compared to control.

* = $P < 0.001$ compared to control.

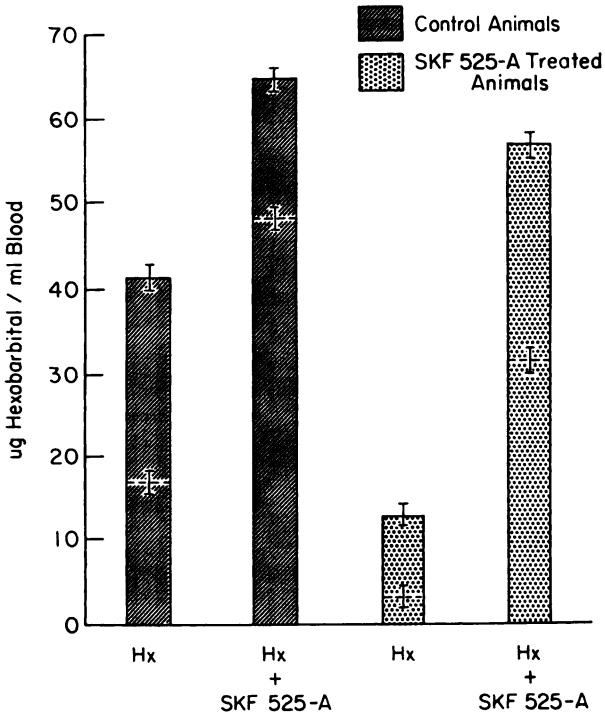

FIG. 1. *Effect of SKF 525-A on the rate of metabolism of hexobarbital (Hx) by SKF 525-A treated rats in vivo*

Treated rats were given 50 mg of SKF 525-A once daily for 5 days. Control rats received saline. A sixth dose of SKF 525-A (50 mg/kg) was given 24 hr after the fifth dose of SKF 525-A or saline. Forty-five minutes later 82.5 mg/kg of hexobarbital sodium was given. All compounds were administered by the intraperitoneal route. Bars represent the mean ± standard error for blood levels measured 30 (———) and 90 (- - - -) minutes after hexobarbital administration. The means are based on the values obtained from at least 5 rats.

1). It can also be seen that SKF 525-A was still a very effective inhibitor of hexobarbital metabolism in these animals. The differences between the corresponding hexobarbital blood levels at 90 min are about equal in the SKF 525-A treated and the control rats, and at 30 min, when these differences are compared, SKF 525-A would appear to be even a more effective inhibitor of hexobarbital metabolism in the SKF 525-A treated rat than in the nontreated rat.

Effect of SKF 525-A, SKF 8742-A, and AEDV on the Rate of Hexobarbital Metabolism by Rats Treated Repeatedly with Phenobarbital

The inhibitory effects of SKF 525-A and its N-dealkylated metabolites on the rate of disappearance of hexobarbital from the blood of untreated rats and rats that had received phenobarbital for five successive days are shown in Fig. 2. The three compounds are seen to be about equally effective as inhibitors of hexobarbital metabolism in both the phenobarbital treated and the control animals.

DISCUSSION

These studies clearly demonstrate that the repeated administration of SKF 525-A type compounds to rats results in increased hepatic microsomal enzyme activity. Not only are the rates of ethylmorphine and hexobarbital metabolism increased, but these inhibitors enhance their own rates of metabolism. SKF 525-A type compounds are still effective prolonging agents in ani-

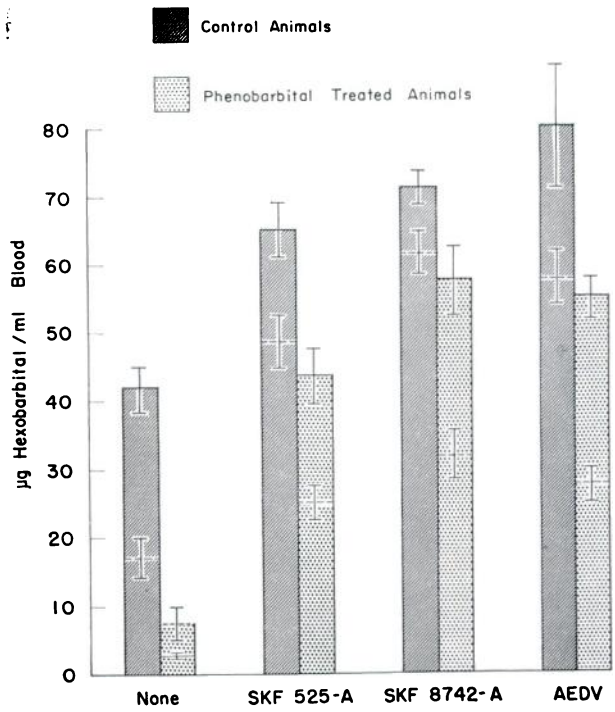

FIG. 2. *Effects of SKF 525-A, SKF 8742-A, and AEDV on the hexobarbital blood levels of phenobarbital treated rats.*

Treated rats were given 40 mg/kg of phenobarbital sodium once daily for 5 days. Control rats received saline. The inhibitors (130 μmoles/kg) were given 24 hr after the last administration of phenobarbital or saline. Forty-five minutes later 82.5 mg/kg of hexobarbital sodium was given. All compounds were administered by the intraperitoneal route. Bars represent the mean ± standard error for hexobarbital blood levels measured 30 minutes (———) and 90 minutes (- - - -) after hexobarbital administration. The means are based on the values obtained from 6 rats.

mals whose rates of drug metabolism have been stimulated by these inhibitors or by phenobarbital because they inhibit the *de novo* microsomal enzymes as well as the enzymes which were present originally. That the metabolism of SKF 525-A is increased as a result of the inductive process does not distract from the effectiveness of this inhibitor because the N-dealkylated products of its metabolism are about as effective as SKF 525-A itself in inhibiting drug metabolism (8, 9, 11). This is not always the case when inhibitors are employed. Kramer and Arrigoni-Martelli (12) showed that while ethyl 2-diethylaminoethyl 2-ethyl-2-butylmalonate HBr (Sch 5712) is an effective inhibitor of hexobarbital metabolism in normal animals, it is not effective as an inhibitor in phenobarbital induced rats, an observation confirmed in this laboratory. It is quite likely that the induction process causes the increased metabolism of Sch 5712 to a compound that is not a good inhibitor and/or one which is excreted rapidly.

The observation that the Michaelis constants for the N-dealkylation of SKF 525-A or Lilly 18947 remained unchanged after induction with either of these two compounds or with phenobarbital is consistent with the view that the inductive process involves a quantitative rather than a qualitative change in enzyme protein. This agrees with Rubin and co-workers (13), who studied the kinetics of the metabolism of ethylmorphine, hexobarbital, and chlorpromazine as influenced by phenobarbital induction, and with Remmer (14), who

found no change in the Michaelis constant for the hydrolysis of procaine after induction. A similar conclusion was drawn by Gillette (15) from his studies of the aminopyrine metabolizing enzyme from normal and phenobarbital induced animals.

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