Inhibition of Drug Metabolism

VI. Inhibition of Hexobarbital Metabolism in the Isolated Perfused Liver of the Rat

R. E. STITZEL,[1] T. R. TEPHLY,[2] AND G. J. MANNERING

Department of Pharmacology, University of Minnesota, Minneapolis, Minnesota 55455

(Received August 11, 1966 and in revised form September 5, 1967)

SUMMARY

A number of structurally unrelated drugs are known to be metabolized by microsomal enzymes systems of such limited specificity that one drug will competitively inhibit the metabolism of another (1). However, of several drugs tested in *in vivo* studies, only codeine and ethylmorphine inhibited hexobarbital metabolism (2). Drugs that inhibited *in vitro*, but not *in vivo*, conceivably failed in the latter case to reach effective concentrations at the metabolic site. The isolated perfused liver was used to circumvent this problem. Ethylmorphine, codeine, morphine, levomethorphan, dextromethorphan, chlorpromazine, and 2-diethylaminoethyl 2,2-diphenylvalerate (SKF 525-A) inhibited hexobarbital metabolism in the perfused liver. Some correlation was found between the inhibitor potency of a drug and the Michaelis constant (K_m) for its own metabolism by hepatic microsomes.

INTRODUCTION

The knowledge that many structurally unrelated drugs may compete for microsomal enzyme systems of the liver (1) led to the testing of several drugs for their possible inhibitory effects on hexobarbital metabolism *in vivo* (2). Ethylmorphine and codeine retarded hexobarbital metabolism, whereas morphine, norcodeine, levomethorphan, dextromethorphan, meprobamate, and acetanilide were without effect. All these drugs are metabolized by hepatic microsomes, and whereas the enzyme systems involved are not thought to be identical in all cases, it is likely that the same enzyme system is responsible for the *N*-dealkylation of ethylmorphine, codeine, morphine, levomethorphan, and dextromethorphan as well as for the side-chain oxidation of hexobarbital (1). If this assumption is correct, and all other things being equal, each of these drugs should have inhibited hexobarbital metabolism *in vivo* to a degree that would relate to its Michaelis constant for the reaction involved in its own metabolism. Of course, all other things are not always equal *in vivo*, and factors that affect the accumulation of the drugs at the site of metabolism will greatly influence the overall effectiveness of the inhibiting drug. This could explain why certain of the drugs tested in the *in vivo* studies were effective inhibitors of hexobarbital metabolism while others were not. In order to circumvent some of the factors that would limit the degree of ac-

[1] This work was performed while the author was a Postdoctoral Fellow of the U.S. Public Health Service. Present address: Department of Pharmacology, West Virginia University, Morgantown, West Virginia.

[2] This work was performed while the author was a Postdoctoral Research Scholar of the American Cancer Society. Present address: Department of Pharmacology, University of Michigan, Ann Arbor, Michigan.

cumulation in *in vivo* studies and yet provide a milieu which is more "physiological" than that which can be achieved with liver fractions, isolated perfused livers were used to determine what effect one drug might have on the rate of metabolism of another drug. Perfused livers permit the use of quantities of drugs that frequently would not be tolerated by whole animals.

METHODS

Isolated rat livers were perfused as described previously (3, 4) with a recirculating perfusate (100 ml of heparinized blood diluted to 150 ml with 0.9% saline) under an atmosphere of 5% CO_2, 95% O_2. Male Sprague-Dawley strain rats (400–500 g) served as liver and blood donors. An equilibrium interval of 30 minutes was allowed between the installation of the liver in the apparatus and the introduction of drugs into the perfusion reservior. The perfusion pressure (18 cm of H_2O) was maintained constant and the hepatic blood flow was restricted to 2–3 ml per gram of liver per minute. Oxygen determinations were performed on arterial and venous perfusate samples drawn simultaneously using a Beckman physiological gas analyzer (Model 160). Varying amounts of ethylmorphine hydrochloride, codeine phosphate, morphine sulfate, dextromethorphan hydrobromide, levomethorphan hydrobromide, chlorpromazine hydrochloride, or 2-diethylaminoethyl 2,2-diphenyl valerate (SKF 525-A) in 1.0 ml of saline solution were added to the perfusate 1 min before the introduction of hexobarbital. Hexobarbital analyses (5) were performed in duplicate on 1.5-ml samples of the perfusion fluid taken 10, 20, 30, 45, 60, and 90 minutes after introduction of the hexobarbital. Perfusion fluid taken before the addition of hexobarbital was used for blank determinations. None of the drugs used as inhibitors interfered with the hexobarbital analysis.

Each liver served as its own control. The rate of disappearance of 96.7 μmoles (25 mg) of hexobarbital sodium from the perfusate in the absence of other drugs was determined for an initial 90-min period. During this time the hexobarbital concentration declines essentially to zero. A volume of perfusion fluid equal to that removed for hexobarbital analyses was added and the inhibitor was introduced. This was followed 1 min later by a second addition of 96.7 μmoles of hexobarbital, and the rate of hexobarbital disappearance was again measured for 90 min. When the rate of hexobarbital disappearance during the first 90-min period was compared with that during the second 90-min period, a statistically significant difference was seen. However, the difference, 0.081 as compared to 0.076 mg of hexobarbital metabolized per gram of liver per minute (Table 1), was not great enough to offset the advantage of allowing each liver to serve as its own control.

RESULTS

The inhibitory effects of ethylmorphine, codeine, morphine, dextromethorphan, levomethorphan, chlorpromazine, and SKF 525-A on the rate of hexobarbital metabolism by the perfused liver are shown in Table 1. SKF 525-A is seen to be the most potent inhibitor and chlorpromazine is more effective than the other drugs. Figure 1 shows the relative inhibitory effects of the morphine and morphinan drugs on hexobarbital disappearance at a 1:1 molar ratio of inhibitor to hexobarbital over 60-min periods of perfusion. Ethylmorphine, codeine, and levomethorphan are seen to be about equally effective as inhibitors, dextromethorphan is somewhat less effective and morphine is the least potent of the compounds tested. The rate of hexobarbital disappearance from the perfusate is not exponentially linear throughout the 60-min perfusion period, but is approximately linear through the times taken for removal of half of the hexobarbital.

In the quantities used to obtain the values shown in Table 1 and Fig. 1, none of the drugs reduced the perfusate flow rate, which had been previously stabilized between 2 and 3 ml per gram of liver per minute. However, when the largest quantities of each of the drugs used in those studies were doubled, dextromethorphan, levomethorphan, chlorpromazine, and SKF

TABLE 1

Inhibitory effects of various drugs on hexobarbital metabolism in the isolated perfused liver of the rat

Drug	Molar ratio of drug to hexobarbital[a]	Number of experiments	Rate of hexobarbital metabolism[b]		Difference[c]	SE of the difference	P
			Without drug	With drug			
None	—	5	0.081	0.076	0.005	0.0016	<0.05
Ethylmorphine	0.4	3	0.089	0.061	0.028**	0.0044	<0.05
	0.8	4	0.085	0.056	0.029**	0.0023	<0.01
	1.6	4	0.080	0.049	0.031*	0.0081	<0.05
Codeine	0.4	3	0.080	0.079	0.001	0.0056	<0.9
	0.8	4	0.084	0.058	0.026*	0.0060	<0.05
	1.6	4	0.078	0.057	0.021**	0.0039	<0.02
Morphine	0.8	5	0.092	0.074	0.018**	0.0027	<0.01
	1.6	4	0.075	0.056	0.019*	0.0051	<0.05
Dextromethorphan	0.4	3	0.096	0.068	0.028**	0.0040	<0.02
	0.8	3	0.078	0.054	0.024**	0.0038	<0.05
Levomethorphan	0.4	3	0.086	0.079	0.007	0.0042	<0.3
	0.8	5	0.079	0.051	0.028**	0.0046	<0.01
Chlorpromazine	0.075	3	0.091	0.072	0.019*	0.0039	<0.02
	0.15	3	0.084	0.065	0.019**	0.0013	<0.01
SKF 525-A	0.033	4	0.078	0.064	0.014	0.0048	<0.1
	0.06	3	0.073	0.047	0.026**	0.0012	<0.01
	0.13	6	0.079	0.039	0.040**	0.0031	<0.01

[a] Initial concentration of hexobarbital in perfusate = 0.167 mg/ml = 0.65 mM.

[b] Mean values (milligrams of hexobarbital metabolized per gram of liver per minute) calculated for the period of time during which the hexobarbital concentration of the perfusate was reduced to one-half of its initial concentration.

[c] Asterisks refer to P values with regard to control difference (0.005); * $P < 0.05$, ** $P < 0.01$.

525-A caused a marked diminution of flow rate. The possibility existed that, although in the amounts used the drugs employed in these experiments did not alter perfusion flow rates, oxygen uptake by the liver might be impaired in some way with a consequent reduction in the rate of hexobarbital oxidation. Using the largest quantities employed previously, each of the drugs was monitored for any change it might produce in the difference between the oxygen tension of arterial and venous perfusates. It was concluded that the drugs did not decrease the oxygen uptake.

DISCUSSION

This study demonstrates that drugs which fail to retard hexobarbital metabolism *in vivo* can be effective inhibitors of hexobarbital biotransformation in the isolated perfused liver. It is not possible to determine from these studies whether or not the observed inhibitions were competitive. However, if they were acting competitively as alternative substrates for the microsomal enzymes responsible for drug metabolism, each drug should inhibit hexobarbital metabolism to a degree that would relate to the Michaelis constant (K_m) for its own metabolism. The following K_m values have been reported for the drugs used in this study (M $\times 10^{-4}$): SKF 525-A, 0.36 (6), chlorpromazine, 1.2 (1), levomethorphan, 1.3,[3] codeine, 2.0 (2), dextromethorphan, 3.5,[3] ethylmorphine, 5.8 (1), and morphine, 6.3.[3] There is a rough correlation between inhibitory effectiveness and K_m value, with SKF 525-A having both the lowest K_m value and the greatest inhibitory potency, and morphine having the lowest inhibitory

[3] Determined in this laboratory using procedures described previously (6).

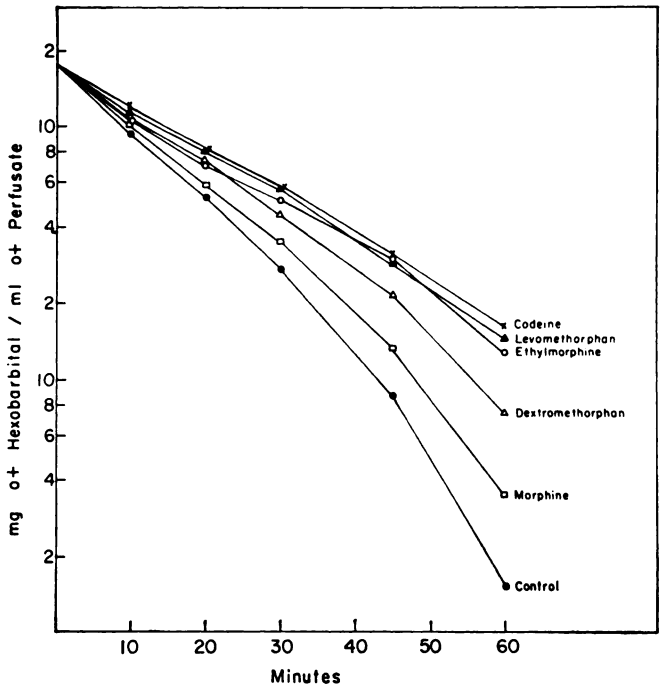

FIG. 1. *Inhibitory effects of several morphine and morphinan drugs on the metabolism of hexobarbital by the isolated, perfused liver.*

Equimolar (97.6 μmoles) amounts of hexobarbital and the narcotic drugs were employed. Control values represent the means of control values obtained from the five studies. The number of experiments employed in each study can be found in Table 1.

potency and the highest K_m value. However, correlation throughout the series is imperfect, as might be expected because of the similarities of K_m values for several of the drugs and the limitation imposed by the method in the determination of small differences in inhibitory potency.

While the assumption remains that these drugs inhibited hexobarbital metabolism by competing as alternative substrates for the microsomal drug-metabolizing system, this study does not provide information as to the site of action. Indeed, the failure of certain of these drugs to inhibit hexobarbital metabolism *in vivo*, while succeeding as inhibitors in the perfused liver, may be due not to a lack of sufficient accumulation at the metabolic site in the intact animal, but to a shift in the rate-limiting component of the drug-metabolizing enzyme system. The isolated perfused liver may give the gross appearance of complete viability without performing optimally at the metabolic level. One example of this is seen in the oxidation of ethanol. Unlike in the intact rat, where the rate of ethanol disappearance is constant as long as saturating levels of ethanol are maintained, in the isolated perfused liver the rate of ethanol oxidation declines rapidly with time. This is most probably due to the inability of the perfused liver to maintain optimal DPN:DPNH ratios (7). In this connection it would be of interest to know whether the perfused liver has the same difficulty maintaining the TPN:TPNH ratio during the metabolism of hexobarbital that it has maintaining the DPN:DPNH ratio during the metabolism of ethanol.

The mean rate of hexobarbital disappearance from the 69 perfusates during the first 90-min period (control perfusions) was 0.082 mg per gram of liver per minute. Anders (8) found the half-life of a dose of 82.5 mg of hexobarbital/kg of rat (80–120 g), as reflected by blood levels of the

drug, to be 35.8 min. Assuming that the disappearance of hexobarbital is due entirely to biotransformation, that hexobarbital is metabolized only in the liver, and that the weight of the liver is about 4% of the body weight, then hexobarbital was metabolized at the rate of about 0.028 mg/g of intact liver per minute. In the *in vivo* studies the extrapolated initial hexobarbital concentration in the blood was 80 μg/ml. In Fig. 1 it may be seen that 11.5 minutes were required for the control livers (mean weight, 12.5 g) to reduce the hexobarbital concentration in the perfusate from 80 to 40 μg/ml. Thus, during this period, when the concentrations of hexobarbital in the perfusate were comparable to those reported by Anders in the blood of intact rats, hexobarbital was metabolized at the rate of about 0.042 mg per gram of perfused liver per minute. This greater rate of hexobarbital metabolism in the perfused liver is somewhat unexpected, particularly since the perfused livers were from very old rats, which might be expected to metabolize hexobarbital somewhat more slowly than the livers of the younger animals used in the *in vivo* studies (9).

ACKNOWLEDGMENTS

This research was supported by USPHS grant no. GM-12543. Part of this material appeared in abstract form (10).

The authors gratefully acknowledge the able technical assistance of Mrs. Shirley Green.

REFERENCES

1. A. Rubin, T. R. Tephly and G. J. Mannering, *Biochem. Pharmacol.* **13**, 1007 (1964).
2. A. Rubin, T. R. Tephly and G. J. Mannering, *Biochem. Pharmacol.* **13**, 1053 (1964).
3. D. R. Van Harken, T. R. Tephly and G. J. Mannering, *J. Pharmacol. Exptl. Therap.* **149**, 36 (1965).
4. R. E. Stitzel, M. W. Anders and G. J. Mannering, *Mol. Pharmacol.* **2**, 335 (1966).
5. J. R. Cooper and B. B. Brodie, *J. Pharmacol. Exptl. Therap.* **114**, 409 (1955).
6. M. W. Anders and G. J. Mannering, *Mol. Pharmacol.* **2**, 319 (1966).
7. D. R. Van Harken, T. R. Tephly and G. J. Mannering, *Pharmacologist* **8**, 220 (1966).
8. M. W. Anders, *Anal. Chem.* **38**, 1945 (1966).
9. R. Kato, E. Chiesara and G. Frontino, *Biochem. Pharmacol.* **11**, 221 (1962).
10. R. E. Stitzel, T. R. Tephly and G. J. Mannering, *Federation Proc.* **24**, 153 (1964).