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INHIBITION OF HEXOBARBITAL METABOLISM BY ETHYLMORPHINE AND CODEINE IN THE INTACT RAT*

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Abstract—The knowledge that chemically unrelated drugs may compete for microsomal enzymes of the liver *in vitro* led to the testing of several drugs for their inhibitory effects on hexobarbital metabolism *in vivo*. Ethylmorphine and codeine were shown to retard the metabolism of hexobarbital *in vivo*, as measured by the rate of hexobarbital disappearance from the blood of rats, whereas morphine, norcodeine, dextromethorphan, levomethorphan, meprobamate, and acetanilide were without effect.

IN THE preceding study, in which hepatic microsomes were employed, it was shown that various drugs competitively inhibited each other's metabolism.[1] Absorption, distribution, excretion, and alternative pathways of drug detoxication may so influence the amount of a given drug reaching the endoplasmic reticulum of the liver cell that many of the drug relationships seen *in vitro* may not have the opportunity to occur in the whole animal. Nevertheless, it seemed probable that in some cases overall conditions would be such that observations made *in vitro* would find some application *in vivo*.

Determination of the rate of disappearance of hexobarbital from the blood has been employed frequently to study factors affecting drug metabolism.[2–4] In the studies to be reported here, several drugs were investigated for their ability to retard the rate of disappearance of hexobarbital from the blood of rats.

METHODS

Sodium hexobarbital was administered intraperitoneally alone or simultaneously with other drugs in 0·9% NaCl solutions to male Holtzman rats weighing 70–100 g. At selected time intervals after injection rats were anesthetized with ether, and 1·5 ml of blood was drawn from the abdominal aorta into heparinized syringes. The hexobarbital content of the blood was determined by the method of Cooper and Brodie.[5] Blood from rats receiving only saline was used to provide control values for the analysis. Before any drug was given in combination with hexobarbital it was administered alone to establish that its presence in the blood, or the presence of one of its metabolites, did not interfere with the barbiturate analysis.

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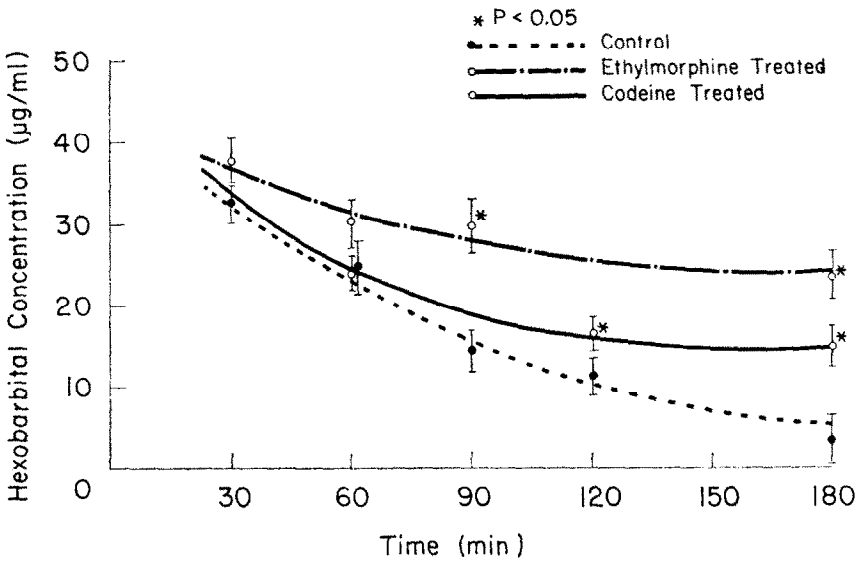

FIG. 1. Inhibition of metabolism of hexobarbital *in vivo* by ethylmorphine (52 µmoles/kg) or codeine (52 µmoles/kg). Hexobarbital was administered at a dose level of 320 µmoles/kg. The values given represent the means (±S.E.) obtained from at least 4 rats.

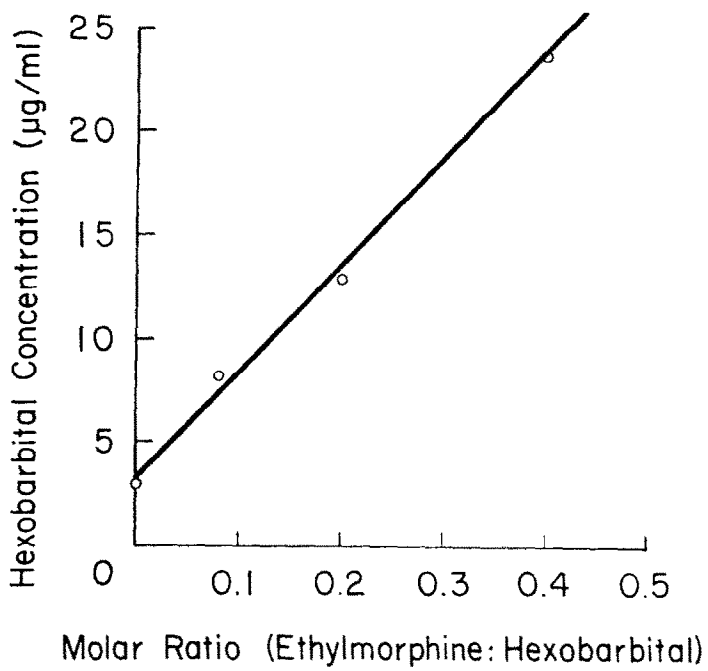

FIG. 2. Effect of ethylmorphine on the blood level of hexobarbital. Blood was drawn 3 hr after drug administration. The values given represent the means obtained from at least 4 rats. Hexobarbital was administered at a dose level of 320 µmoles/kg.

RESULTS

Figure 1 illustrates the retarding effects of ethylmorphine and codeine on the rate of hexobarbital disappearance from the blood of rats. Figure 2 shows the relationship between the dose of ethylmorphine and the hexobarbital blood level.

To exclude the rather unlikely possibility that ethylmorphine might exert its effect by decreasing renal excretion of hexobarbital, the study was repeated in rats that were 'nephrectomized' by bilateral renal vascular occulsion. Rats were anesthetized with ether, their kidneys were exposed by retroperitoneal incisions, and the renal hilus was ligated without enclosing the adrenal glands. The rats were allowed to recover from surgery for 1 hr before drugs were injected. Two hours after drug administration, the mean hexobarbital blood level of four rats receiving hexobarbital alone (320 μmoles/kg) was 13·8 μg/ml; that of four rats receiving the same dose of the barbiturate plus 64 μmoles of ethylmorphine was 20·0 μg/ml. The levels are significantly different ($P < 0{\cdot}02$). Equivalent values in normal rats were 13·0 and 21·5 μg/ml of blood.

Of the variety of drugs tested, only ethylmorphine and codeine exerted a prolonging effect on the blood level of hexobarbital (Table 1). The hexobarbital level observed after codeine administration is significantly different from that seen after ethylmorphine injection ($P < 0{\cdot}05$).

TABLE 1. EFFECTS OF VARIOUS DRUGS ON HEXOBARBITAL BLOOD LEVELS IN THE RAT*

Treatment	Molar ratio (drug/hexobarbital)	Blood level (μg/ml)†
Hexobarbital		10·7 (8)
Hexobarbital plus ethylmorphine	0·08	16·0 (3)
	0·02	21·0‡ (3)
	0·40	25·1‡ (8)§
Hexobarbital plus codeine	0·40	17·0‡ (4)
Hexobarbital plus norcodeine	0·40	12·6 (4)
Hexobarbital plus morphine	0·40	11·0 (6)
Hexobarbital plus dextromethorphan	0·40	13·0 (5)
Hexobarbital plus levomethorphan	0·05	11·4 (4)
Hexobarbital plus meprobamate	0·40	7·2 (4)
Hexobarbital plus acetanilide	0·83	9·8 (3)

* Sodium hexobarbital was administered intraperitoneally at a dose level of 320 μmoles/kg.

† Blood levels of hexobarbital were determined 2 hr after drug administration. Values in parentheses indicate the number of animals employed.

‡ Values significantly different from blood levels of rats receiving hexobarbital alone ($P < 0{\cdot}05$).

§ Value derived from Fig. 1.

The marked central depression caused by the combined administration of hexobarbital and ethylmorphine could conceivably affect barbiturate metabolism indirectly as a consequence of prolonged hypoxia or hypothermia. The signs of central depression observed when morphine was given with hexobarbital were more profound than those seen with either ethylmorphine or codeine. In fact, 30% of these animals died of respiratory arrest during the experiment. Thus the finding that morphine had no effect on the hexobarbital blood level shows that central depression does not account for

the reduced rate of hexobarbital metabolism seen after ethylmorphine or codeine administration.

DISCUSSION

The knowledge that chemically unrelated drugs may compete for microsomal enzymes *in vitro*[1] led to the testing of several drugs for their inhibitory effects on hexobarbital metabolism *in vivo*. Ethylmorphine and codeine were shown to retard the rate of disappearance of hexobarbital from the blood of rats, whereas morphine, norcodeine, dextromethorphan, levomethorphan, meprobamate, and acetanilide were without effect. All these drugs are known to be metabolized by microsomal enzymes. If the presumption is correct that ethylmorphine and codeine are exerting their effects *in vivo* by competing with hexobarbital for a microsomal enzyme which is responsible for the oxidation of all three drugs, then some explanation is needed to account for the ineffectiveness of the other drugs used in this study in retarding hexobarbital disappearance from the blood. On the other hand, so many factors complicate the overall distribution and disposition of drugs *in vivo* that direct correlations between effects seen in the isolated system and those observed in the whole animal frequently cannot be made. For a drug to inhibit the metabolism of another drug at the level of the endoplasmic reticulum of the liver cell several criteria must be met:

1. Absorption and transport of the drug must favor its accumulation in the liver cell.

2. The pharmacologic activity of the inhibiting drug must be such that toxic levels of the drug are not reached in vital organ systems before effective inhibitory concentrations are attained in the liver. In the current study, acute central nervous depression prevented the use of molar ratios of levomethorphan to hexobarbital greater than 0·05.

3. When the inhibiting drug is acting as an alternative substrate, its Michaelis constant (K_m) should not greatly exceed that of the inhibited drug. Inhibitory capacity is increased as the ratio of the K_m of the inhibiting drug to the K_m of the inhibited drug is decreased. The K_m's for the oxidation of ethylmorphine and hexobarbital by the microsomal system are $5{\cdot}8 \times 10^{-4}$ M[1] and $1{\cdot}2 \times 10^{-3}$ M[1] respectively, values that clearly favor the inhibition of hexobarbital by ethylmorphine. The K_m for the demethylation of codeine, as determined in this laboratory, is $2{\cdot}0 \times 10^{-4}$ M.

4. The maximal velocity (Vm) of the inhibiting drug should not greatly exceed that of the drug being inhibited, or its presence at the site of metabolism will not be maintained long enough to produce a substantial effect. Other factors being equal, the inhibitory capacity of a drug is increased as the Vm approaches zero.

5. Alternative routes of metabolism of the inhibiting drug which circumvent the enzyme involved in the metabolism of the inhibited drug should not exist or should play only a minor role in the total metabolism of the drug.

6. The rate of excretion of the inhibiting drug should not greatly exceed that of the inhibited drug, and preferably it should be excreted less rapidly.

It is not known where certain of the drugs used in this study failed to meet these requirements, but in view of the complexity of the problem, it is perhaps more surprising than not that any of the drugs inhibited hexobarbital metabolism. Diethylaminoethyl-2,2-diphenylpentanoate (SKF-525A), the prototype of a growing

list of compounds possessing little pharmacologic action of their own, but which effectively inhibit the metabolism of many other drugs, may well satisfy the above list of criteria. SKF-525A inhibits hexobarbital metabolism *in vitro* and *in vivo*.[3] The K_m for the de-ethylation of SKF-525A by hepatic microsomes from rats ($3{\cdot}2 \times 10^{-5}$ M)* is of a magnitude that would permit SKF-525A to inhibit hexobarbital metabolism as an alternative substrate. Imipramine, a drug known to be N-demethylated,[6] inhibits pentobarbital metabolism *in vivo*.[4] It is perhaps more than coincidental that SKF-525A, imipramine, ethylmorphine, and codeine, all compounds known to undergo metabolic N-dealkylation, also perform as inhibitors of barbiturate oxidation *in vivo*.

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