Inhibition of Drug Metabolism

V. Inhibition of Drug Metabolism by Steroids

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SUMMARY

In a study employing hepatic microsomes from rats, estradiol-17β, testosterone, androsterone, progesterone, and hydrocortisone inhibited competitively the oxidation of ethylmorphine and hexobarbital. Inhibitor constants for each steroid were the same whether ethylmorphine or hexobarbital served as substrates. Results are consistent with the concept that certain drugs and steroids are alternative substrates for a common microsomal mixed function oxidase system. The inhibitory effects of steroids on chlorpromazine metabolism were both qualitatively and quanitatively different from those observed when ethylmorphine and hexobarbital metabolism were studied. Not only were the steroids less potent inhibitors of chlorpromazine oxidation, but inhibition was not competitive.

INTRODUCTION

Conney and Klutch (1) reported that several androgens were metabolized by a TPNH-dependent mixed function oxidase system in hepatic microsomes and Mueller and Rumney (2) described a similar microsomal enzyme system which employed estradiol-17β as a substrate. Kuntzman *et al.* (3) demonstrated a great similarity in the properties of the enzyme system that metabolized steroids in the liver and that which is responsible for the metabolism of many drugs and other compounds. They showed that age, sex, 2-diethylaminoethyl 2,2-diphenylvalerate (SKF 525-A), phenobarbital, chlordane, and 3-methylcholanthrene affect steroid hydroxylase and hexobarbital activities in the same direction and suggested that steroid hormones may be the natural substrates for the oxidative drug-metabolizing enzymes found in hepatic microsomes. If certain steroids and drugs are alternative substrates for a common enzyme system, these steroids should inhibit the metabolism of appropriate drugs in a competitive manner. A study was made of the inhibitory properties of several steroids on the metabolism of ethylmorphine, hexobarbital, and chlorpromazine.

METHODS

Male rats (200–250 g) of the Holtzman strain were used. Microsome plus soluble (9000 *g*) fractions were prepared from their livers as described previously (4). This fraction was diluted so that each milliliter contained microsomes and supernatant from 250 mg of liver. Complete reaction mixtures (5 ml), incubated in open 25-ml Erlenmeyer flasks in a Dubnoff metabolic shaker at 37°, contained the following constituents (mM): nicotinamide (4), magnesium chloride (2.5), TPN (0.04), glucose 6-phosphate (4), various amounts of substrate and steroid and enzyme preparation. When ethylmorphine or chlorpromazine

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was the substrate, 1 ml of enzyme preparation was used; twice this amount was used when hexobarbital was the substrate. Steroids in 95% (w/v) ethanol were added to reaction mixtures in 0.005 or 0.01 ml volumes using Hamilton injector syringes. These quantitites of ethanol did not affect reaction rates. When the *N*-demethylation of ethylmorphine was studied, semicarbazide (15 mM) was added to trap formaldehyde.

The *N*-demethylation of ethylmorphine was measured as described by Takemori and Mannering (5). The methods of Cooper and Brodie (6) and Salzman and Brodie (7) were employed to study the metabolism of hexobarbital and chlorpromazine, respectively. Steroids did not interfere with any of these assay procedures. An incubation time of 15 min was used when ethylmorphine and hexobarbital were the substrates and 12 min when chlorpromazine was the substrate. Data were plotted by the method of Lineweaver and Burk (8) and subjected to the statistical analysis described by Wilkinson (9). Calculations of kinetic constants were performed by a digital computer as described previously (10).

Steroids were purchased from California Corporation for Biochemical Research.

RESULTS

Inhibition of Ethylmorphine and Hexobarbital Metabolism by Steroids

The oxidation of ethylmorphine and hexobarbital by hepatic microsomes was inhibited by estradiol-17β, testosterone, androsterone, progesterone, and hydrocortisone. In all cases the inhibition was competitive. Figures 1 and 2 illustrate the inhibitory effects of androsterone on ethylmorphine and hexobarbital metabolism. Similar curves were obtained with the other steroids. Cholesterol showed no inhibitory effect on the metabolism of either drug. The inhibitor constants (K_i) are

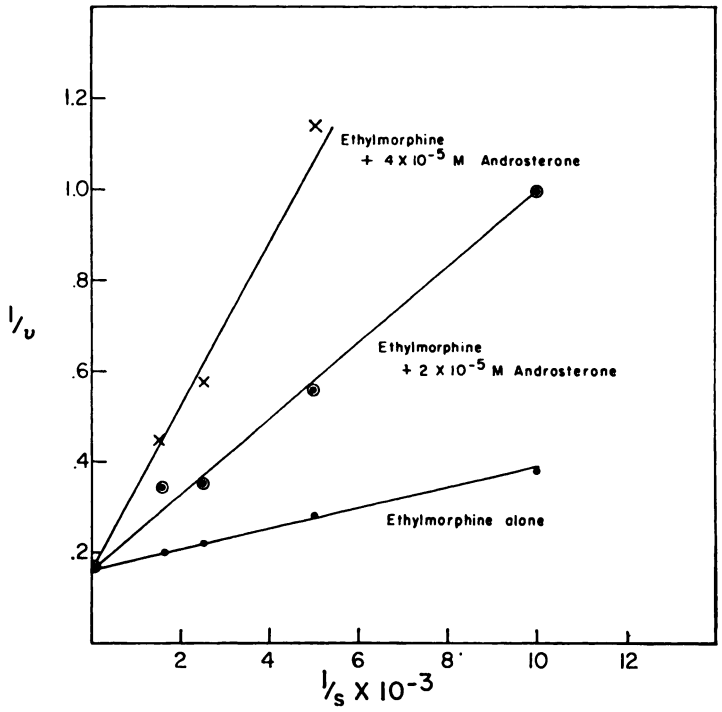

FIG. 1. *Inhibition of the microsomal N-demethylation of ethylmorphine by androsterone*

v = micromoles of formaldehyde formed per gram of liver per hour. The lines were drawn with the aid of the statistical analysis provided by Wilkinson (9).

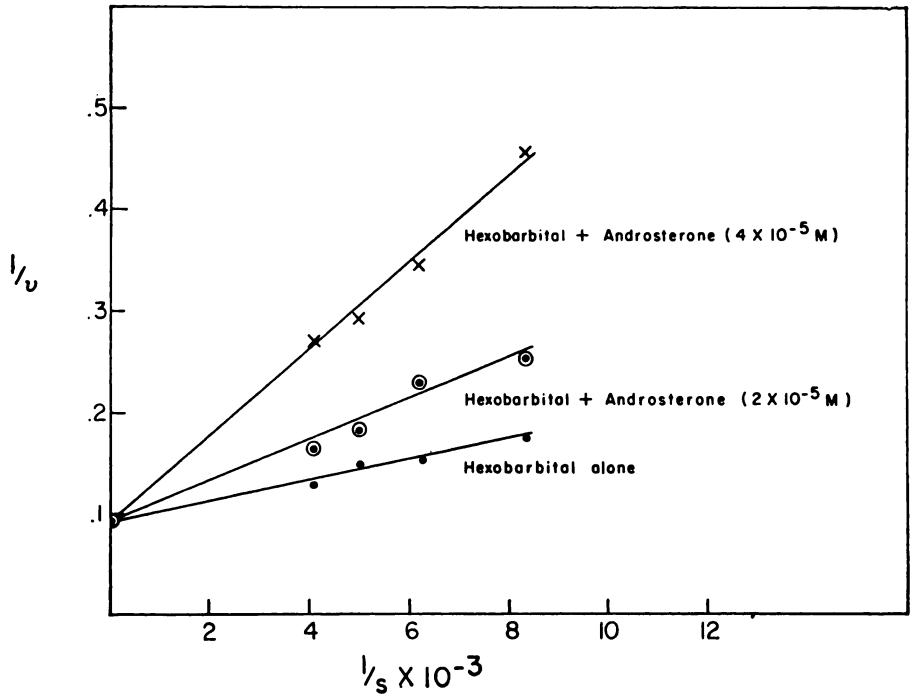

FIG. 2. *Inhibition of the microsomal oxidation of hexobarbital by androsterone*

v = micromoles of hexobarbital disappearing from the medium per gram of liver per hour. The lines were drawn with the aid of the statistical analysis provided by Wilkinson (9).

listed in Table 1. For any given steroid, no significant difference ($P > 0.05$) was found between the K_i, when ethylmorphine was the substrate, and the K_i, when hexobarbital was the substrate. Considered collectively, the correlation coefficient was 0.92 when the inhibitor constants determined for ethylmorphine were compared with the inhibitor constants determined for hexobarbital metabolism.

Effects of Steroids on Chlorpromazine Metabolism

The inhibitory effects of steroids on chlorpromazine oxidation were both qualitatively and quantitatively different from those observed when ethylmorphine and hexobarbital metabolism were studied. Not only were the steroids less potent inhibitors of chlorpromazine oxidation, but inhibition was not competitive. In most cases inhibitions were of a mixed competitive and noncompetitive type and, where inhibitor constants were estimated, they were higher than 10^{-4} M. Androsterone, a potent inhibitor of both ethylmorphine and hexobarbital metabolism, was without effect on chlorpromazine metabolism. In accordance with expectations, cholesterol was also ineffective as an inhibitor when employed in concentrations between 0.04 and 0.1 M.

DISCUSSION

In a previous study from this laboratory (4) where a number of structurally unrelated drugs were shown to inhibit ethylmorphine and hexobarbital metabolism competitively, kinetic data indicated that the metabolism of both drugs was mediated through the action of a single enzyme system. The competitive inhibition of the microsomal oxidation of ethylmorphine and hexobarbital by estradiol-17β, testosterone, androsterone, progesterone, and hydrocortisone, and the good agreement between inhibitor constants for each steroid, whether used as an inhibitor of ethylmorphine or hexobarbital oxidation, suggest that ethyl-

TABLE 1

Inhibition constants (K_i) of steroid inhibitors of the metabolism of ethylmorphine and hexobarbital

Concentrations of steroids (mM): estradiol-17β, testosterone, androsterone and hydrocortisone, 0.02 and 0.04; progesterone, 0.0125 and 0.025; cholesterol, 0.04 and 0.1. Ethylmorphine and hexobarbital concentrations ranged from 0.1 to 1.0 mM and 0.1 and 0.5 mM, respectively. Each value represents the mean of at least three determinations.

Inhibitor	Ethylmorphine *N*-demethylation $K_i \times 10^5$ (M)	Hexobarbital oxidation $K_i \times 10^5$ (M)
Estradiol-17β	4.3	3.7
Testosterone	1.7	1.8
Androsterone	1.0	1.4
Progesterone	0.6	0.6
Hydrocortisone	9.3	21
Cholesterol	No inhibition	No inhibition

morphine, hexobarbital and these steroids all serve as substrates for a common mixed function oxidase system. Further support for the alternative substrate concept of the inhibition of drug metabolism by steroids would be provided if it could be demonstrated that the Michaelis constants (K_m) of the metabolism of the steroids are not different from their inhibitor constants (K_i) when employed as inhibitors of the oxidation of ethylmorphine or hexobarbital. Kuntzman *et al.* (11) recently published kinetic data for the oxidation of a number of steroids by hepatic microsomes. They reported K_m values for estradiol-17β, testosterone, and progesterone of 4.9×10^{-5} M, 2.6×10^{-5} M, and 1.6×10^{-5} M, respectively. These values are in reasonable agreement with the corresponding K_i values for these steroids given in Table 1.

That the inhibition of chlorpromazine metabolism by steroids should differ from that seen with hexobarbital and ethylmorphine metabolism is not entirely unexpected. A previous study (4) showed that although chlorpromazine inhibited the metabolism of ethylmorphine and hexobarbital competitively, kinetic data did not suggest an alternative substrate mechanism of inhibition.

The concept is rapidly developing that the membrane-bound enzymes of the hepatic microsomal drug metabolizing system behave as a complex in which the various proteins involved in the system function together as a unit of integrated enzymic activity, much as has already been established for the electron transfer chain of mitochondria (13). TPNH-cytochrome *c* reductase, a nonheme iron protein, and cytochrome P-450 have been implicated in the microsomal electron transfer chain leading to drug and steroid metabolism (13, 14). The question whether a single enzyme complex capable of producing an active hydroxylating radical is involved in drug metabolism or whether the hydroxylating radical is directed to various drugs through the activity of several hydroxylases of limited specificity is currently a subject that has stimulated considerable speculation. The present study, which shows that steroids inhibit the microsomal drug-metabolizing system competitively, and the previous work from our laboratory (4) which showed that several structurally unrelated drugs competitively inhibit the metabolism of each other, suggest the existence of a common enzyme system of extraordinary nonspecificity. This need not be the case. If, for the sake of argument, one assumes that cytochrome P-450 is coupled to a number of enzymes involved in drug metabolism, and then assumes further that cytochrome P-450 is rate limiting with respect to the performance of these various enzyme systems, then drugs that employ cytochrome P-450 in their metabolism would compete with the metabolism of each other regardless of how many other enzymes are involved. This may very well be the case. There is considerable evidence that the enzyme system that oxidizes ethylmorphine is not identical with that which oxidizes acetanilide and zoxazolamine, yet acetanilide and zoxazolamine inhibit the *N*-demethylation of ethylmorphine competitively (4). This is compatible with the view that two different enzyme systems may share a common component. The possibility must therefore be considered that the enzyme systems responsible for ethyl-

morphine and hexobarbital metabolism may not be identical with each other or with the enzyme system employed in the oxidation of steroids, but that a rate-limiting component common to more than one enzyme system is implicated.

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REFERENCES

1. A. H. Conney and A. Klutch, *J. Biol. Chem.* **238**, 1611 (1963).
2. G. C. Mueller and G. Rumney, *J. Am. Chem. Soc.* **79**, 1004 (1957).
3. R. Kuntzman, M. Jacobson, K. Schneidman and A. H. Conney, *J. Pharmacol. Exptl. Therap.* **146**, 280 (1964).
4. A. Rubin, T. R. Tephly and G. J. Mannering, *Biochem. Pharmacol.* **13**, 1007 (1964).
5. A. E. Takemori and G. J. Mannering, *J. Pharmacol. Exptl. Therap.* **123**, 171 (1958).
6. J. R. Cooper and B. B. Brodie, *J. Pharmacol. Exptl. Therap.* **114**, 409 (1955).
7. N. Salzman and B. B. Brodie, *J. Pharmacol. Exptl. Therap.* **118**, 46 (1956).
8. H. Lineweaver and D. Burk, *J. Am. Chem. Soc.* **56**, 658 (1934).
9. G. N. Wilkinson, *Biochem. J.* **80**, 324 (1961).
10. M. W. Anders and G. J. Mannering, *Mol. Pharmacol.* **2**, 319 (1966).
11. R. Kuntzman, D. Lawrence and A. H. Conney, *Mol. Pharmacol.* **1**, 163 (1965).
12. T. R. Tephly and G. J. Mannering, *Pharmacologist* **6**, 186 (1964).
13. D. H. Maclennan, A. Tzagoloff and D. G. McConnell, *Biochim. Biophys. Acta* **131**, 59 (1967).
14. T. Omura, R. Sato, D. Y. Cooper, O. Rosenthal and R. W. Estabrook, *Federation Proc.* **24**, 1181 (1965).