Michaelis Constants for the Hydroxylation of Steroid Hormones and Drugs by Rat Liver Microsomes

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SUMMARY

The Michaelis constants (K_m values) for the oxidative metabolism of progesterone, testosterone, and estradiol-17β by liver microsomes from adult male rats were determined. These values ranged from 1.6×10^{-5} M to 4.9×10^{-5} M. Previous studies have suggested that oxidative drug-metabolizing enzymes have steroids as normal body substrates. This concept is consistent with the results presented here indicating that the K_m values for the oxidative metabolism of steroid hormones are about 10 times lower than the K_m values for the metabolism of several drugs.

INTRODUCTION

Enzymes in liver microsomes oxidatively metabolize drugs in the presence of TPNH to compounds which are more polar than the substrate. Factors such as sex, age, species, and the administration of foreign compounds have been shown to alter *in vitro* drug metabolism (1, 2). We have recently shown that these same factors also affect the oxidative metabolism of naturally occurring steroids to polar hydroxylated compounds and have suggested that steroid hormones are normal substrates for drug-metabolizing enzymes in liver microsomes (3). Further support for this concept comes from experiments presented here which show that the Michaelis constants (K_m values) for the oxidative metabolism of steroids by liver microsomes are lower than the K_m values for the metabolism of several drugs.

METHODS

Liver microsomes from adult male Sprague-Dawley rats were incubated in air with various amounts of ^{14}C-labeled steroid and a TPNH generating system as previously described (3, 4). After the incubation, substrate and metabolites were extracted into dichloromethane and were chromatographed as described in Fig. 1. Liver microsomes from adult male rats were used in this study because they contain high levels of enzyme systems which metabolize steroids to hydroxylated derivatives (3, 5–7). This contrasts with liver microsomes from adult female rats which metabolize Δ^4-3-ketosteroids predominantly via ring A reduction (5–9).

The amount of metabolism to oxidized compounds more polar than the substrate, and substrate disappearance, were determined as previously described (3, 4). These values were identical since metabolites less polar than the substrate were not formed by liver microsomes from adult male rats. The polar metabolites formed under these conditions were not formed when incubations were done under nitrogen and have been shown to be a mixture of hydroxylated derivatives such as 2-, 6-, 7-, and 16-hydroxylated steroids (4, 7, 10–12). The metabolism of testosterone to 6β-, 7α-, and 16α-hydroxytestosterone was measured as previously described (13).

The Michaelis constant and maximum

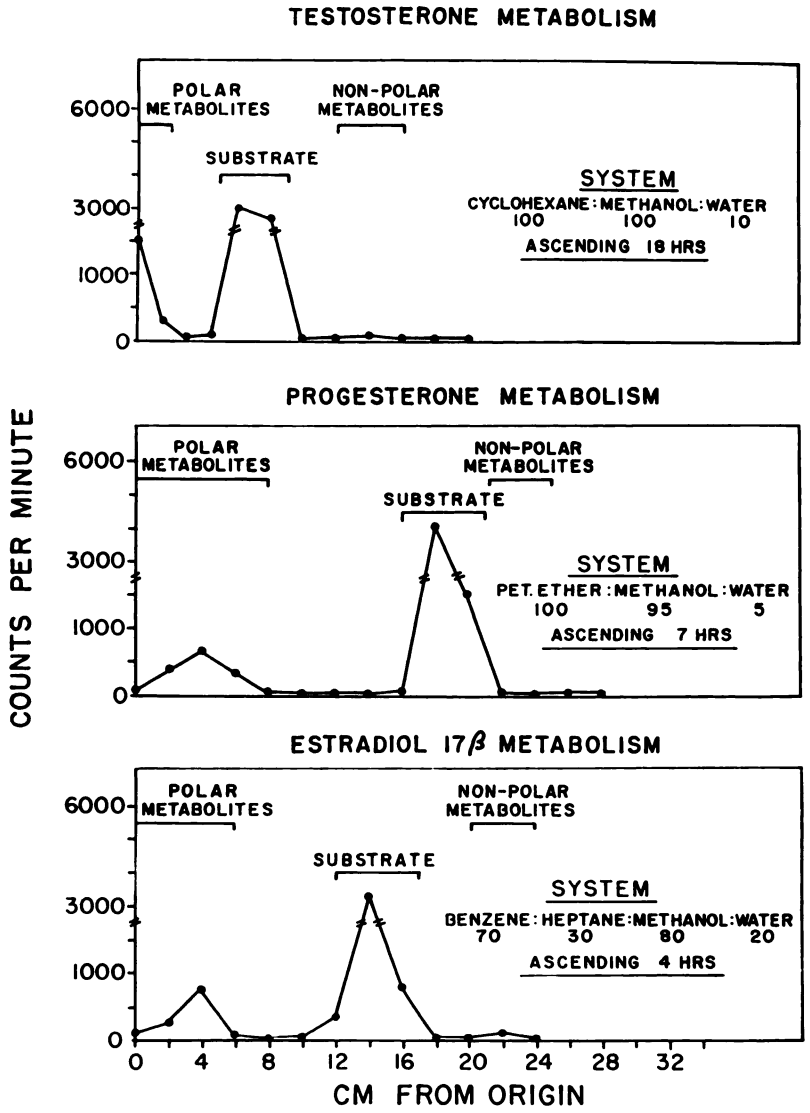

FIG. 1. Hepatic metabolism of testosterone, progesterone, and estradiol-17β to polar metabolites. Microsomes from 165 mg of adult male rat liver were incubated aerobically with 700 mμmoles (0.1 μC) of testosterone 4-^{14}C, progesterone 4-^{14}C, or estradiol-17β-4-^{14}C for 7.5, 7.5, and 15 min, respectively, at 37° in the presence of a TPNH-generating system as previously described (4). After the incubation, a 5 ml aliquot of the incubation mixture (5.7 ml) was taken and extracted with 30 ml of dichloromethane. The organic solvent was evaporated under nitrogen and the residue was dissolved in 1.0 ml of methanol. An exact aliquot (0.1 ml) was applied to Whatman no. 1 filter paper and chromatographed as indicated in the figure. Each chromatogram was cut into 2 cm sections, and each section was counted in a liquid scintillation spectrometer. Reference samples of monohydroxylated substrate had the same mobility as the polar metabolite fraction.

velocity (V_{max}) for the oxidative metabolism of the various steroids by liver microsomes were determined as previously described (14). It would have been desirable to use a more purified steroid hydroxylating system from liver microsomes, but unfortunately, these oxidative enzymes which metabolize steroids and drugs have resisted

all attempts at solubilization and purification. Therefore, the K_m values presented in this paper represent maximum values which may be influenced by nonspecific binding (2) and by the transport of the steroid to the enzyme.

RESULTS

Figures 2A, 2B, and 2C show the determination of the Michaelis constants for the formation of polar metabolites from estradiol-17β, progesterone, and testosterone, respectively. The rate of reaction is expressed in terms of micrograms of polar metabolites formed. When the reaction rate was expressed as micrograms of steroid substrate which disappeared during the incubation, the K_m values were not significantly different. As can be seen, the K_m values for the three steroids studied ranged from 1.6 $\times 10^{-5}$ M to 4.9 $\times 10^{-5}$ M. The lowest value found was for progesterone which is the most lipoid-soluble of the three steroids studied. The highest value found was for estradiol-17β which is the least lipoid-soluble of the steroids studied. The V_{max} values for the oxidative metabolism of progesterone, testosterone, and estradiol were 21, 24, and 12 mμmoles/min, respectively, when liver microsomes from 165 mg

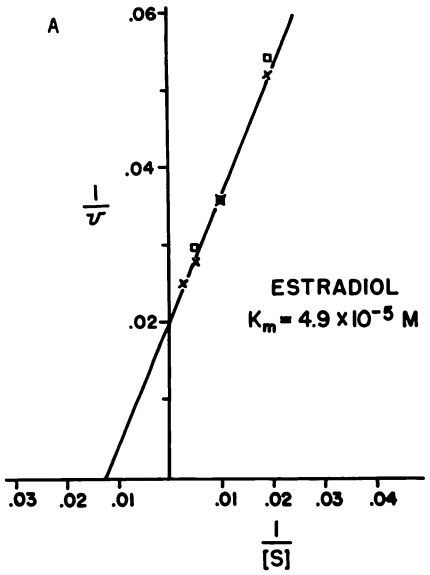

FIG. 2A

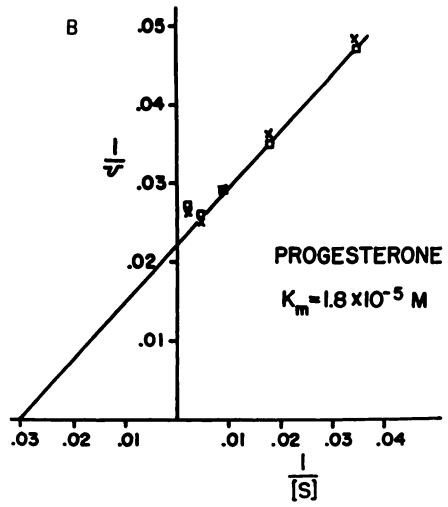

FIG. 2B

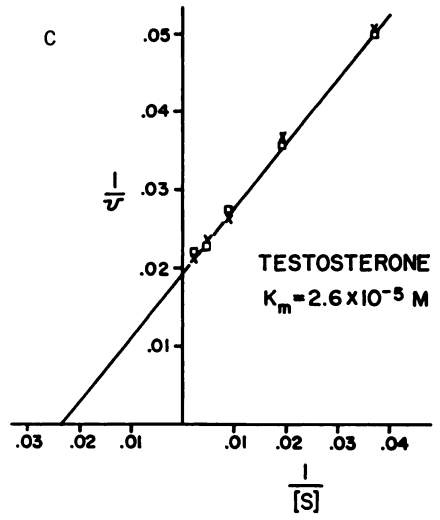

FIG. 2C

FIG. 2. $1/v$ *versus* $1/S$ diagram of the oxidation of estradiol-17β (Fig. 2A), progesterone (Fig. 2B), and testosterone (Fig. 2C) by liver microsomes from adult male rats. v = μg of polar metabolites formed in 15, 7.5, and 7.5 min from estradiol-17β, progesterone, and testosterone, respectively. S = μg of substrate/5.7 ml incubation mixture. Estradiol-17β: 100 μg/5.7 ml is equal to 6.5 $\times 10^{-5}$ M. Progesterone: 100 μg/5.7 ml is equal to 5.6 $\times 10^{-5}$ M. Testosterone: 100 μg/5.7 ml is equal to 6.1 $\times 10^{-5}$ M. Each series of points represents a separate experiment.

TABLE 1

Michaelis constants for the metabolism of drugs and steroids by rat liver microsomal enzymes

Liver microsomes from adult male rats were incubated with various amounts of ^{14}C steroids and a TPNH-generating system as described in Fig. 1. Lineweaver-Burk plots were made of the data (Fig. 2) and the K_m values were then obtained from these graphs. The K_m values for chlorpromazine, *p*-nitroanisole, ethyl morphine, and hexobarbital have been described elsewhere as indicated by the references in parentheses. The K_m value for pentobarbital was determined by Kuntzman *et al.* (unpublished observations).

Substrate	K_m
Progesterone	1.6×10^{-5}
Testosterone	2.6×10^{-5}
Estradiol-17β	4.9×10^{-5}
Chlorpromazine (23)	1.2×10^{-4}
Pentobarbital	2.2×10^{-4}
p-Nitroanisole (24)	2.3×10^{-4}
Ethyl morphine (23)	5.8×10^{-4}
Hexobarbital (23)	1.2×10^{-3}

of tissue were used. Table 1 compares the K_m values obtained here for steroid oxidation with the K_m values obtained elsewhere for the oxidation of several drugs by liver microsomal enzyme systems. Table 1 shows that the K_m values for the oxidative metabolism of steroids are about 10 times lower than the K_m values for several drug oxidations.

Michaelis constants described here for the hydroxylation of steroids are average values since, as mentioned in the Methods section, more than one hydroxylated product is formed, and recent studies with testosterone indicate that more than one enzyme system is required for the 6β-, 7α-, and 16α-hydroxylation of testosterone (6). When the Michaelis constants for the formation of these individual hydroxylated metabolites of testosterone were determined, they were found to be of the same order of magnitude as that found for the total testosterone metabolism to polar metabolites.

DISCUSSION

The K_m values for the hydroxylation of progesterone, testosterone, and estradiol are about 10 times lower than the K_m values for several drug oxidations, thereby suggesting that the steroid hormones are better than drugs as substrates for the TPNH-dependent oxidative enzymes. It is of interest that the K_m values reported for the reduction of the A ring of Δ^4-3-ketosteroids by enzymes in liver microsomes are in the same range as those reported here for the steroid hydroxylases (15–17). Since the physiological importance of ring A reductases for the *in vivo* metabolism of steroids is well established, the low K_m values observed for the steroid hydroxylases suggest that these enzymes can also play a physiological role in the metabolism of steroids in the body.

Evidence that steroid hydroxylases in liver microsomes are important for the *in vivo* oxidation of steroids comes from the following studies which indicate that factors which influence the *in vitro* activity of hydroxylating enzymes in liver microsomes also influence the *in vivo* hydroxylation of steroids: (a) Treatment of rats with the phenyldiallylacetic acid ester of diethylaminoethanol (CFT 1201), a potent inhibitor of TPNH-dependent oxidative enzymes in liver microsomes (18), resulted in a decreased *in vivo* 6β-hydroxylation of corticosterone (19); (b) Treatment of rats with phenobarbital, a potent stimulator of enzymes in liver microsomes that hydroxylate Δ^4-androstene-3,17-dione, resulted in an increased urinary excretion of polar metabolites of this steroid following a dose of Δ^4-androstene-3,17-dione-4-^{14}C (Kuntzman *et al.*, in preparation); (c) Treatment of guinea pigs with phenobarbital or diphenylhydantoin markedly stimulated the activity of cortisol-6β hydroxylase in liver microsomes (20), and this effect was correlated with enhanced urinary excretion of 6-hydroxycortisol in human subjects treated with phenobarbital or diphenylhydantoin (21, 22). Although it appears from these results that steroid hydroxylases in liver microsomes have importance *in vivo*, the physiological importance of altering the activity of these enzymes must await further investigation.

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