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EFFECTS OF SYNTHETIC PROGESTAGENS ON DRUG METABOLISM IN RAT LIVER MICROSOMES*†

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Abstract—The inhibitory action of four progestagens on oxidative drug metabolism was studied. *p*-Nitroanisole demethylation and aniline hydroxylation in phenobarbital stimulated rat liver microsomes were inhibited by norethisterone acetate, *d*-norgestrel, lynestrenol and allylestrenol. Inhibitor constants were between 19 μM and 49 μM for *p*-nitroanisole demethylation and between 45 μM and 112 μM for aniline hydroxylation. The four steroids tested exhibited type I difference spectra in microsomes under aerobic conditions. The spectral dissociation constants were in the same order of magnitude as the inhibitor constants.

STEROID inactivation in liver endoplasmic reticulum depends on the same enzyme system as that responsible for the oxidation of foreign compounds.[1] Interactions between steroid and drug metabolism are therefore likely to occur, and observations have been published on both interference of drugs with steroid inactivation[2,3] and interference of steroids with drug oxidation.[4–6]

There are numerous reports on the influence of female sex hormones on the drug-metabolizing capacity of liver microsomes. It has been suggested that physiological estrogens[7] and progesterone have a regulatory function in drug metabolism. Progesterone has been widely investigated as an inhibitor[4,8,9] and inducer[9,10] of drug oxidation.

Since many women are now using contraceptive steroids for long periods of time, increasing interest is directed to the effects of these synthetic estrogenic and gestagenic compounds on drug elimination. Few observations have been published on drug metabolism in women taking hormonal contraceptives. They suggest that the drug-oxidizing capacity of the liver may be impaired under such conditions.[11,12] In animal experiments mestranol and ethinylestradiol have been shown to inhibit a number of drug-metabolizing processes.[13] Lynestrenol[14] and medroxyprogesterone acetate[10] are able to induce microsomal drug-metabolizing enzymes, and norethynodrel has been found to induce[9,10] and to inhibit[9] drug oxidation in the liver. This paper presents evidence that norethisterone acetate, *d*-norgestrel, and lynestrenol, all of which are typical gestagenic components of hormonal contraceptives, and the progestagen allylestrenol inhibit the oxidative metabolism of *p*-nitroanisole and aniline in rat liver microsomes at concentrations between 5×10^{-5} M and 5×10^{-4} M.

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MATERIALS AND METHODS

Liver microsomes were prepared from male Sprague-Dawley rats weighing approximately 150 g. The animals were injected intraperitoneally with 80 mg sodium phenobarbital/kg once daily for 3 days and were sacrificed 24 hr after the last injection of phenobarbital and 12 hr after food had been withdrawn. The livers were perfused *in situ* with ice-cold saline to remove blood, excised and homogenized in 2 volumes of 0·25 M sucrose solution containing 0·02 M Tris–HCl buffer pH 7·4 and 0·005 M EDTA.[15] Nuclear fragments and mitochondria were sedimented by centrifugation of the homogenates at 1600 *g* for 15 min and at 9000 *g* for 30 min. The microsomal pellet was obtained by centrifugation of the supernatant at 105,000 *g* for 1 hr and was washed once. Protein was determined according to Lowry *et al.*[16] using Dade clinical sera as references.

Spectrophotometric measurements were made with an Aminco–Chance split beam spectrophotometer at 30° using microsomal suspensions (1 mg protein/ml) in 0·067 M phosphate buffer pH 7·4. These suspensions were confirmed to be essentially hemoglobin-free by recording difference spectra after saturation of the sample with carbon monoxide. Difference spectra for the steroids were induced by adding aliquots of steroid dissolved in 2–20 μl dimethylformamide, the reference cuvette receiving solvent alone. This solvent alone did not induce a binding spectrum. The apparent spectral dissociation constants (as defined by Schenkman *et al.*[17]) were derived from Lineweaver–Burk plots[18] of the trough-to-peak difference in absorption against steroid concentration.

Incubation mixtures for the determination of drug-metabolizing activity contained 0·9 μmole NADP, 15 μmoles glucose-6-phosphate, 1·4 units glucose-6-phosphate dehydrogenase, and 2·5 mg microsomal protein in 2·5 ml 0·067 M phosphate buffer pH 7·8. Incubations were carried out at 30°. The reactions were started by the addition of substrates and stopped with 20% (w/v) trichloroacetic acid after 2 min (*p*-nitroanisole demethylation) or 20 min (aniline hydroxylation). *p*-Nitrophenol was determined at 405 nm after adding excess alkali.[19] *p*-Aminophenol formation was measured by the indophenol method of Brodie and Axelrod[20] omitting the ether extraction.[21]

In inhibition experiments the steroids, dissolved in dimethylformamide, were introduced into the test tubes and the solvent was evaporated before the incubation mixture was added. Inhibitor constants were determined by plotting the reciprocal reaction rate against the inhibitor concentration (Dixon plot).[22]

NADP, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were obtained from Boehringer Mannheim. Lynestrenol and allylestrenol were kindly donated by Organon, München; norethisterone acetate and *d*-norgestrel were kindly donated by Schering, Berlin.

RESULTS

p-Nitroanisole demethylation and aniline hydroxylation in phenobarbital-stimulated liver microsomes were inhibited by norethisterone acetate, *d*-norgestrel, lynestrenol, and allylestrenol. The results are shown in Figs. 1 and 2. Fifty per cent inhibition of *p*-nitrophenol production from *p*-nitroanisole was obtained with about 5×10^{-5} M progestagen. To obtain 50 per cent inhibition of aniline metabolism at a substrate concentration of $2{\cdot}5 \times 10^{-4}$ M, inhibitor concentrations of 10^{-4} M (*d*-

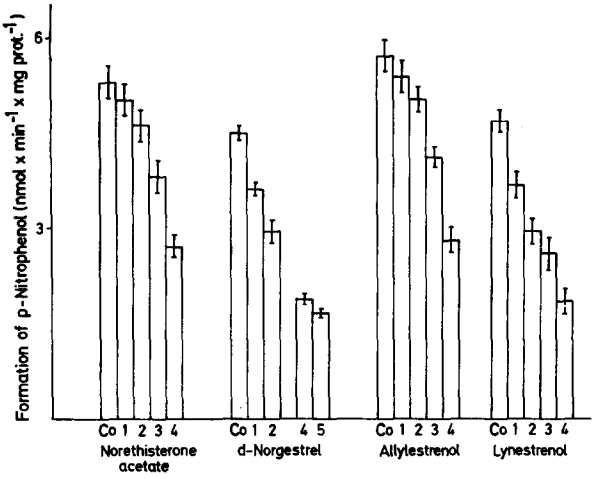

FIG. 1. Inhibition of *p*-nitroanisole demethylation by synthetic progestagens. The ordinates show the amount of *p*-nitrophenol formed per minute in an incubation mixture containing 1 mg protein/ml, a NADPH generating system and 50 μM *p*-nitroanisole. Control incubations (Co) without gestagen; inhibitor concentrations: $1:5\times10^{-6}$ M, $2:10^{-5}$ M, $3:2\times10^{-5}$ M, $4:5\times10^{-5}$ M, $5:10^{-4}$ M. Values are means $\pm$ S.E.M. (n = 4–7). Significance was determined by Student's *t* test. At an inhibitor concentration of 5×10^{-5} M, the differences between control activity and inhibited activity were highly significant for all gestagens ($P < 0.001$).

norgestrel and allylestrenol) and 5×10^{-4} M (norethisterone acetate and lynestrenol) were required.

Inhibitor constants were determined from Dixon diagrams; for both reactions linear plots were only obtained with low inhibitor concentrations possibly because the solubility of the inhibitors within the microsomal membranes was limited. Figures

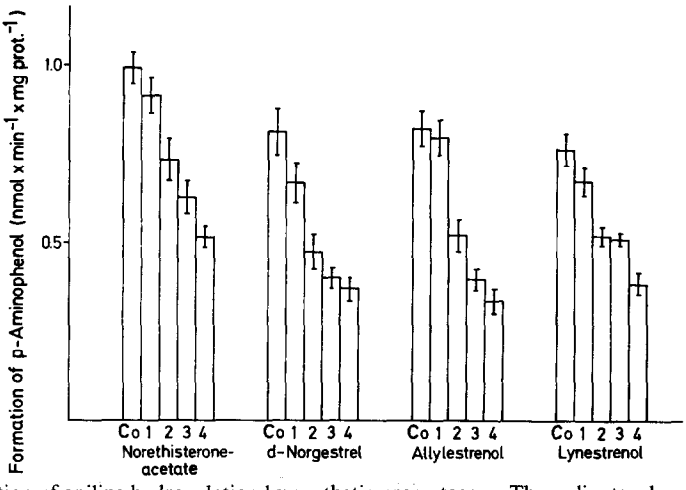

FIG. 2. Inhibition of aniline hydroxylation by synthetic progestagens. The ordinates show the amount of *p*-aminophenol formed per minute in an incubation mixture containing 1 mg protein/ml, a NADPH generating system and $2{\cdot}5\times10^{-4}$ M aniline. Control incubations (Co) without gestagen; inhibitor concentrations: $1:10^{-5}$ M, $2:5\times10^{-5}$ M, $3:10^{-4}$ M, $4:5\times10^{-4}$ M. Values are means $\pm$ S.E.M. (n = 6–10). Significance was determined by Student's *t* test. At an inhibitor concentration of 10^{-4} M the differences between control activity and inhibited activity were highly significant for all gestagens ($P < 0.001$).

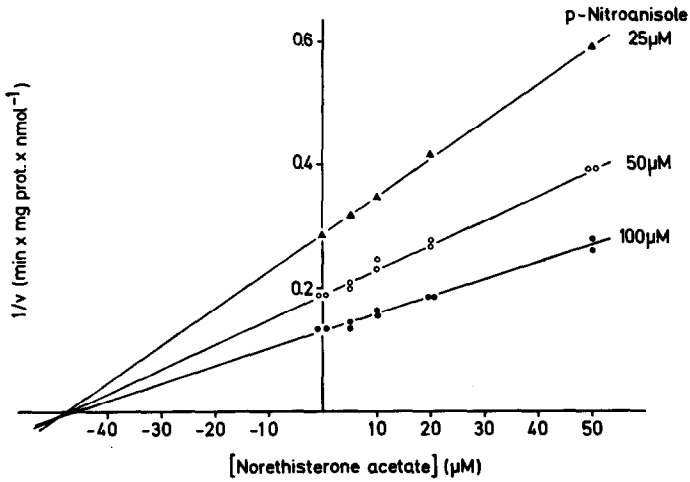

FIG. 3. Dixon analysis of the inhibition of *p*-nitroanisole demethylation by norethisterone acetate. $1/_v$ is expressed as the reciprocal value of the amount of *p*-nitrophenol formed per minute in an incubation mixture containing 1 mg protein/ml.

3 and 4 show Dixon plots of single experiments representing norethisterone acetate inhibition of *p*-nitroanisole demethylation and *d*-norgestrel inhibition of aniline hydroxylation. The inhibitor constants derived from these plots are about 5×10^{-5} M. The K_i values are listed in Table 1.

The binding of the progestagens to oxidized cytochrome P-450 was determined by recording difference spectra under aerobic conditions. Figure 5 shows a titration of the spectral changes after addition of lynestrenol to microsomes which results in the formation of type I difference spectra with maxima at 392 nm and minima at 422 nm. Similar spectra were obtained with all steroids tested. Apparent spectral dissociation constants derived from Lineweaver–Burk plots are given in Table 1.

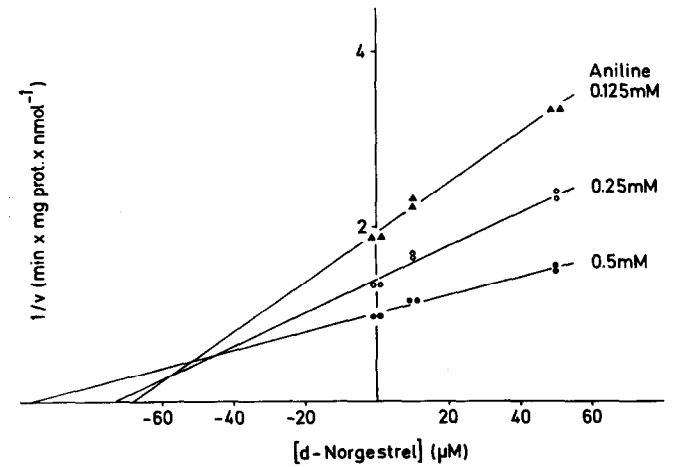

FIG. 4. Dixon analysis of the inhibition of aniline hydroxylation by *d*-norgestrel. $1/_v$ is expressed as the reciprocal value of the amount of *p*-aminophenol formed per minute in an incubation mixture containing 1 mg protein/ml.

TABLE 1. INHIBITOR CONSTANTS (K_i) AND APPARENT SPECTRAL DISSOCIATION CONSTANTS (K_S) OF PROGESTAGENS

	K_i (μM) (*p*-Nitroanisole demethylation)	K_i (μM) (Aniline hydroxylation)	K_S (μM)
Norethisterone acetate	49 ± 3	45 ± 19	36 ± 10
d-Norgestrel	22 ± 1	46 ± 3	46 ± 7
Lynestrenol	19 ± 1	112 ± 16	22 ± 2
Allylestrenol	47 ± 8	82 ± 8	17 ± 1

Values are means ± S.E.M. from three to five different microsomal preparations.

DISCUSSION

The results presented in this paper show that some of the commonly used gestagenic components of hormonal contraceptives are inhibitors of the hepatic microsomal drug-oxidizing activity. However, the poor solubility of these steroids in aqueous solution prevents exact calculation of the actual concentration of steroid in

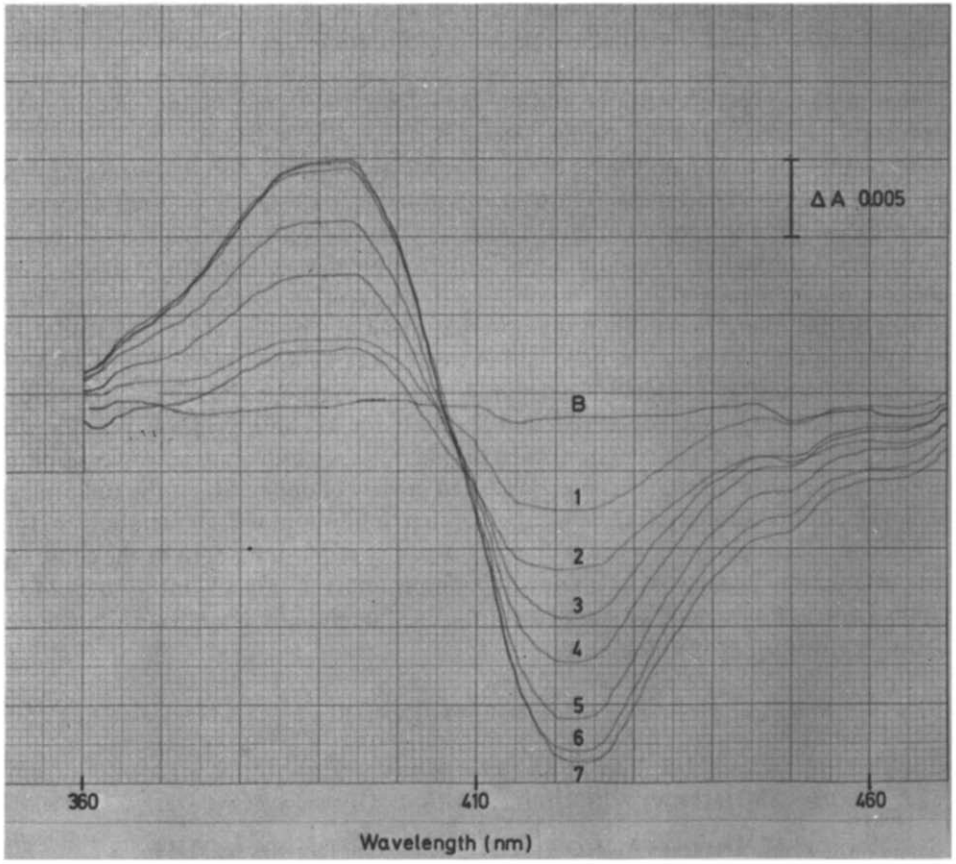

FIG. 5. Titration of the spectral changes produced by lynestrenol. Difference spectra from rat liver microsomes were recorded under aerobic conditions (1 mg protein/ml, pH 7·4). B: Baseline of equal light absorption. 1–7: Cumulative additions of lynestrenol in 2–10 μl dimethylformamide, $1{:}10^{-5}$ M, $2{:}2 \times 10^{-5}$ M, $3{:}5 \times 10^{-5}$ M, $4{:}10^{-4}$ M, $5{:}2 \times 10^{-4}$ M, $6{:}5 \times 10^{-4}$ M, $7{:}10^{-3}$ M lynestrenol.

the membrane. Inhibition was dose-dependent up to a steroid concentration of 1×10^{-4} M if complete solubility is assumed. Further increases in steroid concentration resulted in little increase in inhibition, suggesting that a saturation concentration of steroid was present in the membranes.

The concentrations of the progestagens necessary to obtain the inhibitory effects are similar to those for other gestagenic compounds,[8,9] but greater by an order of magnitude, than those concentrations of, for example metyrapone, which inhibit drug oxidation.[23] The progestagens inhibit drug metabolism when the concentrations of substrate and inhibitor are similar.

The progestagens used in this study exhibit type I difference spectra in microsomes, suggesting that they are themselves substrates of the mixed function oxidase. Their spectral dissociation constants are of the same order of magnitude as their inhibitor constants for *p*-nitroanisole demethylation. Some evidence of metabolites being formed from these progestagens under the experimental conditions employed for the inhibition experiments was obtained by chromatographing the incubation mixtures (unpublished observations). However, there was no apparent change in the inhibitory capacity of the progestagens when the steroids were incubated under the same conditions as in the normal experiments, prior to determining their inhibition of the drug metabolizing enzymes. Thus, unlike progesterone, which is metabolized to the more potent inhibitors pregnanedione and pregnanolone,[8] these metabolites are no more potent as inhibitors than the original steroids.

The situation in women taking oral contraceptives is quite different to the *in vitro* conditions of our experiments. The dose of synthetic progestagen ingested daily does not exceed 5 mg, and even if combined with synthetic estrogens, the concentrations of these agents in the liver should not markedly inhibit the metabolism of drugs given concomitantly if judged by the inhibitory potency of the steroids *in vitro*. These results do not, therefore, explain the observations made by Crawford and Rudofsky who found that in pregnant women and in women taking contraceptive preparations increased amounts of unmetabolized pethidine and promazine were excreted.[11] More recently O'Malley *et al.* observed that the half life of antipyrine in plasma was increased by about 30 per cent in women taking different oral contraceptive preparations over a control group.[12] It must be asked in view of our results if the pill attacks antipyrine elimination by a mechanism other than inhibition of drug metabolism. It is unlikely that changes in the protein binding capacity of plasma might be the cause for this interaction in view of the known poor protein binding of antipyrine. More clinical research is needed to supplement the existing data on the effect of hormonal contraceptives on drug elimination.

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