

Induction of a Deficiency of Steroid Δ^4 -5 α -Reductase Activity in Liver by a Porphyrinogenic Drug

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ABSTRACT The hepatic enzymes that catalyze drug oxidations and the reductive metabolism of steroid hormones to 5 α -derivatives are localized in membranes of the endoplasmic reticulum. Phenobarbital, which exacerbates acute intermittent porphyria in man, induces drug-oxidizing enzymes in liver. Additionally, patients in whom the primary gene defect (uroporphyrinogen-I-synthetase deficiency) of acute intermittent porphyria has become clinically expressed have low levels of hepatic steroid Δ^4 -5 α -reductase activity. This 5 α -reductase deficiency in acute intermittent porphyria leads to the disproportionate generation of 5 β -steroid metabolites from precursor hormones; such steroid metabolites have significant porphyria-inducing action experimentally. In this study the effects of phenobarbital on drug oxidation and steroid 5 α -reduction in man were examined to determine if this drug could produce changes in steroid 5 α -reductase activity which mimicked those seen in patients with acute intermittent porphyria. Metabolic studies with [¹⁴C]-testosterone and 11 β -[³H]hydroxyandrostenedione were carried out in five normal volunteers. In all five subjects phenobarbital administration (2 mg/kg/per day for 21 days) enhanced plasma removal of the test drugs antipyrine and phenylbutazone as expected; but in four subjects phenobarbital also substantially depressed 5 α -metabolite formation from [¹⁴C]testosterone and resulted in a pattern of hormone biotransformation characterized by a high ratio of 5 β /5 α -metabolite formation. Studies with 11 β -[³H]hydroxyandrostenedione in three subjects confirmed that phenobarbital produced this high 5 β /5 α ratio of steroid metabolism by depressing 5 α -reductase activity for steroid hormones in liver. The high ratio of 5 β /5 α -metabolites formed in normals after drug treatment

mimicks the high 5 β /5 α -steroid metabolite ratio formed from endogenous hormones in acute intermittent porphyria. The proximate mechanism by which phenobarbital induces reciprocal changes in activities of the microsomal enzymes which catalyze drug oxidations and steroid 5 α -reductions is not known. This action of phenobarbital raises the possibility, however, that certain drugs which provoke exacerbations of human porphyria may do so, in part, by producing deleterious shifts in the patterns of endogenous steroid hormone metabolism.

INTRODUCTION

The present study was undertaken to examine the effect of phenobarbital on steroid Δ^4 -5 α -reductase activity in normal subjects. Phenobarbital is a prototype of drugs known to provoke human as well as experimental hepatic porphyria and to induce the cytochrome P-450-dependent mixed function oxidase system in liver (1, 2). This NADPH-requiring enzyme complex localized in the membranes of the endoplasmic reticulum mediates the oxidation of numerous, structurally diverse chemicals, including natural steroid hormones (3).

The NADPH-dependent 5 α -reductase system for steroids is also localized in the endoplasmic reticulum, but the effects of phenobarbital or other porphyrinogenic drugs on its activity have not been studied in normal individuals. Such studies could be relevant to the interplay of drug, endocrine, and gene factors which results in exacerbation of acute intermittent porphyria (AIP)¹ since (a) individuals in whom the gene defect for this disorder, uroporphyrinogen I synthetase (UROS) deficiency (4-9) has become clinically expressed also have a significant deficiency of hepatic steroid Δ^4 -5 α -reductase activity (10); and (b) this 5 α -reductase deficiency leads to the disproportion-

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¹ Abbreviations used in this paper: AIP, acute intermittent porphyria; 11-OHAD, 11-OH-androstenedione; UROS, uroporphyrinogen I synthetase.

TABLE I

Effect of Phenobarbital Treatment on Plasma Half-Lives of Antipyrine and Phenylbutazone in Normal Subjects

Subject	Antipyrine-plasma $t_{1/2}$, h			Phenylbutazone-plasma $t_{1/2}$, days		
	Pre-treatment period	Post-treatment period	Decrease in $t_{1/2}$	Pre-treatment period	Post-treatment period	Decrease in $t_{1/2}$
			%			%
1	10.0	6.3	37	2.6	2.1	19
2	5.7	3.7	35	4.5	2.0	55
3	16.5	11.0	33	2.7	1.8	33
4	18.0	10.5	42	2.4	2.0	17
5	7.8	6.0	23	3.7	2.3	38

ate generation of 5β -metabolites from certain steroid hormones and precursors in such patients (11, 12). Steroids derived from the 5β -pathway of hormone metabolism are known to be more potent in their ability to induce porphyria in avian liver cells growing in primary cultures than their 5α -epimers (13–15).

We have earlier shown that AIP patients who have the clinically and biochemically expressed syndrome generate substantially more 5β -, than 5α -steroid metabolites from endogenous as well as isotopically labeled precursor hormones (11, 12) than do normal subjects; in addition Paxton et al. (16) and Goldberg et al. (17) have demonstrated that AIP patients have considerably higher levels of 5β -steroids in their urine and in plasma than do normal individuals. These findings suggest that the patterns of endogenous steroid metabolism, particularly the relative proportion of 5β -metabolites formed from gonadal and adrenal steroid hormones, as well as the production rates of such hormones may have considerable relevance to the clinical expression of the UROS-gene defect in AIP individuals.

The results of the present study show that short-term treatment of normal individuals with the porphyria-inducing drug, phenobarbital, leads not only to enhanced rates of oxidative drug metabolism in liver as expected; but also to the concomitant and significant depression of activity of the hepatic Δ^4 - 5α -reductase system for natural steroids and therefore to an increase in the ratio of $5\beta/5\alpha$ -metabolites formed from precursor hormones. Since 5β -steroids have potent porphyrinogenic properties, the possibility is thus raised that certain drugs or other exogenous chemicals which provoke exacerbations of AIP may do so, in part, by inducing deleterious shifts in the patterns of metabolism of endogenous steroid hormones.

METHODS AND RESULTS

Five normal volunteers (three males, two females, age range 25–35 yr) participated in the study; each gave informed consent and the experimental protocol was reviewed and approved by the Rockefeller University Institutional Review Board. None had received drugs within the 4 wk preceding the experiments. Erythrocyte UROS activities were assayed in each volunteer by the method of Sassa et al. (8) and shown to be within the normal range. $[4\text{-}^{14}\text{C}]$ Testosterone and 11-OH- $[1,2\text{-}^3\text{H}]$ androstenedione were prepared and administered and metabolites were isolated to radiochemical purity as described in our previous studies (11, 12, 18–20). Briefly, on day 1 of the study, an intravenous infusion of 5% glucose in water was begun and a weighed amount of radioactive testosterone was injected into the rubber tubing immediately adjacent to the needle and washed in with the intravenous infusion over a 10-min period. Two complete 24-h urine collections were obtained from each subject and metabolites were hydrolyzed, extracted, and isolated. On day 4 of the study radioactive 11-OH-androstenedione (11-OHAD) was similarly administered intravenously and a subsequent 2-day urine collection was obtained and analyzed as described. After these infusions the plasma half-lives of antipyrine and phenylbutazone were determined in each subject. Antipyrine was dissolved in a flavored syrup and administered orally at a dose of 11 mg/kg. Blood samples were obtained at 0, 3, 6, 9, 12, and 15 h after ingestion and antipyrine measured in plasma samples according to the method of Brodie et al. (21). Phenylbutazone was administered 1 day later at a dose of 6 mg/kg orally and plasma samples were obtained on days 0, 1, 2, 3, 4, 5, and 7. Phenylbutazone levels were determined according to the method of Burns et al. (22). Phenobarbital was then administered orally at night in a dose of 2 mg/kg for 21 consecutive days and each steroid and drug metabolism study was then repeated in the same sequence except for two subjects in whom the 11-OHAD metabolism studies were omitted. All studies were carried out in The Rockefeller University Hospital.

Antipyrine and phenylbutazone metabolism. The results of the plasma half-life studies of these drugs in the five normal subjects before and after the phenobarbital treatment period are shown in Table I. Each subject responded to phenobarbital induction of the microsomal enzymes which mediate oxidation of these drugs by increasing their hepatic metabolism and thus their rates of plasma removal. The extent of the decreases in plasma half-lives varied for each drug from subject to subject, as did the changes in rates of oxidation of the two drugs by each individual subject in keeping with the findings of Vesell and Page (23, 24) and Davies et al. (25).

Reductive metabolism of $[4\text{-}^{14}\text{C}]$ testosterone. Table II provides quantitative data on the recovery of isotopically labeled metabolites from $[^{14}\text{C}]$ testosterone in each of the five subjects. The total recovery of labeled derivatives varied from individual to individual as expected but there were no significant or consistent differences in total isotope recovery between the phenobarbital and control periods. However, in each individual the absolute amount of 5α -metabolite (androsterone) formed in the phenobarbital period declined from the control period and the amount of isotope recovered in the 5β -(etiocholanolone) metabolite or "polar" metabolite fraction increased. In four of the five subjects these changes in metabolite formation resulted in a significant increase in ratio of $5\beta/5\alpha$ -metabolites formed from the hormone in the liver (26) as shown in Fig. 1. The 5β - and 5α -compounds isolated constitute the predominant fraction of

recoverable metabolites derived from the biotransformation of testosterone in man (18). The left portion of Fig. 1 shows for comparative purposes the $5\beta/5\alpha$ -metabolite ratios in a group of 13 normal subjects and 15 AIP patients studied earlier in identical metabolic studies with the radiolabeled hormone (11). One subject (subject 5, Table II) showed no significant change in the ratio of $5\beta/5\alpha$ -metabolites formed from testosterone after drug treatment although a significant increase in polar metabolites did result from phenobarbital administration. These polar compounds are a mixture primarily of C18 and C7 (β) hydroxy derivatives of testosterone, the bulk of which are 5β -compounds (27). Such metabolites normally comprise less than 4% of the total recoverable urinary radioactivity from administered $[4-^{14}\text{C}]$ -testosterone in man (see Table II). In these studies the percent of polar compounds increased above 7% in the drug treatment period as compared to the control period in only one subject (Table II).

Reductive metabolism of $[1,2,3\text{-}^3\text{H}]11\text{-OHAD}$. To determine whether the increase in $5\beta/5\alpha$ -ratio of metabolites formed from $[4-^{14}\text{C}]$ testosterone (Fig. 1) after phenobarbital administration may have resulted from a relative deficiency of steroid Δ^4 - 5α -reductase activity, studies of $[1,2,3\text{-}^3\text{H}]11\text{-OHAD}$ disposition in three of the four normal subjects who demonstrated this shift in metabolism (subjects 1-3, Fig. 1) were carried out. The principal metabolite recovered in the urine from 11-OHAD in man is the 5α -compound 11-hydroxyandrosterone. The 5β -metabolites, 11-hydroxy-etiocholanolone, and 11-keto-etiocholanolone form only a small fraction of transformation products from 11-OHAD (usually less than 5-10%) (19, 20). The latter compounds do not increase in conditions in which steroid Δ^4 - 5α -reductase is depressed; instead a direct reduction of the C3 ketone to a C3-hydroxyl takes place and the double bond at C4-5 remains chemically intact (12, 20). This pattern of

TABLE II
Effect of Phenobarbital Treatment on the Metabolism of $[^{14}\text{C}]$ Testosterone to Androsterone, Etiocholanolone,* and Polar Metabolites

Subject	Period	Steroid metabolites isolated		
		A†	E†	Polar†
		%	%	%
1	C	32.7	20.8	2.9
	P	9.0	32.0	3.4
2	C	24.9	26.5	2.7
	P	11.8	35.6	6.8
3	C	16.9	18.0	3.2
	P	9.7	20.1	3.7
4	C	14.7	19.2	0.5
	P	10.2	19.2	4.1
5	C	15.9	25.8	3.9
	P	12.2	17.6	20.4

* A, 5α , androsterone; E, 5β , etiocholanolone; C, control period; P, phenobarbital period.

† Percent of administered dose as recovered metabolite.

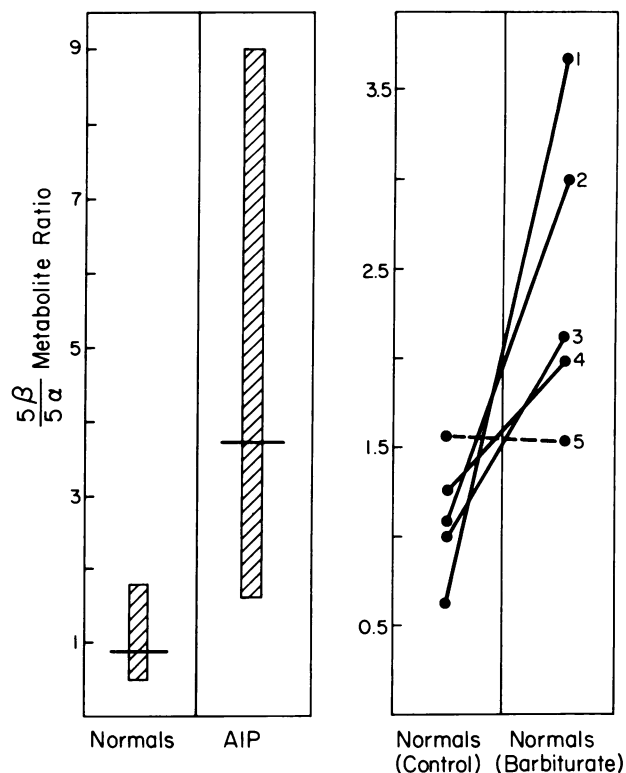


FIGURE 1 Ratios of $5\beta/5\alpha$ -metabolites formed from $[^{14}\text{C}]$ -testosterone. The ranges of $5\beta/5\alpha$ -metabolite ratios found in groups of normal individuals and in patients with clinically expressed AIP are shown on the left. These data were obtained in earlier studies. The specific ratios in the five normal subjects in this study during control periods and after phenobarbital are shown on the right. Subjects 1-4 showed a substantial increase in the fraction of hormone metabolized along the 5β -pathway; the $5\beta/5\alpha$ -ratio in subject 5 did not change.

11-OHAD metabolism reflects the fact that the C11-hydroxy substituent of this C19 hormone appears to inhibit its reduction by the 5β -steroid reductase in contrast to the ability of C11-deoxy steroids such as testosterone and dehydroisoandrosterone to serve equally as substrates for both the 5α - and the 5β -steroid reductases.

The results of the metabolic studies with 11-OHAD in the three normal subjects before and after phenobarbital are shown in Fig. 2, with the data expressed, as in previous studies, as the percent 5α -metabolism of 11-OHAD of the total fraction of identifiable metabolites formed from the hormone. Earlier data from similar metabolic studies in 13 normal and 7 AIP subjects (12) are shown in Fig. 2 for comparative purposes. All three subjects studied showed a decrease in 5α -reductive metabolism of 11-OHAD after phenobarbital (Fig. 2).

These data parallel our earlier findings in AIP patients except that in the latter subjects, the 5α -reductase deficiency for 11-OHAD and for testosterone was more profound (and was demonstrated in the absence of drug treatment); and the output of Δ^4 - 3α -OH-metabolites was greater (12). Erythrocyte UROS activity did not change in any subject after phenobarbital treatment.

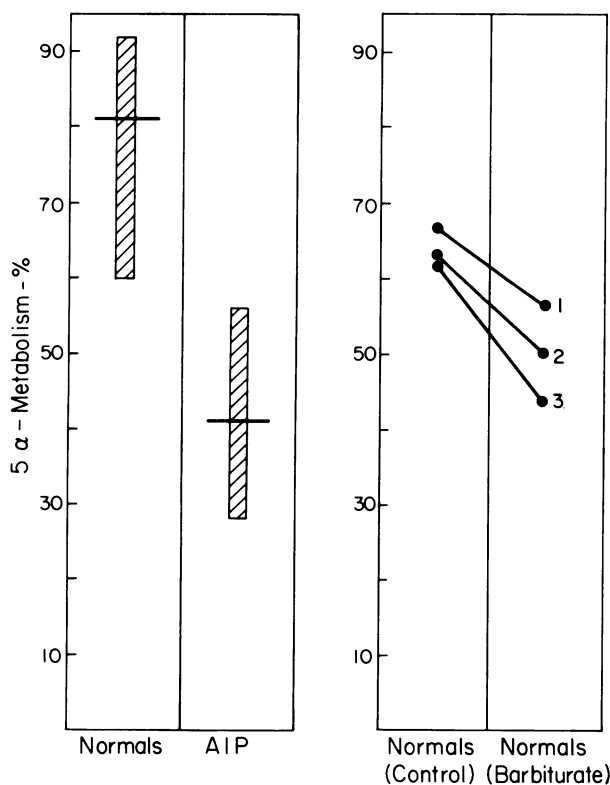


FIGURE 2 Percent 5α -metabolism of $[^3\text{H}]11\text{-OH}$ -androstenedione. The ranges of values for the percent 5α -metabolism of this hormone in groups of normal individuals and AIP patients obtained in earlier studies are shown on the left. The specific values obtained in this study before and after phenobarbital treatment of subjects 1-3 are shown on the right. In all three subjects, phenobarbital decreased the rate of formation of 5α -metabolism of the hormone.

DISCUSSION

The present study demonstrates that administration of the porphyria-inducing drug, phenobarbital, to normal subjects can significantly alter the pattern of reductive metabolism of natural steroid hormones, while simultaneously enhancing the mixed function oxidation of drugs such as antipyrine and phenylbutazone. In the case of testosterone, phenobarbital induced a shift in the pattern of reductive metabolism of the C4-5 double bond such that the ratio of $5\beta/5\alpha$ -metabolites formed from the hormone increased significantly. This higher $5\beta/5\alpha$ -metabolite ratio resulted principally from depressed activity of the 5α -reductase for the hormone; as a consequence of this effect, a relatively greater fraction of the C4-5-reductive metabolism of the hormonal substrate took place via the steroid Δ^4 - 5β -reductase in cytosol as compared with the membrane-bound steroid Δ^4 - 5α -reductase. This was confirmed by the studies with 11-OHAD an adrenal hormone whose reductive metabolism takes place largely

along the 5α -pathway. Depression of 5α -reductase activity by phenobarbital resulted, in the case of this hormone, in a decrease in formation of its main metabolite, 11 β -hydroxyandrosterone and an increase in excretion of 11-OHAD metabolites in which the C4-5 double bond remained intact. The C11-hydroxyl substituent of this hormone as noted above blocks its compensatory metabolism by the 5β -steroid reductase. These findings provide firm evidence that the activity of steroid Δ^4 - 5α -reductase in liver is depressed by phenobarbital.

The data recorded in Table I and Fig. 1 indicate that reciprocal changes in the rates of steroid reduction along the 5α -pathway and the rates of drug oxidation occurred in four of the five subjects studied after treatment with phenobarbital. Phenobarbital is known to induce activity of the hepatic mixed function oxidase system for drugs but the reason why the drug concurrently produces depression of steroid Δ^4 - 5α -reductase activity in man is not known. This effect could result from a variety of mechanisms but it cannot wholly be due to competitive diversion of hormonal substrate from the 5α -reductase to the steroid hydroxylase system in liver since in only one of the five individuals studied (Table II) did the fraction of polar metabolites increase substantially.

The ability of phenobarbital to depress the 5α -reduction of steroid hormones in normals and thus to produce a higher ratio of $5\beta/5\alpha$ -metabolite formation from certain hormone substrates mimicks the pattern of excessive generation of 5β -steroid metabolites which characterizes AIP patients in whom the porphyric syndrome has become clinically and biochemically expressed (11, 12). This drug effect may thus have some relevance to the mechanism of phenobarbital-induced exacerbations of AIP. In tissue cultured avian embryo liver 5β -steroid metabolites are more potent porphyrinogenic agents than their 5α -epimers, although the latter compounds do display clear porphyrin-inducing activity in this preparation as we have shown earlier (13, 14). In rat liver 5α -metabolites have equal or greater potency in inducing δ -aminolevulinatase synthetase (28) thus suggesting the possibility of a species dependence of the potency of 5β - and 5α -steroids in inducing experimental hepatic porphyria. Nevertheless, in patients with clinically expressed AIP high ratios (ranging from 50 to 1,000% above the mean normal ratio [11]) of $5\beta/5\alpha$ -metabolism of endogenous as well as tracer-labeled steroid hormones have been demonstrated in our earlier studies; and Goldberg and associates (16, 17) have shown that AIP patients excrete excessive amounts of 5β -steroids in urine and have considerably greater than normal levels of 5β -steroids in their plasmas. These findings thus

conform with our earlier suggestion that the greatly increased production of steroid hormones as well as the ratio of 5 β - and 5 α -metabolites formed from these precursors in the liver may bear significantly on the mechanism of endocrine-related activation of AIP (29) at or near puberty. While phenobarbital, as a prototypic porphyrinogenic agent, clearly can provoke experimental hepatic porphyria directly in tissue culture (1), the fact that it can also shift endogenous steroid metabolism in man towards a presumably deleterious pattern (i.e. relatively more 5 β -steroid as compared with 5 α -steroid formation) suggests an additional mechanism by which this and related drugs might provoke AIP in genetically susceptible individuals.

In considering the possibility of some type of interaction between chemical, hormonal, and genetic factors in activating AIP it should be recalled that while clinically manifested AIP occurs only at or after puberty, the UROS-deficiency characterizing patients with this disease can be identified in utero (9) as well as in all prepubertal and adult gene carriers of the AIP trait even though the defect remains entirely latent (8, 29). Since it is extremely unlikely that completely latent AIP gene carriers escape all exposure to drugs and other porphyrinogenic chemicals it is reasonable to suppose that clinical penetrance of the UROS-gene defect is determined, in part, by a congeries of "metabolic" (including environmental and nutritional) factors, of which the chemical-endocrine interaction described in this study may be an example.

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