STUDIES IN PORPHYRIA

II. Evidence for a Deficiency of Steroid Δ^4-5α-Reductase Activity in Acute Intermittent Porphyria*

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It was demonstrated in a preceding study from these laboratories that patients with the genetic liver disease acute intermittent porphyria (AIP)[1] display a significant defect in the reductive transformation of prototype steroid hormones such as testosterone and dehydroisoandrosterone (1). This defect is manifest by the marked preferential metabolism of these hormones along the 5β-pathway—the pathway through which endogenous steroids having a potent ability to induce the mitochondrial enzyme δ-aminolevulinate synthetase (ALAS) are generated (1–3). ALAS controls the rate-limiting step in porphyrin-heme synthesis (4) and has been found at high levels of activity in the livers of AIP patients (5). This excessive activity of hepatic ALAS accounts for the overproduction of porphyrin precursors which characterizes this genetic disorder (6–8).

Reduction of the double bond at the A:B ring junction of neutral steroid hormones is catalyzed in the liver by two classes of enzymes: steroid Δ^4-5α-reductases localized in the membranes of the endoplasmic reticulum and steroid Δ^4-5β-reductases found in the cytosol. The preferential metabolism of testosterone and dehydroisoandrosterone along the 5β-pathway in AIP could therefore be due to a decrease in the activity of the 5α-reductase for these hormones, an increase in the activity of the 5β-reductase, or both. To examine the enzymatic basis for the abnormal steroid hormone metabolism in AIP patients further, we have studied the metabolic disposition in them of tracer doses of the adrenocortical hormone, 11β-hydroxyandrostenedione (11-

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[1] *Abbreviations used in this paper:* AIP, acute intermittent porphyria; ALAS, δ-aminolevulinate synthetase; Δ^4-30H, a mixture of Δ^4-androstene 17-ketosteroid metabolites of 11-OHAD containing 3α- or 3β-hydroxy, and 11β-hydroxy or 11-keto substituents; 11=OE, 5β-androstane-3α-hydroxy-11,17-dione; 11-OHA, 5α-androstane-3α-11β-dihydroxy-17-one; 11-OHAD, Δ^4-androstene-11β-hydroxy-3,17-dione; 11-OHE, 5β-androstane-3α-11β-dihydroxy-17-one; PCT, porphyria cutanea tarda; URO-S, uroporphyrinogen synthetase.

OHAD). This C-19 steroid has an oxygenated substituent at the C-11 position that greatly hinders its biotransformation along the 5β-pathway (9); thus the metabolites formed from the hormone in man are predominantly of the 5α type. Examination of its metabolic fate in AIP patients affords, therefore, an extremely sensitive test of whether steroid Δ^4-5α-reductase activity is normal or depressed in such individuals.

The results of the present study indicate that AIP patients have a markedly decreased ability to effect the reductive transformation of 11-OHAD along the 5α-pathway, as compared with normal subjects. The extent of this impairment of steroid 5α-reductase activity is sufficient to explain the compensatory diversion of hormones such as testosterone and dehydroisoandrosterone, towards metabolism via the 5β-pathway in this disease (1). To establish the specificity of this enzymatic deficiency in AIP similar studies were carried out in patients with the acquired form of hepatic porphyria, porphyria cutanea tarda (PCT). In this disorder, normal levels of activity of the steroid Δ^4-5α-reductase for 11-OHAD were demonstrated.

Materials and Methods

A total of nine normal individuals, seven AIP patients, and three PCT patients were studied. All seven of the AIP patients belonged to the group A category defined in our previous report (1) in which the disorder though clinically manifest was symptomatically mild; all patients also had abnormally low levels of activity of uroporphyrinogen synthetase (URO-S) (10) in erythrocytes as determined by the method of Granick et al. (11). The normal subjects were age and sex matched with the AIP patients. All studies were carried out on the wards of The Rockefeller University Clinical Research Center.

Steroid Studies.—Isotopically labeled [4-^{14}C or 1,2-^{3}H]11β-hydroxyandrostenedione (11-OHAD), whose structure is shown in Fig. 1, was prepared by bismuthate oxidation of [4-^{14}C]-cortisol or [1,2-^{3}H]cortisol (12). Crude 11-OHAD was purified by paper chromatography and shown by radioisotopic dilution with carrier 11-OHAD to have a purity greater than 98%. 1–10 μl of the purified steroid [4-^{14}C or 1,2-^{3}H] was dissolved in a small volume of alcohol, diluted with approximately 100 ml of 5% glucose, and a weighed amount of this solution then administered intravenously to the subjects over 15–20 min as previously described (13). Complete urine collections, determined from the constancy of the creatinine content, were made for 2 days after intravenous injection of the compound. Urease and phosphate buffer were added in most cases to remove urea (13). The 2-day collections were combined and enzymic and acid hydrolysis of the steroid conjugates was carried out by hydrolytic, extraction, and chromatographic methods reported in detail elsewhere (13).

Two major extracts containing the glucuronidated metabolites of 11-OHAD were prepared by extensive hydrolysis with β-glucuronidase (Ketodase^{R}); the numbers shown in Table I for percent radioactivity of glucuronide extracts represent the combined sum of the recovery values for these two glucuronide extracts.

The principal metabolite of 11-OHAD in man is the 5α-steroid 11-OHA (Fig. 1). Rechromatography as well as reverse isotope dilution with carrier 11-OHA established that the radioactive peak identified as containing this metabolite after appropriate chromatography was, in fact, substantially all authentic 11-OHA.

Additional 11-OHA-containing conjugates were still present in the urine treated with β-glucuronidase and extensively extracted since subsequent hot acid hydrolysis resulted in the liberation of the dehydration product of this metabolite, $\Delta^{9(11)}$-androsterone. The recovery of this radioactive steroid, in terms of percent counts, after such hot acid treatment of urine

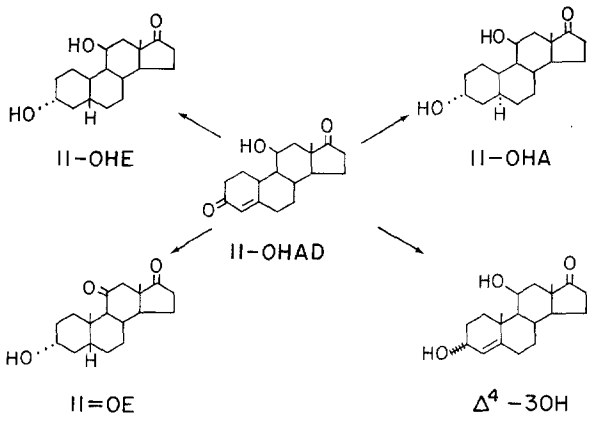

FIG. 1. Structures of 11β-hydroxy-Δ^4-androstenedione (11-OHAD) and its metabolites. The abbreviations used are defined in footnote 1. The principal metabolite of 11-OHAD is the 5α-compound, 11-OHA shown in the upper right. The 5β-steroids shown at the left represent only minor transformation products of 11-OHAD. The C-3 hydroxyl of Δ^4-30H is designated (-|-|-|-|-|-|-) to indicate a mixture of 3α- and 3β-hydroxy compounds.

is shown as the number under "acid extract" in Table I; and the specific amount of isolated $\Delta^{9(11)}$-androsterone found is included with the total amount found of its precursor 11-OHA in Table II. Reverse isotope dilution analysis showed that the radioactive peaks migrating as the 5β-steroids 11-OHE and 11=OE (Fig. 1) contained in addition to the authentic compounds, acid labile ring A unsaturated components (i.e. Δ^4-3 hydroxylated steroids [Fig. 1]) similar to those excreted by myxedematous patients (13). The results of these isolation procedures are summarized in the data shown in Table II; in this table, acid labile steroids are designated "Δ^4-30H" compounds. All samples were counted in a Packard 3320 scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.) using Diatol as the scintillant.

RESULTS

Table I summarizes the mean values for urinary excretion of radioactivity after administration of labeled 11-OHAD to normal individuals and to the porphyric patients, and the amount of radioactivity recovered in the form of the combined "glucuronide extracts" and the "acid extract" of urine from each group of subjects. The excretion of labeled steroids was rapid and recovery of administered isotope was similar and nearly quantitative in the three groups of individuals studied. The pattern of metabolites produced from 11-OHAD in the AIP patients, however, was considerably different from the normal subjects, as show in Table II. In the AIP patients there was a markedly decreased formation of the principal 5α-metabolite, 11-OHA, formed from 11-OHAD as compared with normals. This decreased formation of 5α-metabolite was not compensated for by an enhanced production of 5β-steroids (i.e. 11-OHE Table II); rather there was a very large increase in output of steroids (i.e. Δ^4-3OH, Table II) in which the C^{4-5} double bond was chemically unaltered. The

TABLE I

*Urinary Excretion and Hydrolysis of Metabolites of ^{14}C-Labeled 11-OHAD**

Subjects	(No.)	Urine	Glucuronide extracts	Acid extracts
Normals	(9)	93	58	19
AIP	(7)	81	54	15
PCT	(3)	92	59	22

* Mean values for each group, expressed as percent of the administered radioactivity.

TABLE II

*Metabolism of ^{14}C-Labeled 11-OHAD in Normal Subjects and Porphyric Patients**

Subjects	(No.)	Metabolites formed		
		11-OHA	11=OE	Δ^4-3OH‡
Normals	(9)	63	12	1
		(55–71)	(7–16)	(0–2)
AIP	(7)	33	12	33
		(26–38)	(4–19)	(10–58)
PCT	(3)	52	6	10
		(35–61)	(4–9)	(8–14)

Includes small amounts of the related 5β-metabolite, 11-OHE.

* Mean and range of values for each group expressed as percent radioactivity of the total neutral extracts.

‡ Δ^4-30H includes a mixture of 3α- and 3β-OH Δ^4-metabolites of 11-OHAD.

PCT patients (Table II) produced normal amounts of the 5α-metabolite, 11-OHA. The slightly increased output of ring A unsaturated steroids in these patients may result from a diversion of the small fraction of 11-OHAD, which is normally metabolized via the 5β-pathway, since the amounts of 11-OHE formed by PCT patients were slightly lower than those produced by the control group.

Fig. 2 depicts the individual values for percent 5α-metabolite formed from 11-OHAD in each of the AIP and normal subjects. The mean value for metabolism of 11-OHAD along the 5α-pathway in the AIP patients was 43% or approximately one-half the mean value for controls, with the range in AIP patients extending from 34 to 70% below the average value for 5α-metabolism in normals. The mean value for percent 5α-metabolism of 11-OHAD by the three PCT patients was slightly lower than the mean for normals (Table II) but the difference was not statistically significant and the specific values for these patients are not depicted in Fig. 2.

DISCUSSION

The data reported here establish that patients with AIP have a major deficiency in activity of the hormone-metabolizing enzyme(s), steroid-Δ^4-5α-

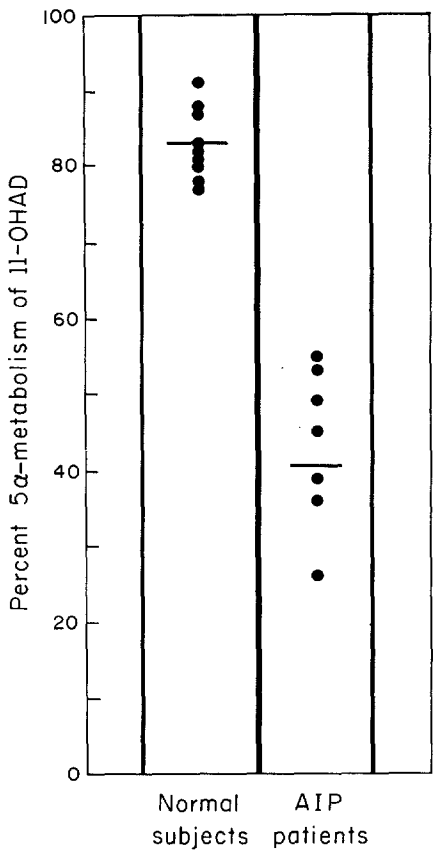

FIG. 2. Percent 5α-metabolism of 11-OHAD in nine normal subjects and seven AIP patients. This pathway for the reductive metabolism of steroid hormones is impaired in AIP subjects in whom the disease has become clinically expressed.

reductase. This deficiency was demonstrated in all seven of the patients studied, and ranged in degree from 34 to 70% less than the mean 5α-reductase activity characterizing normal subjects. The 5α-reductase deficiency was defined in terms of the adrenal hormone 11-OHAD as substrate for the enzyme; but it is reasonable to assume that the deficient 5α-metabolism of other steroid hormones, which we demonstrated earlier in AIP (1), results from comparable enzymic abnormalities. The ability of triiodothyronine to normalize the defect in 5α-metabolism of steroids such as testosterone in AIP (14) supports this view since thyroid hormone is a potent inducer of 5α-reductase activity in the liver. There presumably is some degree of substrate specificity for steroid 5α-reductases but this question has not been examined in man; in any event, the impaired 5α-metabolism of 11-OHAD, testosterone, and dehydroisoandrosterone demonstrated in this and earlier studies (1) indicates that as a class,

these endoplasmic reticulum-bound enzymes are clearly deficient in activity in AIP patients as compared with normal subjects. Whether the deficiency is attributable to direct defects in the enzyme(s) itself or in its synthesis and metabolism, to limitations on the availability of cofactors, or results from the presence of inhibitors of 5α-reductase in AIP patients, etc., is not presently known. The assumption that the defect in 5α-reductase activity shown here primarily reflects deficient liver enzyme activity is, however, undoubtedly valid. Hepatic extraction of circulating steroids is an extremely rapid and efficient process; and it is well known that intravenously administered tracer doses of steroid hormones undergo metabolism almost exclusively in liver cells in man. The possibility that other tissues of AIP patients may also display deficient 5α-reduction of hormones should nevertheless be explored since identification of this steroid metabolic defect by less complex and costly means than those required for these studies would be useful.

It should be noted that these data, while clearly defining a major deficiency of steroid-5α-reductase activity in AIP, do not completely exclude the existence of a concurrent elevation in activity of steroid-5β-reductase in this disease. The AIP patients showed no increased production of the 5β-metabolites of 11-OHAD in this study. However, the limited formation of such metabolites in normal subjects reflects the steric restriction imposed by the C-11 hydroxyl substituent of 11-OHAD on its 5β-reductase; this enzymatic constraint could also preclude a compensatory diversion of 11-OHAD metabolism from the 5α- to the 5β-pathway in the AIP patients studied. Steroid hormones such as testosterone and dehydroisoandrosterone, which lack a C-11 substituent, present no steric restriction to the 5β-reductase; and the 5β-pathway thus remains accessible, as shown previously (1) for the reductive transformation of that fraction of such hormones that cannot otherwise be metabolized to 5α-derivatives. The extent to which shunting of hormones from the 5α- to the 5β-reductase can take place in human liver without requiring an increase in the specific activity of the latter enzyme is not known. Thus while the deficiency in steroid-5α-reductase activity demonstrated here probably accounts entirely for the impaired hormone metabolism seen in AIP patients, a reciprocal increase in 5β-reductase activity cannot be completely ruled out.

The formation of Δ^4-30H metabolites in substantial quantity in AIP patients is a phenomenon that we have previously observed only in myxedematous subjects (13). It is of interest that this metabolic pattern was characteristic only of the biotransformation of 11-OHAD in the AIP subjects; studies with testosterone in this disease as in myxedema (15) showed no excess production of ring A unsaturated metabolites. Another unusual feature of 11-OHAD metabolism in AIP is the occurrence of Δ^4-30H C-11-ketone metabolites; these were not previously observed with myxedematous patients who tend rather to have a relative increase in C-11 hydroxyl over C-11-ketonic derivatives. Indeed a relative increase in C-11-ketonic metabolites is a characteristic not of hypo-

thyroid, but of hyperthyroid, individuals. These findings are consistent with earlier observations that indicate that the relative formation of 5β- and Δ^4-30H steroid metabolites is dependent on the effect of the C-11 substituent on 5β-reductase activity (9).

The AIP subjects described in this report were all patients in whom the disease, though only mildly active at the time of study, was nevertheless well defined on the basis of the usual symptomatic and biochemical criteria (6–8). The 5α-reductase deficiency demonstrated here thus may typify only those individuals whose gene defect for AIP has become clinically expressed. It is not known whether subjects in whom the AIP trait has remained completely latent, i.e. has never become clinically manifest, display a similar enzyme deficiency. Such subjects presumably comprise those individuals identified as having the trait by low levels of erythrocyte URO-S activity, but who have never had symptoms or evidence of increased excretion of porphyrin precursors.[2]

It is also not clear to what extent the deficient 5α-reductase activity in AIP reflects the relative influence of genetic and extragenetic determinants on this enzyme activity. We have previously demonstrated the existence of major genetic influences on steroid hormone metabolism in man (16), and there may be significant heritable determinants of the patterns of 5α- and 5β-metabolism of steroids in AIP patients as well. Extragenetic factors, such as chemicals, hormones, etc., also affect the patterns of steroid metabolism in humans (17–19), and recent studies from these laboratories have shown that porphyrinogenic drugs can, in normal individuals, depress 5α-reductase activity and increase 5β/5α metabolite ratios in a manner analogous to that found in untreated AIP patients (17).

The possible relation of the 5α-reductase deficiency to the transformation of AIP from a latent to an active disorder at puberty merits particular consideration. It is well established that this inherited disorder rarely manifests itself clinically before puberty. However, as Sassa et al. have shown (20, footnote 2), the erythrocyte URO-S defect that characterizes all subjects carrying the gene abnormality for AIP can be identified in children as young as 5 mo of age. The URO-S defect is thus not sufficient in itself to evoke clinical expression of AIP and additional biochemical or metabolic factors must underlie the transition of this disorder from the latent to the active state.

In this regard the reductive biotransformation of steroids in prepubertal children is known to preferentially favor the 5α-pathway (21–23); this pattern changes near puberty, with a shift towards greater metabolism of hormones via the 5β-pathway, taking place with increasing age (24). This change in the pattern of steroid metabolism, together with the dramatic increase in synthesis of hormones and precursors that occurs at puberty, may be critical in determining clinical expression of the latent gene defect for AIP in many affected individuals.

[2] Sassa, S., S. Granick, D. R. Bickers, and A. Kappas. 1973. Manuscript in preparation.

SUMMARY

Patients with the genetic liver disease, acute intermittent porphyria (AIP), have a defect in the reductive transformation of steroid hormones that is manifest by the disproportionate generation of 5β-steroid metabolites from precursor hormones. 5β-steroid metabolites were earlier shown to be potent inducers experimentally of δ-aminolevulinate synthetase (ALAS), the mitochondrial enzyme that is rate-limiting in porphyrin synthesis, and that is found at high levels of activity in the livers of AIP patients.

In this report, the basis for the defective steroid metabolism in AIP has been shown, through studies with the ^{14}C-labeled adrenal hormone 11β-hydroxy-Δ^4-androstenedione, to reside in a substantial deficiency of hepatic steroid Δ^4-5α-reductase activity. This enzymic deficiency was found in all seven AIP patients studied, and ranged from 34% to as much as 70% below the mean enzyme activity characterizing normal subjects.

The functional consequence of the low levels of 5α-reductase activity in AIP is to divert the reductive transformation of certain natural hormones from the 5α- to the 5β-pathway; the latter is the metabolic route through which endogenous steroids having the potential for inducing hepatic ALAS are generated. It is not presently known whether the 5α-reductase deficiency in AIP is acquired in some fashion or whether it has partial genetic determinants. It seems probable, however, that this enzymatic abnormality, coupled with the dramatic increase in hormone synthesis that occurs at puberty, may be of major importance in determining clinical expression of the latent gene defect for AIP in many individuals. The 5α-reductases for steroid hormones are known to be localized in the endoplasmic reticulum of hepatic cells and the present findings in AIP thus represent the first demonstration that an enzymic component of these membranous structures is functionally abnormal in this hereditary liver disease.

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