STEROID CONTROL OF PORPHYRIN AND HEME BIOSYNTHESIS: A NEW BIOLOGICAL FUNCTION OF STEROID HORMONE METABOLITES*

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Three classes of chemicals are capable of inducing the formation of porphyrins in chick embryo liver cells grown in primary culture.[1-3] These classes are the barbiturates, the dihydrocollidines, and the steroids. In contrast to certain chemicals of the first two classes which induce strongly, the steroids which had been tested earlier were only weakly active. However, of these classes of inducing agents, only the steroids are natural products. In view of the physiological origin of the steroids, and their potential relation to the pathogenesis of hepatic porphyria in man, it was decided to study their porphyrinogenic activity in more detail.

We have now examined over 120 steroids for their porphyrin-inducing effect in liver cell culture. The steroids tested consisted of hormones, physiological intermediates, metabolites, bile acids, and so on. We have found that a number of 5β-H (A:B *cis*) C-19 and C-21 steroids have potent porphyrinogenic activity. In collaboration with Dr. R. Levere we have found that these same steroids also stimulate heme synthesis in embryonic erythroid cells.[4]

In this paper data are presented to support the idea that a new "hormonal" activity, the stimulation of porphyrin and heme synthesis, characterizes certain steroids which were previously considered to be waste products of hormone metabolism.

Methods and Results.—The tissue culture method used for studying porphyrin induction has been described in detail previously.[3] It consists, briefly, in growing liver cells from 16–17-day old chick embryos in primary culture for 24 hr on coverslips on which they form a monolayer of colonies; on the second day the culture medium is replaced and steroids and other chemicals are added. Approximately 20 hr later the coverslips are examined with fluorescence microscopy and semiquantitative estimates are made[3] of the excess porphyrins which have been induced in the cells.

Structure of inducing steroids: Some of the most potent porphyrin-inducing steroids we have found are listed in Table 1. The inducing activity of these compounds was consistently demonstrable in concentrations as low as 10^{-6} to 10^{-8} M, thus characterizing these natural substances as having inducing action equal to or exceeding that of the most potent foreign chemicals and drugs tested previously.[1-3]

TABLE 1

STEROIDS WITH STRONG PORPHYRIN-INDUCING ACTIVITY

C-19 steroids	C-21 steroids
Etiocholanolone (5β Androstan-3α-ol, 17-one)	Pregnandiol (5β Pregnan-3α, 20α-diol)
Etiocholandiol (5β Androstan-3α, 17β-diol)	Pregnanolone (5β Pregnan-3α-ol, 20-one)
Etiocholandione (5β Androstan-3, 17-dione)	11-Ketopregnanolone (5β Pregnan-3α-ol, 11,20-dione)
Etiocholanolone-17β (5β Androstan-3-one, 17β-ol)	17α-OH Pregnanolone (5β Pregnan-3α,17α-diol, 20-one)

The steroids listed belong to the C-19 and C-21 neutral series; they all share the basic nuclear structure of 5β-H (A:B *cis*) compounds in which the junction of the A and B rings is highly angular rather than planar; and they are all derived from the *in vivo* biotransformation of steroid hormones or intermediates, rather than being primary endocrine secretions themselves. Several of them such as etiocholanolone, pregnandiol, and pregnanolone are produced in significant amounts daily from the metabolic conversion of such precursor hormones as testosterone and progesterone.

The inducing activity of other steroids was either weak or absent. The primary neutral hormones themselves (i.e., testosterone, progesterone, and so on) were weakly active as were estradiol and the related estrogens, estrone and estriol. The 5α-H (A:B *trans*) series of metabolites was weakly active. All C-21 hydroxylated steroids such as the gluco- and mineralocorticoids were inactive. The glucuronide conjugates of even the most potent inducers were totally inert.

Characteristics of the induction process evoked by steroids: Steroid induction of porphyrins appears to be, in all the characteristics studied, similar to the induction process evoked by foreign chemicals and drugs.[1-3] It seems likely, therefore, that these steroids act at the same cellular site(s) as do porphyrinogenic chemicals and drugs.

Typical effects of certain blocking agents on the steroid-induction process are summarized in Table 2. The initial and rate-limiting enzyme in the heme biosynthetic pathway is δ-aminolevulinic acid (ALA) synthetase. It has been shown that chemicals which induce porphyrin formation act by stimulating the *de novo* synthesis of this enzyme.[5] Agents such as actinomycin D and puromycin which inhibit mRNA and protein synthesis, respectively, prevent this synthesis and thus prevent formation of excess porphyrins.[3] These agents block the porphyrin-inducing action of steroids as shown in Table 2. Heme and certain other metalloporphyrins also block induction of porphyrin synthesis by steroids. This is interpreted to mean that heme, the presumed natural corepressor,[3] displaces steroids from the binding site on the repressor apoprotein; as a consequence, the formation of δ-aminolevulinic acid synthetase is prevented. The repressor hypothesis is depicted graphically in Figure 1. A similar model for induction by drugs and foreign chemicals was proposed earlier.[3] The steroid induction of porphyrins is also inhibited by uridine-diphosphate glucuronic acid (UDPGA), but by no other component of the glucose-glucuronic acid (UPD) pathway; this effect is viewed as reflecting enhanced conversion of the active free steroid to the inactive steroid-glucuronide by the enzyme UDP-glucuronyl transferase.[6] Chick embryo liver has a small amount of transferase

TABLE 2

INHIBITION OF STEROID INDUCTION OF PORPHYRIN SYNTHESIS

Chemicals added to the culture medium	Amount added (μg/ml)	Cellular fluorescence induced by pregnanolone (approx. %)
Control	—	100
Actinomycin D	0.05	25
Mitomycin C	1.0	50
Puromycin	5.0	25
Acetoxycycloheximide	0.01	25
Fe-protoporphyrin	4.5	50
Mn-protoporphyrin	4.5	50
UDPGA	750	25

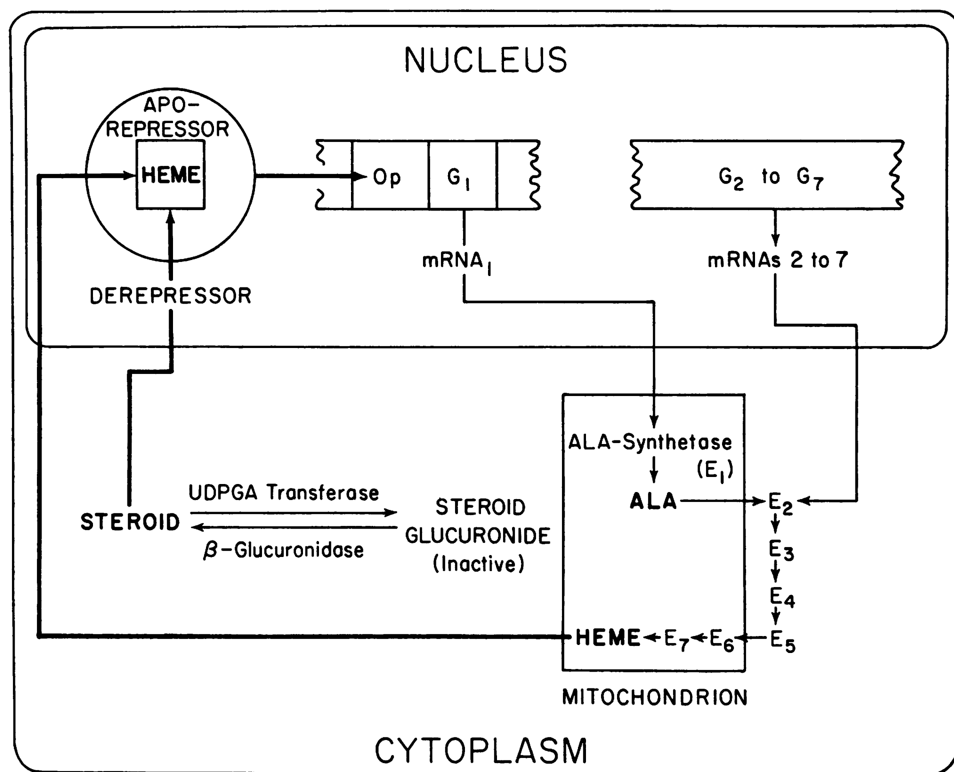

FIG. 1.—Hypothesis on steroid control of heme biosynthesis. δ-Aminolevulinic acid (ALA) synthetase (E_1) is the first and limiting enzyme of the heme biosynthetic sequence. All other enzymes (E_2–E_7) are present in nonlimiting amounts. Synthesis of E_1 is regulated by a repressor-operator mechanism. The repressor consists of an aporepressor protein and a corepressor, heme. When the corepressor, heme, is displaced from its binding site by a steroid (*derepressor*), the repressor is inactivated, the structural gene (G_1) for ALA-synthetase can code for more messenger ribonucleic acid ($mRNA_1$), and enzyme (E_1) is synthesized. The concentration of active steroid (*derepressor*) in the cell depends on the rate of its conversion by UDP-glucuronyltransferase to the inactive glucuronide and the rate of hydrolysis of the glucuronide back to the active form by β-glucuronidase.

activity,[7] and enhanced glucuronidation of appropriate substrates by added UDPGA is well known.[8]

Discussion.—A number of steroid metabolites of the 5β-H type strongly stimulate the production of porphyrins in chick embryonic liver cells grown in primary culture. These metabolites originate from the biotransformation of several adrenocortical and gonadal hormones and precursors in man. They have been generally considered to represent biologically inert degradation products of hormone metabolism, which are en route to elimination from the body. This study implicates these steroid metabolites as one class of physiological control agents in porphyrin and heme biosynthesis.

Hypothetical mechanism of the steroid-induction process: In previous studies of chemical porphyria,[3, 5] the limiting enzyme of heme formation has been identified as ALA-synthetase, the first one of this biosynthetic scheme. Inducing drugs and chemicals act specifically by causing a marked increase in the synthesis of this rate-

limiting enzyme. We have presented a hypothesis that explains this action in terms of a repressor-operator control on the structural gene for ALA-synthetase.[3] The essential features of this hypothesis are presented in Figure 1. The repressor is considered to comprise an aporepressor protein and a corepressor heme. When the repressor is active (i.e., combined with heme), it blocks the operator of the ALA-synthetase operon (Op), thus preventing the reading-out of the code of the structural gene; ALA-synthetase production then decreases and heme formation diminishes. The primary action of an inducing chemical (i.e., derepressor, Fig. 1) is to block or displace the heme from its binding site on the aporepressor protein; the repressor is then rendered inactive, ALA-synthetase production is enhanced, and more porphyrins and heme are formed.

On the basis of this hypothesis, steroids would induce ALA-synthetase by competing with heme for a binding site on the aporepressor protein (Fig. 1). To do this the steroids would have to be in the free and not the glucuronidated form. Thus any process which led to (*a*) impaired glucuronidation of the steroids such as inhibited or defective UDP-glucuronyl transferase, limited UDPGA formation, and so on, or (*b*) enhanced intracellular hydrolysis of formed conjugates by excessive β-glucuronidase activity, or (*c*) steroid production in amounts exceeding the body's capacity to dispose of them would lead to induction of ALA-synthetase and, as a consequence, to increased production of ALA and other intermediates, as well as heme.

Steroid participatation in control mechanisms for heme synthesis: It has been shown that in drug-induced experimental porphyria, those chemicals which in whole animals induce the formation of ALA-synthetase or increase porphyrin and heme production are the same ones which induce excessive porphyrin formation in chick embryo liver cells *in vitro*.[3] In the whole animal only liver, and no other tissue, appears to become induced by porphyrinogenic agents such as the barbiturates or the dihydrocollidines.[5] Similarly, in *in vitro* cultures of various chick embryo tissues, only the liver appears to respond to these chemicals, as determined by fluorescence microscopy.[3] In liver, this induction of excess heme by drugs and chemicals may reflect a control mechanism which has as one of its physiological purposes the use of heme to detoxify these substances.

It has been found in a collaborative study with Dr. R. Levere[4] that certain 5β-H steroid metabolites which stimulate porphyrin formation in embryonic liver cells also strongly stimulate the formation of hemoglobin in chick blastoderm erythroid cell culture. It has been shown in a previous study from this laboratory[9] that the activity of ALA-synthetase is the rate-controlling step in hemoglobin formation in this embryonic tissue. According to the hypothesis presented here (Fig. 1), steroids in erythroid cells would function to inactivate the repressor of the ALA-synthetase operon; ALA-synthetase would then be made in increased amounts leading to the formation of additional heme, and once heme was made, additional hemoglobin would be formed. The fact that both liver cells and the pro-erythroblasts in embryonic tissues are induced to form more porphyrin and heme by certain steroids thus suggests that these physiological substances may, to some degree, regulate heme synthesis in all body cells.

Clinical significance of the porphyrin-inducing action of steroids: The chemical nature of the physiological substances which lead to the episodic, spontaneous (i.e., not drug-induced) exacerbations of hepatic porphyria in man is not known although

certain clinical and experimental observations imply that such agents may be endocrine in origin.[10] It is suggested that steroids, produced endogenously, may be one class of such agents which periodically exacerbate hepatic porphyria in certain patients carrying the genetic lesion[3] for this disorder. The relation of spontaneous attacks of porphyria to puberty, pregnancy, and the menstrual cycle support this idea, as do recent observations on the deleterious effects of administered steroids,[11] such as contraceptive steroid mixtures,[12] on this disease. The most potent exacerbating steroids presumably would be compounds of the structures characterising the steroids listed in Table 1. It would further be expected that the porphyrin-inducing effects of these steroids would be enhanced by any process, such as liver damage, glucose deprivation or starvation, and so on, which inhibited steroid glucuronidation or led to intracellular accumulations of unconjugated steroids in concentrations sufficient to further impair repressor control of the already mutated operator gene in hereditary hepatic porphyria.[3] The relation between these chemical events and the abdominal and neurological symptoms of hepatic porphyria has not yet been established.

With respect to one clinical symptom, fever, which is common during acute attacks of porphyria, it may be of potential interest to note that certain 5β-H steroids which have strong porphyrinogenic action in liver cell culture (Table 1) also have strong fever-producing action in man.[13, 14]

The stimulation of heme biosynthesis by certain steroids suggests the possibility of their use clinically to increase hemoprotein formation in liver in order to facilitate drug-detoxifying processes, or to increase hemoprotein formation in erythropoetic or other tissues in some disease states.

Summary.—A number of 5β-H (A:B *cis*) steroids of the C-19 and C-21 series strongly stimulate porphyrin synthesis in chick embryo liver cells in culture. These steroids are metabolic products of steroid hormones. The present studies suggest that these metabolites have a hormone function—the stimulation of heme biosynthesis.

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