GLUCOCORTICOID POTENTIATION OF δ-AMINOLEVULINIC ACID SYNTHETASE IN CHICK EMBRYO LIVER CELLS: A "PERMISSIVE" EFFECT

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Summary: Glucocorticoids at physiologic concentrations do not induce δ-aminolevulinic acid synthetase, the rate-limiting enzyme in the heme biosynthetic pathway, in cultured chick embryo hepatocytes. Etiocholanolone-mediated induction of this enzyme is however markedly potentiated by cortisol (2.6-fold vs. 4.4-fold). The order of effectiveness in this "permissive" effect is dexamethasone ≥ cortisol > corticosterone > progesterone. The addition of 17α-methyltestosterone, an "anti-glucocorticoid", substantially decreased the action of cortisol. This "permissive" action is specific for steroid hormones possessing glucocorticoid activity and may play an important role in heme biosynthesis.

The rate-limiting enzyme in heme biosynthesis (1), δ-aminolevulinic acid synthetase (EC 2.3.1.37), is inducible in liver by various drugs (2) and steroids (3). This regulatory enzyme is also sensitive to both feedback inhibition (4) and to repression by heme (5). In the genetic disease acute intermittent porphyria (AIP)**, excessive ALA synthetase activity and diminished uroporphyrinogen I synthetase activity is associated with an overproduction of ALA and porphobilinogen (6). Sex steroids or their metabolites appear to be involved in the pathogenesis of AIP since the clinically manifest disease appears only post-pubertally, more frequently in females (7), and is often exacerbated by the hormonal changes associated with menstruation, pregnancy, and parturition. Additionally, the activity of a hepatic steroid 5α-reductase is also decreased in individuals with AIP (8).

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**The abbrevations used are: AIP, acute intermittent porphyria; ALA, δ-aminolevulinic acid; cAMP, adenosine 3':5'-monophosphate.

The induction of ALA synthetase by 5β-H steroids such as etiocholanolone, a metabolite of testosterone, is mediated at the transcriptional level (5,9) in chick embryo liver cells and is associated with the stimulation of a DNA-dependent RNA polymerase (10). In this study, we show that glucocorticoids at physiologic concentrations exert a "permissive" or potentiating effect on the steroid-mediated induction of ALA synthetase in these cells. Thus, glucocorticoids augment the action of other inducing steroids but are themselves non-inducing. These observations suggest that glucocorticoids may play an important role in the regulation of heme biosynthesis and, possibly, in the pathogenesis of AIP.

MATERIALS AND METHODS

Materials: Etiocholanolone was purchased from Steraloids, Inc. All other steroids were obtained from the Sigma Chemical Co. All chemicals were reagent grade and were used without further purification.

Cell culture and incubation: Monolayer cultures of liver cells from fertilized, 17-day-old chicken eggs were maintained as previously described (11). Perfused livers were minced and incubated for 10 min at room temperature in a 2.5% solution of Viokase (1.7 mg/g liver). The cells were resuspended at approximately 10^5 cells/ml in Eagle's basal medium with Earle's salts enriched 2-fold with essential amino acids and containing 10% fetal calf serum (heat-inactivated). The cell suspension was filtered through gauze and plated in 100 mm plastic tissue culture dishes. After 24 hr of incubation (37°) in 5% CO_2 in air, the medium was replaced with a serum-free medium***, the steroids added, and the cells cultured for an additional 24 hr.

ALA synthetase assay: Mitochondrial ALA synthetase activity was assayed spectrophotometrically (11,12). Aliquots of mitochondrial suspensions, prepared from cells obtained from two replicate plates, were incubated with a buffered medium containing glycine. Enzymatically formed ALA was detected after conversion to a pyrrole which was subsequently reacted with a modified Ehrlich-Hg reagent (13) to produce a colored derivative. The amount of ALA formed was calculated using an extinction coefficient of 58 x 10^3 M^{-1} cm^{-1} at 555 nm for the colored derivative; one unit of ALA synthetase is defined as the amount of enzyme catalyzing the formation of 1 nmole of ALA in 90 min at 37°. Protein was determined by the method of Lowry et al (14) using bovine serum albumin (Fraction V) as a standard.

***In the presence of fetal calf serum [Granick, S. (1966) J. Biol. Chem. 241, 1359-1375] or insulin containing serum-free medium [Granick, S., Sinclair, P., Sassa, S., and Grieninger, G. (1975) J. Biol. Chem., 250, 9215-9225] induced chick liver cells contain increased levels of intracellular porphyrins. Although cellular porphyrin levels are markedly diminished in serum-free medium, we have found that there is no decrease in the production of ALA synthetase under these conditions.

Table I. Effect of cortisol and tetrahydrocortisol on the etiocholanolone-mediated stimulation of ALA synthetase activity.

Additions	ALA Synthetase Activity* (units/mg protein)	Fold Stimulation
none	0.079±0.003	-
etiocholanolone ($3x10^{-5}M$)	0.204±0.004+	2.6
cortisol ($10^{-8}M$)	0.079±0.001	0
etiocholanolone ($3x10^{-5}M$) + cortisol ($10^{-8}M$)	0.351±0.01 ‡	4.4
tetrahydrocortisol ($10^{-8}M$)	0.074±0.003	0
tetrahydrocortisol ($5x10^{-7}M$)	0.075±0.004	0
etiocholanolone ($3x10^{-5}M$) + tetrahydrocortisol ($10^{-8}M$)	0.207±0.007	2.6
+ tetrahydrocortisol ($5x10^{-7}M$)	0.208±0.006	2.6

* Mean ± SEM of four separate determinations
\+ Significantly different from control values ($P<.05$).
‡ Significantly different from the etiocholanolone treated cells ($P<.05$).

RESULTS AND DISCUSSIONS

Table I shows that the addition of $3 \times 10^{-5}M$ etiocholanolone to the cells produced a 2.6-fold stimulation in enzyme activity. There was no stimulation of the enzyme by cortisol at $10^{-8}M$. When these two steroids were added simultaneously, however, the increase in ALA synthetase activity was approximately 72% greater than that seen in the presence of only etiocholanolone. Tetrahydrocortisol, an inactive metabolite of cortisol (15), has no glucocorticoid-like activity and did not potentiate the effect of etiocholanolone (Table I). This cortisol-mediated synergistic effect is not limited to etiocholanolone, since the concurrent addition of this glucocorticoid and any of several other naturally occurring steroid inducers of ALA synthetase resulted in a similar "permissive" phenomenon (16).

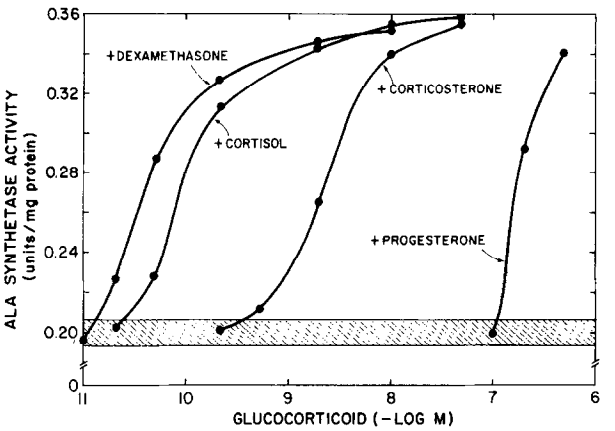

Figure 1. Dose-response relationship for glucocorticoids and etiocholanolone in the stimulation of ALA synthetase activity. The horizontal hashed region represents the stimulation of ALA synthetase by 3 x 10^{-5}M etiocholanolone. Each point represents the mean value of at least three separate cultures.

Figure 1 depicts the enhancement of ALA synthetase activity in etiocholanone treated cells by various glucocorticoids. In this experiment, each culture dish contained a constant amount of etiocholanolone and a variable concentration of glucocorticoid; the horizontal hash marks indicate the enzyme level produced by cells treated with only etiocholanolone (3 x 10^{-5}M). The order of effectiveness of the steroids in this "permissive" effect was dexamethasone $\geq$ cortisol > corticosterone > progesterone. Dexamethasone and cortisol exerted an effect at concentrations of about 5 x 10^{-11}M; at concentrations greater than 10^{-8}M these steroids resulted in a new steady-state level of ALA synthetase. Potentiation by corticosterone, the major glucocorticoid in chickens, was first detected at about 10^{-9}M. Progesterone, a compound which possesses some glucocorticoid-like activity in several other systems, was also active here but only at concentrations greater than 10^{-7}M.

The initial event in steroid-mediated enzyme induction usually involves the interaction of the steroid hormone with a specific cytoplasmic receptor protein (17). In this connection, at least one high affinity binding protein for glucocorticoids is present in chick embryo liver (18). In order to

Table II. Effect of the antiglucocorticoid, 17α-methyltestosterone, on the "permissive" action of cortisol.

Additions	ALA Synthetase Activity* (units/mg protein)
none	0.077±0.003
17α-methyltestosterone ($5x10^{-8}M$)	0.075±0.002
etiocholanolone ($3x10^{-5}M$)	0.206±0.005+
etiocholanolone ($3x10^{-5}M$) + 17α-methyltestosterone ($5x10^{-8}M$)	0.209±0.007
etiocholanolone ($3x10^{-5}M$) + cortisol ($2x10^{-10}M$)	0.308±0.008‡
etiocholanolone ($3x10^{-5}M$) + cortisol ($2x10^{-10}M$) + 17α-methyltestosterone ($5x10^{-8}M$)	0.258±0.008**

* Mean ± SEM of three separate determinations.
\+ Significantly different from control values ($P<.05$).
‡ Significantly different from the etiocholanolone treated cells ($P<.05$).
**Significantly different from the etiocholanolone+cortisol addition ($P<.05$).

implicate a glucocorticoid binding protein in the "permissive" effect seen in our system, we employed an "anti-inducing" steroid which interferes with the formation of the glucocorticoid-receptor protein complex and, thus, inhibits glucocorticoid action (15). An effective "anti-glucocorticoid" in cultured rat liver hepatoma cells, 17α-methyltestosterone, added to cultures containing both cortisol and etiocholanolone, results in a substantial decrease (51%) in the "permissive" action of the glucocorticoid (Table II). These data suggest, therefore, that the "permissive" action of the glucocorticoids in these cells requires a cytoplasmic binding event.

The term, "permissive", has been employed to indicate metabolic processes which are either diminished (19,20) or abolished (21) by adrenocortical insufficiency. While most "permissive" phenomena are associated with the humoral regulation of glucose metabolism, particularly gluconeogenesis (22,23),

Marver et al. (24) demonstrated a "permissive" role for cortisol in the induction, in vivo, of hepatic ALA synthetase by the barbiturate analog, allylisopropylacetamide. Although the synergistic actions of androgens and glucocorticoids have been described in vivo (21,25), our study shows that glucocorticoids are also able to effect steroid-mediated ALA synthetase activity in cultured cells. The increase in allantoic corticosteroid concentration during the development of the chick embryo (26) suggests that "permissive" effects may also occur in ovo.

The mechanism by which glucocorticoids influence "permissive" phenomena is not known. cAMP has been implicated in the "permissive" action of glucocorticoids, although the precise role(s) of this nucleotide in this phenomenon is not clear (22,23). In this regard, other studies suggest that the site of the "permissive" effect is distal to the formation of cAMP (27,28) and that cAMP enhances the translation of preexisting mRNA, whereas glucocorticoids stimulate specific gene transcription (29). Additionally, Gospodarowicz (30) has proposed that cortisol may increase the number of receptor sites for fibroblast growth factor in 3T3 cells and, thus, the level of stimulation by a specific concentration of the factor. One may speculate, therefore, that cortisol in the chick liver cells increases the number of cytosol receptors for etiocholanolone and, by this mechanism, potentiates the effect of inducing steroids. Chick embryo liver cells will provide a useful model system for testing this proposal and/or studying other aspects of the "permissive" effect in enzyme induction.

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