CHICK OVIDUCT PROGESTERONE RECEPTOR BINDING OF 15β,17-DIHYDROXYPROGESTERONE AND ITS ANALOGUES

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ABSTRACT

We have tested derivatives of progesterone obtained by fermentation with *Aspergillus giganteus* for relative binding affinity for the progesterone receptor of chick oviduct. Our studies show that hydroxyl and oxo substitutents at C-11 and C-15 of progesterone significantly decrease the hormone's affinity for the progesterone receptor. The loss in affinity on introduction of a C-15 hydroxyl in 17-hydroxyprogesterone is restored by acetylation to 15β-acetoxy-17 hydroxyprogesterone. The latter compound may have some potential as an in vivo agent.

INTRODUCTION

The mechanism by which progesterone binds to its receptor in target tissues can be investigated by competition experiments using analogues of progesterone possessing substituents such as hydroxyl, methyl, acetoxy, etc. This has led to various proposals about progesterone's interaction with its receptor [1-6]. In connection with another project we required a sample of 11α,15β-dihydroxyprogesterone and we therefore carried out fermentation of progesterone with Aspergillus giganteus by a procedure which has been reported to give the 11α,15β-dihydroxy derivative [7, 8]. In addition to the desired compound, we have obtained a number of other derivatives which are either new or uncommon compounds. We now report the use of these compounds to gain further insight into how substituents on progesterone affect the affinity for its receptor in the chick oviduct [9, 10].

MATERIALS AND METHODS

Fermentation of progesterone with Aspergillus giganteus was carried out according to the literature procedure [7, 8]. The product contained several hydroxylated derivatives of progesterone as indicated by high performance liquid chromatography (HPLC). A typical trace is shown in figure 1. Steroids corresponding to the various peaks were isolated by

column chromatography and their identification and purity were established by melting point, thin layer chromatography, ultra violet, infrared, nuclear magnetic resonance and mass spectroscopy.

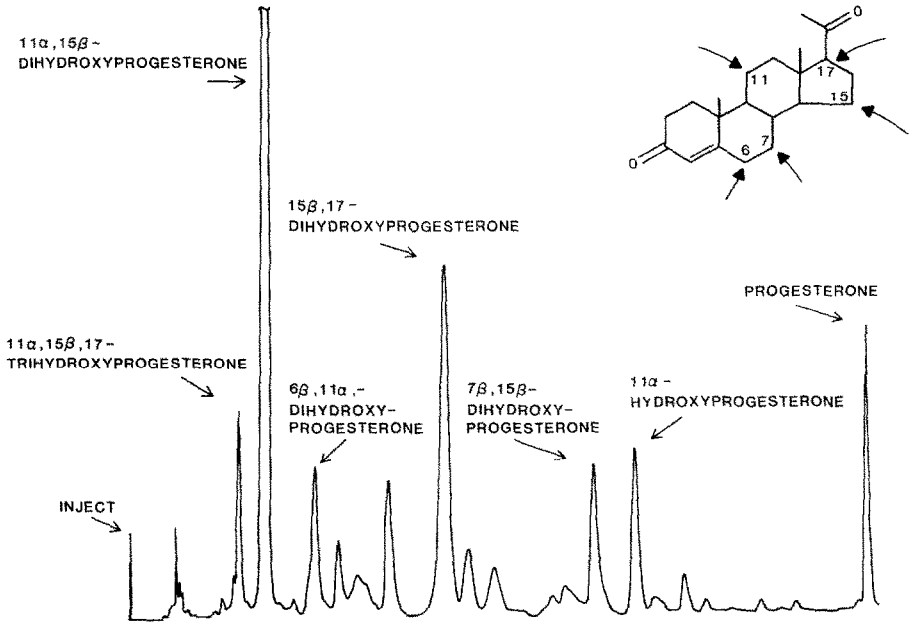

FIG. 1. High Performance Liquid Chromatography of Progesterone metabolites formed by Aspergillus giganteus. A semipreparative μ Bondapack reverse phase column (7.8 mm i.d. x 300 mm) was used with uv detection set at 254 nm; the solvent was methanol-water, initially 55:45 the proportion of methanol being increased steadily during 1 hr to a ratio of 85:15.

Hormone Binding Studies

Preparation of Cytosol. Progesterone receptor was prepared from DES primed white leghorn chicks as described by Schrader [10]. Briefly, we implanted 15 mg DES pellets (Vineland Laboratories) in day old chicks. In addition, we injected 2 mg of DES, dissolved in safflower oil, per chick per day for 5 days prior to sacrificing the chicks. Chicks were sacrificed at ages 28 to 40 days. The oviducts averaged 1.5 to 2 gm wet weight. All further procedures were done at 0-4° C. To reduce the levels of corticosteroid binding globulin (CBG), we washed minced oviduct once with calcium-free phosphate buffered saline and once with the homogenization buffer which consisted of 20 mM TES pH 7.8, 2 mM EDTA, and 12 mM monothioglycerol. Then the oviducts were homogenized (1 gm/4 ml of buffer) with a Brinkman Polytron in the cold room. The homogenate was first centrifuged at 25,000 x g for 15 min in a Sorvall Centrifuge and then the supernatant was centrifuged for 60 min at 190,000 x g in a Beckman Ultracentrifuge. The resulting supernatant was our standard chick oviduct cytosol. The fresh cytosol was assayed for progestin binding activity and aliquots were stored

at -20° C for use up to 2 months later without any loss of progestin binding activity.

Hormone Binding Assay. Steroid hormone binding assays were carried out on the cytosol in glass tubes (on ice) that contained 1.5 x 10^{-9}M ^{3}H-progesterone alone or with the following: competing steroids or a 200-fold concentration of unlabelled progesterone. The cytosol (protein concentration, ~ 20 mg/mL) was diluted 1/30 to 1/40 by incubation buffer which was 20 mM TES pH 7.8, 2 mM EDTA, 50 mM NaCl, 12 mM monothioglycerol, 2 mg/mL ovalbumin and 3 x 10^{-7}M cortisol (to bind to any CBG that was present), just before beginning the assay. Bound steroid was separated from unbound steroid using a dextran-coated charcoal assay [12] which involved incubation of 2 ml of sample solution with 0.2 mL of 100 mg/mL charcoal, 10 mg/mL dextran, 10 mg/mL ovalbumin for 1 min., and then centrifugation at 6000 x g for 15 min. to remove the charcoal. The radioactivity in 1 mL of the supernatant mixed with 10 ml of Beta Phase (Western Chemical Products) scintillation cocktail was determined in a Searle Delta 300 liquid scintillation counter. Specifically bound steroid was determined by subtracting the amount of radioactivity bound in the presence of a 200-fold excess concentration of non-radioactive steroid from the amount of tritiated steroid bound in the absence of the non-radioactive steroid. All determinations were done in duplicate. Variation between determinations was 10% or less.

RESULTS

In competitive binding studies we determined how the different progesterone analogues derived from *A. giganteus* competed with 1.5 x 10^{-9}M ^{3}H-progesterone for binding to chick oviduct cytosol. Our findings are summarized in Table 1. In general we find that hydroxyl or oxo substituents at C-11 or C-15 significantly reduce progesterone's affinity for the chick oviduct receptor (Table I).

Table 1. Effect of Progesterone Analogues on ^{3}H-Progesterone Binding to Chick Oviduct Cytosol.

	Compound	Relative Binding Affinity
a)	Progesterone	100.0
	17-Hydroxyprogesterone	0.8
b)	Derivatives with C-15 substituents	
	15β-Acetoxy-17-hydroxyprogesterone	1.0
	15β,17-Dihydroxyprogesterone	less than 0.08
	15-Oxo-17-hydroxyprogesterone	less than 0.08
	15β-Isobutyryloxy-17-hydroxyprogesterone	less than 0.08
c)	Other compounds with relative binding affinity less than 0.08	
	11α,15β,17-Trihydroxyprogesterone	
	11α,15β-Dihydroxyprogesterone	
	11α,15β-Diacetoxy-17-hydroxyprogesterone	
	15β,17-Diacetoxyprogesterone	
	11,15-Dioxo-17-hydroxyprogesterone	
	11,15-Dioxoprogesterone	

Chick oviduct cytosol was incubated with 1.5 x 10^{-9} ^{3}H-progesterone alone or with different concentrations of unlabelled progesterone or progesterone analogues at 0°, pH 7.8 for 4 hours. Then ^{3}H-progesterone specifically bound to the progesterone receptor was determined using the dextran-coated charcoal assay. 100% specifically bound progesterone = 17,000 cpm = 2 x 10^{-10}M.

DISCUSSION

Our results taken with previous reports [1-6] can provide additional information about the properties of the hormone binding site in the chick oviduct progesterone receptor. In the following analysis we consider the effects of hydrophobic and polar interactions, hydrogen bonding, and van der Waals' interactions between the hormone and the receptor and between different regions of the receptor in interpreting our results. We focus on the substitutions at C-15 which have not previously been studied in the chick oviduct receptor system.

Table 1b shows that introduction of a 15β-hydroxyl substituent reduces the affinity of 17-hydroxyprogesterone to about one tenth of its original value, but this affinity is fully restored by acetylation of the 15β hydroxyl. Possible explanations for this change are:

a) Polar Interactions. Binding to the acetoxy substituent at position 15 could be due to interaction of its electrophilic carbonyl group with nucleophile(s) on the receptor. Baker et al. [12-14] have reported that inhibitors and ester substrates of serine proteases inhibit binding of adrenal and sex steroids to their receptors. The ester substrates competitively inhibit steroid hormone binding to receptors [11, 13]. Based on these findings they proposed: 1) that the chick oviduct progesterone receptor and other steroid hormone binding proteins contain a nucleophilic recognition site that controls steroid hormone binding, 2) that this site is spatially close to and may overlap with the steroid hormone binding site, 3) that this site contains one or more histidine residues [14] and 4) that this site influences steroid hormone binding by interaction with the hormone and other regions of the receptor. Thus, substituents on progesterone that interact with this nucleophilic site or change its spatial orientation could influence the steroid's affinity for the receptor.

b) Effect on orientation of the C-17 side chain. The orientation of the C-17 acetyl side chain, which is important for binding [15], is influenced by the presence of the 15β-hydroxyl group. Dreiding models show that in a conformation having the C-20 to 0-20 bond cis coplanar with the C-15 to 0-15 bond there is a separation of about 3.8 Å between 0-15 and 0-20 so that intramolecular hydrogen bonding is unlikely [16]. However, intermolecular hydrogen bonding, perhaps involving a water molecule with the 15β-hydroxyl and the 20-oxo group, might affect the orientation of the side chain.

The reduced affinity of the corresponding 15β-isobutyrate compared to that of the 15β-acetate (Table 1b) can be attributed to difference in size of the substituents, indicating the proximity of this position of the ring D to the receptor protein.

Acetylation of 17-hydroxyprogesterone causes a ten-fold increase in affinity for the hamster and the human uterine receptor [2, 5] but reduced affinity (to one-third the former value) for the chick oviduct receptor [2]. The present study (Table 1) indicates that there is also a reduction in affinity on converting 15β-acetoxy-17-hydroxyprogesterone to the diacetate. This is further indication that the chick oviduct receptor differs from other receptors in the region of C-17.

Biological Implications

The affinity of 15β-acetoxy-17-hydroxyprogesterone for the progesterone receptor (K_d ~ 50nM) suggests that it may be a progestin agonist or antagonist. Interestingly, 11,15-dioxoprogesterone and the diesters of 11α,15β-dihydroxyprogesterone which have little affinity for the chick oviduct progesterone receptor possess progestational activity in humans [8]. This could be due to in vivo conversion to an active progestin or could reflect differences between the human and other systems. There is precedence for the latter possibility. For example, 17-acetoxyprogesterone is a potent progestin by Clauberg assay and is a fairly good competitive binder for the human receptor but not at all for the chick [2], guinea pig [4] or hamster [5].

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