TESTOSTERONE BINDING IN THE CHICK OVIDUCT

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Summary: A novel androgen receptor was observed in estrogen-stimulated chick oviducts but not in unstimulated oviducts. This binding component showed a preference for androgens and could therefore be distinguished from oviduct receptors for estadiol and progesterone. Testosterone was tightly bound having a dissociation constant (Kd) of 2.7×10^{-10} M. Sucrose gradient centrifugation, under low ionic strength conditions, showed testosterone to be bound as an 8S complex. These binding properties, plus the estrogen dependency of this component, suggest its role as a biological receptor for androgens.

INTRODUCTION

The responsiveness of the chick oviduct to estrogen and progestin administration has been well defined (1). Estrogen administration results in dramatic growth of this tissue and the development of distinctive cell types which secrete characteristic oviductal proteins (2,3). However, the role of testosterone in oviductal growth and development is less clear. Testosterone treatment alone has little demonstrable effect, but when given concurrently with estradiol, the two hormones appear to act synergistically to induce oviductal growth and protein synthesis (3,4). Investigations by Cox et al indicated that testosterone may interact with the estrogen receptor (5); but in our studies we found testosterone to have little affinity for the estrogen receptor[1]. However, in the course of this investigation we identified a testosterone binding component with properties indicative of a hormone "receptor." This receptor is novel because it is found in a female reproductive organ, has a greater affinity for 5 α-dihydrotestosterone than testosterone and is dependent upon estrogen treatment.

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[1] Harrison and Toft, manuscript in preparation

MATERIALS AND METHODS

{1,2^{3}H}-testosterone, 43.5 Ci/mM, ({^{3}H}-T) and {^{14}C}-formaldehyde, 10 mCi/mM, were obtained from New England Nuclear Corp. Unlabeled steroids and other chemicals, except as noted, were obtained from Sigma Chemical Co.

Female Rhode Island red chicks (Acme Farms, Nashville, Tennessee) were obtained at one day of age and were either used unstimulated or after subcutaneous injection of 5 mgm diethylstilbesterol (DES), in sesame oil, daily for at least fourteen days.

The chicks were killed by cervical dislocation, the oviducts removed and placed in cold Tris-buffered saline. All subsequent procedures were performed at 4° C. The oviducts were homogenized using a Polytron PT-10 (Brinkman) in 5 volumes (w/v) of TETG buffer containing 50 mM Tris-HCl, 1 mM EDTA and 10 mM thioglycerol, pH 7.5. The homogenate was centrifuged at 150,000 x g for one hour and the clear supernatant (cytosol) used within twenty-four hours.

Linear 5-20% sucrose gradients (4.6 ml) in TETG buffer were prepared using a Beckman Gradient Former. The gradients also contained either 0.01 M KCl (low salt) or 0.3 M KCl (high salt). Cytosol in these experiments contained 2×10^{-9} M {^{3}H}-T; unlabeled steroids were added, when appropriate, in a final concentration of 4×10^{-8} M (20x). All cytosol was incubated at 4° C for two hours before layering. Cytosol (0.2 ml) and 10 µl of {^{14}C}-BSA (Bovine Serum Albumin, 4.4S) were layered onto the gradients which were centrifuged in a Beckman L2-65B ultracentrifuge with a SW 50.1 rotor at 40,000 rpm for 16 hours. Twenty fractions were then collected by piercing the bottom of each tube. Sedimentation values were calculated as described by Martin and Ames (6) by comparison to the {^{14}C}-BSA peak.

Protein-bound {^{3}H}-T was determined by a charcoal assay based on the method of Korenman (7). When this technique was used unlabeled desoxycorticosterone was

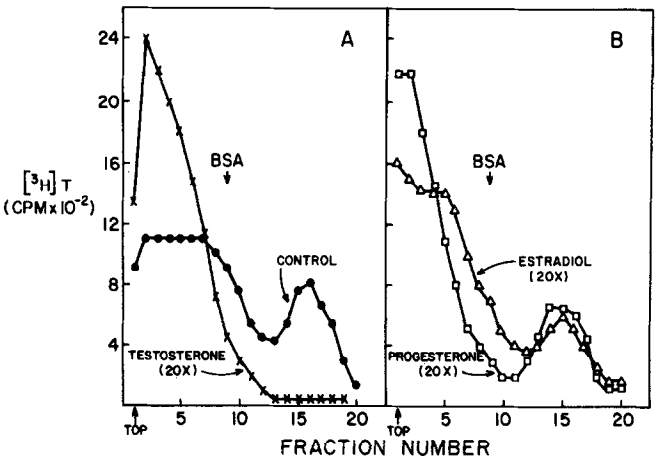

Figure 1 Testosterone binding components of chick oviduct cytosol demonstrated by sucrose gradient centrifugation. The control gradient and the effect of a 20-fold excess of unlabeled testosterone are shown in Panel A, and the effects of similar quantities of unlabeled progesterone and estradiol in Panel B.

added to the cytosol in a concentration 20-fold in excess of {^{3}H}-T in order to reduce non-specific binding. Aliquots of labeled cytosol were treated with equal volumes of a charcoal suspension {0.5% Norite A, 0.05% Dextran-80 (Pharmacia), 10 mM Tris-HCl and 1 mM EDTA, pH 7.5} and allowed to incubate for ten minutes. Centrifugation for ten minutes at 600 x g then yielded a supernatant containing protein-bound {^{3}H}-T.

Quantitative binding analysis was performed by mixing aliquots of cytosol with {^{3}H}-T in concentrations of 1 to 20 x 10^{-9} M. Duplicate aliquots containing a large excess (>1000-fold) of unlabeled testosterone were used to provide a correction for non-specific binding. After a twenty-hour incubation at 4°C, charcoal assays were performed and specific binding analyzed according to Scatchard (8). The equilibrium dissociation constant (Kd) and the concentration of binding sites (n) were calculated using the equation: {bound}/{unbound} = /Kd (n-{bound}).

The relative binding affinity of various steroids was determined, using the charcoal assay technique (7), by comparing the concentration of unlabeled steroid

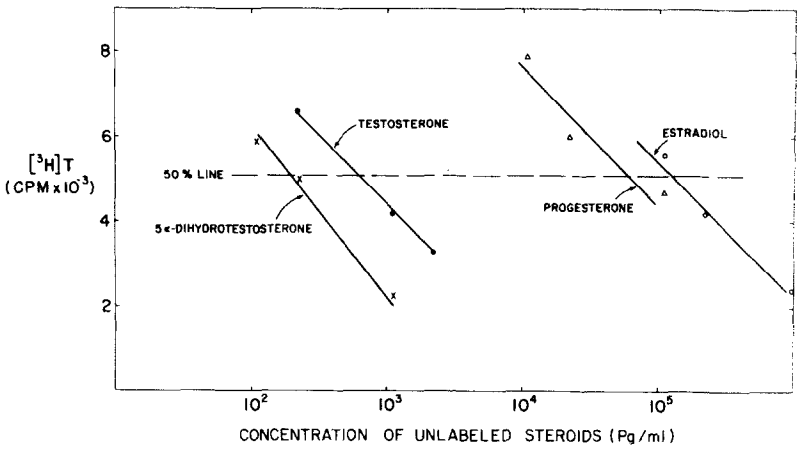

Figure 2 Competitive binding analysis using unlabeled testosterone, 5α-dihydrotestosterone, progesterone and estradiol-17β.

needed to displace 50% of the bound {^{3}H}-T to the concentration of unlabeled testosterone needed to achieve a similar effect.

Radioactivity was determined by combining the aqueous samples with a 5 ml cocktail consisting of Toluene (Baker), Triton X-100 (RPI) and Spectrafluor (Amersham-Searle), 2366: 1320: 100 (v/v). Counting efficiency was 30% in a Beckman LS 233 ambient temperature scintillation counter.

Quantitative measurement of protein was performed according to Lowry et al (9). {^{14}C}-BSA was prepared according to Rice and Means (10).

RESULTS AND DISCUSSION

The binding of {^{3}H}-T in cytosol from estrogen-stimulated oviducts was first examined using sucrose gradient centrifugation. Under low ionic strength conditions {^{3}H}-T is bound as an 8S complex (Fig. 1-A). This binding is abolished by a 20-fold excess of unlabeled testosterone (Fig. 1-A) but not by similar amounts of estradiol-17β or progesterone (Fig. 1-B). The ability of low concentrations of unlabeled testosterone but not other steroids to abolish 8S binding indicates that the steric requirements of binding are specific and that the number of binding sites is low. Binding in the 8S

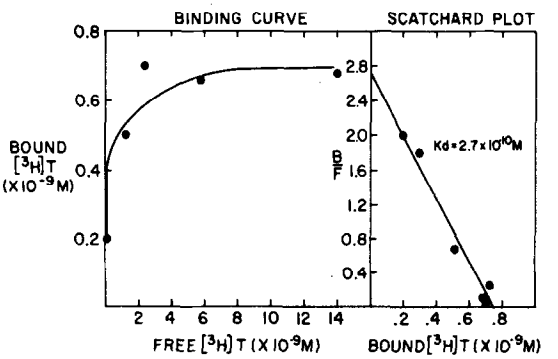

Figure 3 Binding curve (A) and Scatchard plot (B) of oviduct cytosol (18.6 mg/ml protein).

region was not seen on gradients run in high salt conditions but a slower moving 5S component appeared (not shown). The apparent dissociation of the species 8S under high salt conditions and displacement of the radioactive label with a small excess of unlabeled hormone are characteristics shown on gradient analysis of other steroid hormone receptors (11).

Specificity of binding was further examined using the charcoal assay described in Methods. Figure 2 shows that unlabeled progesterone and estradiol were less than 1% as effective as testosterone in displacing the labeled hormone. The data presented in this figure and in figure 1-B serve to distinguish {^{3}H}-T binding to a specific androgen receptor from binding to the oviduct progesterone receptor (12). Figure 2 also shows that 5 α-dihydrotestosterone is 2-3 times more effective than testosterone in displacing labeled hormone. This pattern is similar to that found for androgen binding in reproductive tissues (13) and is distinctly different from the pattern of androgen binding in mammalian uteri (14).

The binding affinity for {^{3}H}-T was determined by measuring the bound hormone over a 20-fold concentration range. Binding saturation was approached at low hormone concentrations (Fig. 3-A). These data can be expressed in a Scatchard plot as a straight line (Fig. 3-B) indicating that, within this range of steroid concentrations, a single class of binding sites are measured. The dissociation constant (Kd)

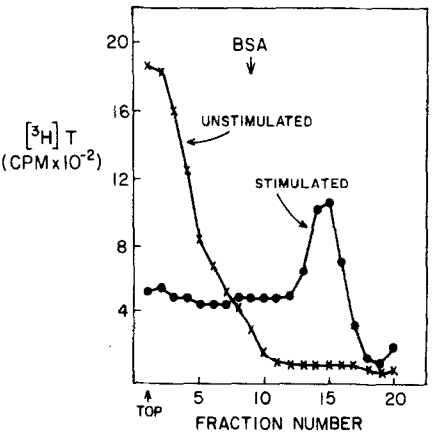

Figure 4 The effect of DES treatment on testosterone binding to oviduct cytosol. Stimulated chicks received DES daily for fourteen days. Unstimulated and stimulated chicks were of the same age. The protein content of unstimulated and stimulated cytosol was 12 and 20 mg/ml respectively.

calculated from the slope of the Scatchard plot is $\sim 2.7 \times 10^{-10}$ M. The concentration of binding sites calculated from the X intercept and corrected for protein content is 4×10^{-14} M/mg protein.

When {^{3}H}-T binding to cytosol prepared from unstimulated oviducts was analyzed by the sucrose gradient technique, no binding was observed (Fig. 4). It therefore appears that the development of androgen receptors in the oviduct requires estrogen pretreatment. This finding offers a tenable explanation for previous results showing that testosterone is effective in stimulating oviductal growth and development only when administered during estrogen treatment (3, 4). It also adds further support for a role of this binding component as a mediator in the mechanism of action of testosterone.

While the extent of testosterone's involvement is female reproduction is uncertain, this hormone is synthesized by ovarian cells (15) and can be identified in the circulating blood of mature hens (16). This, together with the information presented here, points to a possible physiologic role for testosterone in the chick oviduct. The characteristics of testosterone binding to cytoplasmic components of the chick oviduct are consistent with its identity as a steroid hormone receptor.

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