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CHARACTERIZATION AND COMPARISON OF ESTROGEN AND ANDROGEN RECEPTORS OF CALF ANTERIOR PITUITARY

E. GLENN ARMSTRONG, JR. and CLAUDE A. VILLEE*
Laboratory of Human Reproduction and Reproductive Biology, Department of Biological Chemistry, Harvard Medical School, Boston, MA 02115, U.S.A.

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SUMMARY

A comparative study of the characteristics of estrogen and androgen binding in the high speed supernatant fraction (cytosol) of calf anterior pituitary revealed that [^{3}H]-17β-estradiol and [^{3}H]-testosterone were bound to macromolecules which sediment at approximately 9 and 8s, respectively, on sucrose density gradients. The estrogen and androgen binding macromolecules are distinguishable on the basis of their ligand specificity and possess properties which distinguish true steroid hormone receptors from other steroid binding proteins. Competitive binding assays indicate that the androgen receptor has similar affinities for 5α-dihydrotestosterone and for testosterone. The affinity constants for the 17β-estradiol and testosterone binding reactions were $1.2 \times 10^{10}\,M^{-1}$ and $0.8 \times 10^{9}\,M^{-1}$ respectively. The concentration of testosterone receptor sites was determined as 2×10^{-14} mol/mg) cytosolic protein in the pituitaries of both male and female calves. A statistically significant difference was found in the concentration of 17β-estradiol receptor sites in the male (1.6×10^{-14} mol/mg protein) and female calf (3.2×10^{-14} mol/mg protein).

INTRODUCTION

It has been well established that there are specific cytoplasmic receptors for 17β-estradiol in the mammalian anterior pituitary. Such receptors have been identified in the rat [1, 2], bovine [3, 4] and human [5] anterior pituitary. The binding of 17β-estradiol by specific receptors in the anterior pituitary probably plays an important role in both negative and positive feedback control of gonadotropin secretion. Androgens have also been reported to influence gonadotropic release directly [6]. There are contradictory reports as to the occurrence of specific androgen receptors in the anterior pituitary which may be due to differences in the animal models and experimental conditions used. Korach and Muldoon[7] were unable to detect specific androgen binding in anterior pituitary cytosol from intact or long-term castrate rats. Similarly, Kahwanago *et al.*[4] failed to detect specific androgen binding in the anterior pituitaries of steers and adult female cattle. Proteins which specifically bind testosterone [8] and 5α-dihydrotestosterone [9] have been described in the cytosol of the anterior pituitary of immature rats. An androgen binding protein of anterior pituitary cytosol from short-term castrate rats which has properties that are typical of steroid hormone receptors has been well characterized by Naess *et al.*[10, 11].

To our knowledge there is no previous report of specific androgen binding in the bovine anterior pituitary. The 17β-estradiol receptor of this tissue has not been completely characterized. In the present study we have examined and compared the binding of androgens and estrogens within the high speed supernantant (cytosol) of calf anterior pituitary. The sex steroid binding properties of cytosol preparations were evaluated with regard to several criteria which are characteristic of steroid hormone receptors. These properties include ligand and tissue specificity; binding affinity and capacity; sensitivity to heat, proteolytic enzymes, and sulfhydryl group blocking agents; and salt dependent changes in sedimentation coefficients of protein–steroid complexes. Our findings indicate that the cytoplasm of calf anterior pituitary has separate and distinct receptors for estrogens and androgens.

MATERIALS AND METHODS

Materials. [2,4,6,7-^{3}H]-17β-estradiol ([^{3}H]-17β-E_2, 91 Ci/mmol), [1,2,6,7-^{3}H]-testosterone ([^{3}H]-T, 85 Ci/mmol), [1,2,4,5,6,7-^{3}H]-5α-dihydrotestosterone ([^{3}H]-5α-DHT, 80 Ci/mmol), Triton X-100, and Omnifluor were purchased from New England Nuclear. Unlabeled steroids, Trizma base, ethylenediamine tetracetate (EDTA), bovine serum albumin (BSA), protamine sulfate (Grade 1), N-ethylmaleimide (NEM), activated charcoal (untreated powder), and dextran were purchased from Sigma Chemical Company. Pronase (Grade B) was purchased from Calbiochem and ultrapure sucrose from Schwarz–Mann. All chemicals were of reagent grade or better.

Tissue. Tissues were obtained from calves (one to two weeks old) at a local slaughter house. Skulls were opened and pituitaries were removed immediately after the animals were killed by exsanguination. Male

* To whom requests for reprints should be addressed.

and female calf pituitaries were kept separate and placed in ice cold 10 mM Tris–HCl, 1.5 mM EDTA, pH 7.4 buffer (TE buffer) containing 0.9% NaCl and transported to the laboratory within thirty minutes. The anterior pituitary was separated from the posterior pituitary and capsule, weighed; rinsed in 10 mM Tris–HCl, 1.5 mM EDTA, 0.5 mM dithiothreitol, pH 7.4 buffer (TED buffer) at 4°C and minced with surgical scissors. In some experiments calf cerebellum was used as a control tissue and was treated in the manner described above.

Anterior pituitaries from three or four animals were combined for cytosol preparations. Tissues were homogenized in TED buffer which in most experiments also contained 10% glycerol (TEDG buffer) using 10 strokes of a Teflon on glass homogenizer. Tissues were homogenized in 2–3 volumes TEDG buffer for experiments using sucrose density gradient centrifugation and in 5 volumes TED or TEDG buffer for those using the protamine sulfate precipitation technique. A high speed supernatant (cytosol) was prepared by centrifugation of homogenates at 105,000 × *g* for one hour using a Beckman 50 Ti rotor in a Beckman Model L-2 ultracentrifuge at 4°C.

In some experiments blood was taken from calves immediately after death and was allowed to clot at 0°C. The blood was centrifuged at 1000 × *g* for 10 min in a Sorvall RC-2 refrigerated centrifuge. The serum was removed and diluted 1 : 4 (v/v) with TEDG buffer.

Sucrose density gradient centrifugation. Cytosols and serum were incubated at 0°C in the presence of [^{3}H]-17β-estradiol, [^{3}H]-testosterone or [^{3}H]-5α-DHT for one h. In studies of ligand specificity a concentration of unlabeled competitor steroid 100 times that of the radioactive steroid was included in the incubation medium. The effects of the sulfhydryl group blocking agent N-ethylmaleimide (NEM) on ligand binding was investigated by the inclusion of 5×10^{-3} M NEM in the incubation medium. The action of the proteolytic enzyme pronase on steroid receptors was determined by incubations of 0.5 ml aliquots of anterior pituitary cytosol with [^{3}H]-17β-estradiol or [^{3}H]-testosterone in the presence of one mg pronase. The heat sensitivity of steroid receptors was investigated by incubating anterior pituitary cytosol with [^{3}H]-steroid at 0°C for one hour followed by a thirty minute incubation at 37°C.

In studies of the effects of high salt concentration on the sedimentation properties of steroid receptors, unbound steroid was removed from the cytosol by treatment with dextran-coated charcoal. Following incubation with [^{3}H]-steroid at 0°C for one h, the cytosol was treated for 10 min at 0°C with the resuspended pellet from an equal volume of dextran-coated charcoal suspension (0.5% charcoal, 0.05% dextran in TED buffer). Charcoal was cleared from the cytosol by centrifugation for 10 minutes at 1000 × *g*.

Linear 5–15% sucrose density gradients (4.8 ml vol.) were prepared in TEDG buffer using a Buchler Universal mixing chamber (triple outlet) and a Gilson peristaltic pump. In some experiments the gradients were made to contain 0.4 M KCl. Four-tenths ml of radioactive ligand labeled cytosol or serum were layered onto the gradients. The gradients were centrifuged at 190,000 × *g* for 16 h using a Beckman SW 50.1 swinging bucket rotor in the Beckman model L-2 ultracentrifuge at 4°C. Gradients were fractionated by inserting a thin steel tube from the top to the bottom of the gradient and removing the sucrose with a Buchler peristaltic pump. Three drop (~0.2 ml) fractions were collected directly into liquid scintillation vials using an LKB ultrorac fraction collector. Two ml of scintillation fluid containing Toluene–Triton X-100 (2:1 v/v) and 0.6% Omnifluor were added to each vial. Radioactivity was determined in a Packard model 3002 scintillation spectrometer at 34% efficiency for tritium.

The sedimentation coefficients of steroid receptors were estimated by comparison to the rate of migration of ^{14}C labeled bovine serum albumin ([^{14}C]-BSA) added to the same or a different gradient (~ 9000 d.p.m. in 5 μl TE buffer). The [^{14}C]-BSA was prepared according to the method of Siiteri *et al.* [12]. When [^{14}C]-BSA was included in the same gradient with [^{3}H]-steroid labeled cytosol the spill of ^{14}C counts into the tritium window was subtracted.

Protamine sulfate precipitation assay. The protamine sulfate precipitation technique described by Korach and Muldoon[13] with some modification was used as an assay for the binding of [^{3}H]-steroid within anterior pituitary cytosol. Assays of [^{3}H]-17β-estradiol binding were performed in cytosols prepared in TED buffer and assays of [^{3}H]-testosterone binding in those prepared in TEDG buffer. One hundred μl aliquots of cytosol were added to 0.4 ml buffer containing [^{3}H]-17β-E_2 or [^{3}H]-testosterone in the presence or absence of a one hundred-fold excess of unlabeled 17β-E_2 or testosterone respectively. The incubation mixtures had a protein concentration of approximately one mg/ml. Incubations were performed in triplicate in polystyrene tubes (12 × 75 mm, Falcon No. 2025) at 4°C. Specific binding was taken as the difference in bound radioactivity in the presence and absence of a 100-fold molar excess of unlabeled steroid.

Following incubation of cytosol preparations with [^{3}H]-steroid, 0.5 ml of TED buffer containing 1 mg/ml protamine sulfate was added to each tube. The tubes were allowed to stand at 4°C for five min and then centrifuged at 1000 × *g* for ten mins. The aqueous layer was removed by aspiration and the precipitate was washed one time with one ml TED buffer. Washing of the tubes in this manner reduces the amount of non-specific binding, but has no significant effect on the specific binding of steroid hormone by receptor. The bottoms of tubes were cut off with a hot blade and the residue was dissolved in 4 ml of a Toluene scintillation cocktail containing 0.4% Omnifluor. The radioactivity of samples was determined with a 42% efficiency for tritium.

The equilibrium association constants for the binding of 17β-estradiol and testosterone and number of steroid receptor sites within anterior pituitary cytosol were determined by incubating nine different concentrations of [^{3}H]-steroid in the presence or absence of unlabeled steroid at 4°C. The concentrations of [^{3}H]-17β-E_2 ranged from 0.1–1.7 $\times$ 10^{-9} M and of [^{3}H]-T from 0.2–4.3 $\times$ 10^{-9} M. The values for specific binding were graphically analyzed according to the method of Scatchard[14].

Protein determination. The protein concentrations of cytosol preparations were determined according to the method of Lowry *et al.*[15] using bovine serum albumin as a standard.

RESULTS

Tissue specificity. Sucrose density gradient centrifugation of anterior pituitary cytosol previously incubated with [^{3}H]-17β-E_2 and [^{3}H]-T revealed the presence of macromolecules with bound radioactive ligand (Fig. 1). The 17β-E_2 and testosterone binding components sediment at approximately 9*s* and 8*s* respectively. Ultracentrifugation of identically treated cerebellum cytosol failed to show the presence of macromolecules which bind 17β-E_2 or testosterone. Calf serum was found to contain sex steroid binding macromolecules which sediment in the 4*s* region of sucrose density gradients but not in the 8–9*s* region.

Ligand specificity. The specificity of ligand binding by macromolecules of calf anterior pituitary was investigated by incubating cytosol preparations with [^{3}H]-17β-E_2 or [^{3}H]-T in the presence or absence of a 100-fold molar excess of unlabeled steroids (Fig. 2). Analysis by sucrose density gradient centrifugation showed that unlabeled estradiol or diethylstilbestrol (not shown) eliminated the binding of [^{3}H]-17β-E_2 to 9*s* macromolecules whereas testosterone was an ineffective competitor (Fig. 2-a). Conversely, the binding of [^{3}H]-testosterone was completely eliminated by a 100-fold excess of unlabeled testosterone or 5α-DHT (not shown) but was only partially inhibited by the same concentration of 17β-E_2 (Figure 2-b). Androgen binding macromolecules within the anterior pituitary were also detectable by sucrose density gradient centrifugation of cytosols incubated with [^{3}H]-5α-DHT (not shown). The binding of [^{3}H]-5α-DHT was virtually eliminated in the presence of excess concentrations of either unlabeled 5α-DHT or testosterone.

The specificity of androgen binding was further investigated by incubating calf anterior pituitary cytosols with 3 nM [^{3}H]-testosterone in the presence or absence of various concentrations of unlabeled competitor steroids and determining the amounts of specific binding by the protamine sulfate precipitation assay (Fig. 3). Testosterone and 5α-DHT at concentrations 1–100 times that of [^{3}H]-testosterone competitively inhibited specific binding to approximately

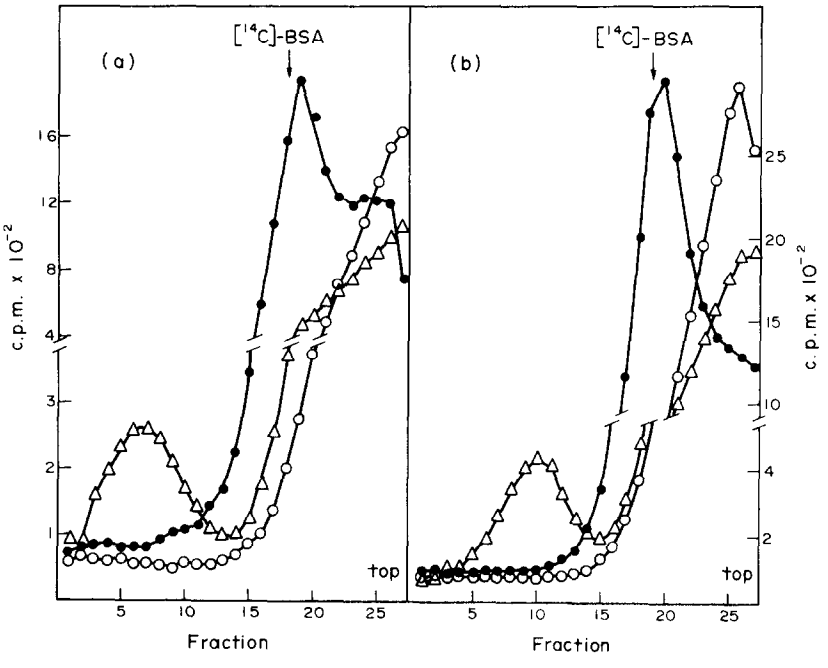

Fig. 1. Tissue specificity: a; sucrose density gradient centrifugation sedimentation profiles of male calf anterior pituitary cytosol (△——△), cerebellum cytosol (○——○), and serum (●——●) incubated with 1 $\times$ 10^{-9} M [^{3}H]-17β-estradiol. b; Sedimentation profiles of male calf anterior pituitary cytosol (△——△), cerebellum cytosol (○——○), and serum (●——●) incubated with 1 $\times$ 10^{-9} M [^{3}H]-testosterone. The protein concentrations of pituitary cytosol, cerebellum cytosol and serum were 10.3, 6.3 and 11.0 mg/ml respectively.

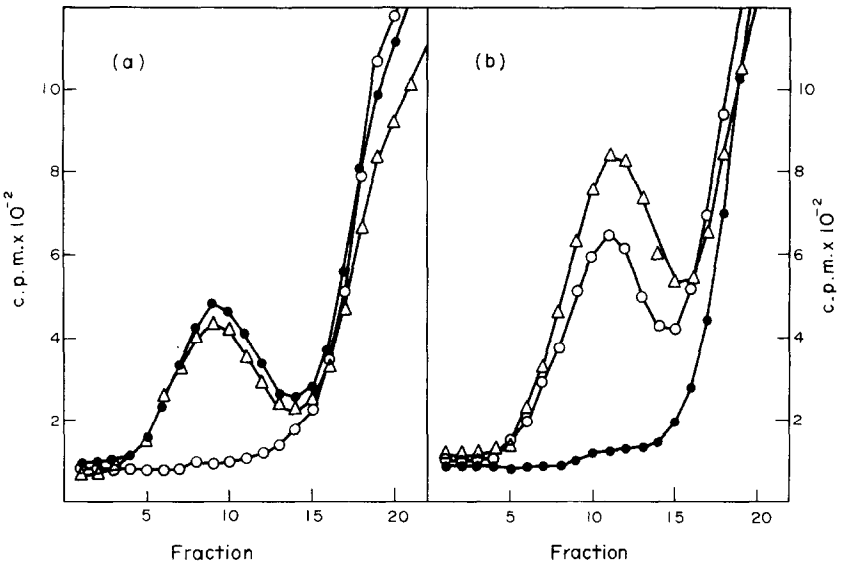

Fig. 2. Ligand specificity: a; Sedimentation profiles of male calf anterior pituitary cytosol (protein conc. = 9.2 mg/ml) incubated with 1×10^{-9} M [^{3}H]-17β-estradiol (△——△) in the absence or presence of 1×10^{-7} M 17β-estradiol (○——○) or testosterone (●——●). b; Sedimentation profiles of male calf anterior pituitary cytosol (protein conc. = 9.2 mg/ml) incubated with 1×10^{-9} M [^{3}H]-testosterone (△——△) in the absence or presence of 1×10^{-7} M 17β-estradiol (○——○) or testosterone (●——●).

the same extent. These results indicate that the androgen receptor has a similar affinity for testosterone and 5α-DHT.

17β-Estradiol was a much less effective competitive inhibitor of [^{3}H]-testosterone binding than were 5α-DHT or testosterone. A 220-fold excess concentration of 17β-E_2 was required to reduce the binding of 3 nM [^{3}H]-T to 50% of control value (Fig. 4). Cortisol concentrations up to 3 μM had no significant effect on the binding of 3 nM [^{3}H]-testosterone.

Effects of proteolytic enzymes, sulfhydryl blocking agents and heat on steroid binding. Incubation of anterior pituitary cytosols with [^{3}H]-17β-E_2 or [^{3}H]-T in the presence of pronase results in the complete loss of [^{3}H]-steroid binding in the 8–9*s* region of sucrose density gradients (data not shown). Treatment of [^{3}H]-steroid labeled cytosols at 37°C for 30 min also completely eliminates the binding of 17β-E_2 and testosterone to macromolecules which sediment at 8–9*s* (data not shown). The addition of 5×10^{-3} M NEM to anterior pituitary cytosol markedly inhibited the binding of [^{3}H]-17β-E_2 or [^{3}H]-T as measured by sucrose density gradient centrifugation (data not shown). These results indicate that the 17β-E_2 and testosterone binding macromolecules of calf anterior pituitary are both heat sensitive proteins which require free sulfhydryl groups for binding activity.

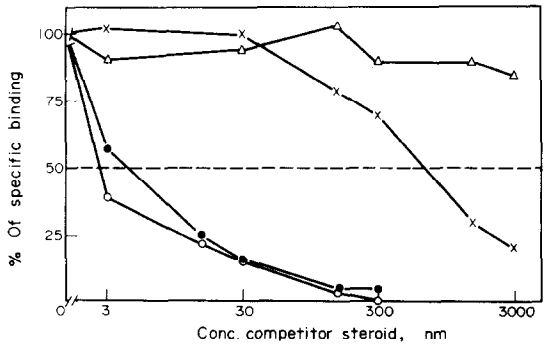

Fig. 3. Competitive Binding: Female calf anterior pituitary cytosol (protein conc. = 0.7 mg/ml) were incubated with 3×10^{-9} M [^{3}H]-testosterone in the absence or presence of various concentrations of testosterone (●——●), 5α-dihydrotestosterone (○——○), 17β-estradiol (×——×), or cortisol (△——△). Binding was analyzed by the protamine sulfate precipitation technique and the data were expressed as the percentage of total specific binding detected in the presence of unlabeled steroid.

Effect of salt on the sedimentation coefficient of the protein–steroid complex. Calf anterior pituitary cytosols previously incubated with [^{3}H]-17β-E_2 or [^{3}H]-T were centrifuged on sucrose density gradients in the presence or absence of 0.4 M KCl (Fig. 4). In both cases the protein–steroid complex observed in the 8–9*s* region of low salt gradients was converted to a 4–5*s* complex on gradients containing 0.4 M KCl. The specificity of the binding observed in the 4–5*s* region was demonstrated by the fact that the binding of both radioactive steroids was virtually eliminated by a 100-fold molar excess of unlabeled steroid.

Binding of steroids with time. Time studies of the binding of [^{3}H]-17β-E_2 and [^{3}H]-T in calf anterior pituitary cytosol were performed using the protamine sulfate precipitation assay in order to determine the amount of time required for the binding reactions to reach equilibrium conditions. It was found that the binding of [^{3}H]-17β-E_2 was maximal after 6 h of incubation at 4°C and the binding remained stable up to 20 h (Fig. 5-a). The binding of [^{3}H]-T was maxi-

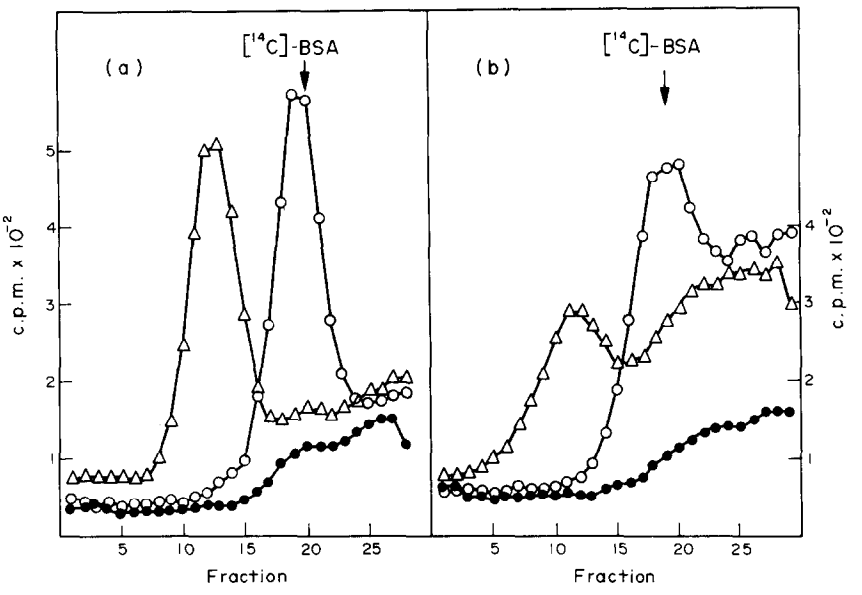

Fig. 4. Effect of salt concentration on sedimentation coefficients: a: Sedimentation profiles of female calf anterior pituitary cytosol (protein conc. = 7.8 mg/ml) incubated with 1×10^{-9} M [^{3}H]-17β-estradiol on sucrose density gradients containing no KCl (△——△) or 0.4 M KCl (○——○). Cytosol incubated with 1×10^{-9} M [^{3}H]-17β-estradiol in the presence of 1×10^{-7} M 17β-estradiol centrifuged on 0.4 M KCl gradients (●——●). b; Sedimentation profiles of male calf anterior pituitary cytosol (protein conc. = 12.9 mg/ml) incubated with 1×10^{-9} M [^{3}H]-testosterone on sucrose density gradients containing no KCl (△——△) or 0.4 M KCl (○——○). Cytosol incubated with 1×10^{-9} M [^{3}H]-testosterone in the presence of 1×10^{-7} M testosterone on 0.4 M KCl gradients (●——●).

mal after 2 h of incubation at 4°C and remained constant up to 6 h (Fig. 5-b). However, at time periods greater than 6 h (12 and 24 h) there was a decrease in specific binding of [^{3}H]-T suggesting instability of the binding protein–steroid complex under these experimental conditions. Therefore, quantitative assays of testosterone binding were conducted using incubation periods of 4–6 h.

Saturation analysis. Calf anterior pituitary cytosol was incubated in the presence of various concentrations of [^{3}H]-17β-E_2 or [^{3}H]-T in the presence or absence of a 100-fold molar excess of unlabeled 17β-E_2 or testosterone, and binding assayed by the protamine sulfate precipitation technique. Representative plots of 17β-E_2 and testosterone binding are shown in Fig. 6. Figure 6-a shows that specific 17β-E_2 binding sites were saturated with approximately a 1 nM hormone concentration. However a several fold greater concentration of testosterone (3–4 nM) was required to saturate the specific androgen binding sites (Fig. 6-b).

Scatchard plots. The data for specific binding of 17β-E_2 and testosterone presented in Fig. 6 were graphically analyzed according to the method of Scatchard [14] and plotted in Fig. 7. The plot for 17β-E_2 binding (Fig. 7-a) indicates a single class of binding sites with a K_a of 6.5×10^9 M^{-1} and a concentration of 1.1×10^{-11} M. This concentration of binding sites is equivalent to 1.2×10^{-14} mol per mg of cytosolic protein. In comparison, testosterone was bound to a single class of binding sites with a K_a calculated as 1.1×10^9 M^{-1} and a concentration of 8.6×10^{-12} M or 1.1×10^{-14} mol/mg protein (Fig. 7-b).

Comparison of sex steroid binding in male and female calves. The data obtained from Scatchard plots for 17β-E_2 and testosterone binding in male and female calf anterior pituitary cytosols were averaged and tabulated for comparison (Table 1). The K_a for the 17β-E_2 binding reaction was an order of magnitude greater than that for the testosterone binding reaction. There were no significant differences in the association constants for 17β-E_2 or testosterone binding in the anterior pituitary cytosols of male and female calves.

The difference in the concentrations of androgen binding sites in male and female calf anterior pituitary cytosols was not statistically significant. However, there was a statistically significant difference ($P < 0.01$) in the concentration of 17β-E_2 binding sites in anterior pituitary cytosols from the two sexes: the concentration of 17β-E_2 binding sites in the female anterior pituitary is twice that of the male.

DISCUSSION

These studies indicate that the cytoplasm of calf anterior pituitary contains specific receptors for both 17β-estradiol and androgens. This conclusion is based on the finding that cytosol preparations of this tissue have two distinctly different macromolecules which bind estrogens and androgens, respectively, and have properties which are characteristic of steroid hormone receptors. These properties include steroid specificity,

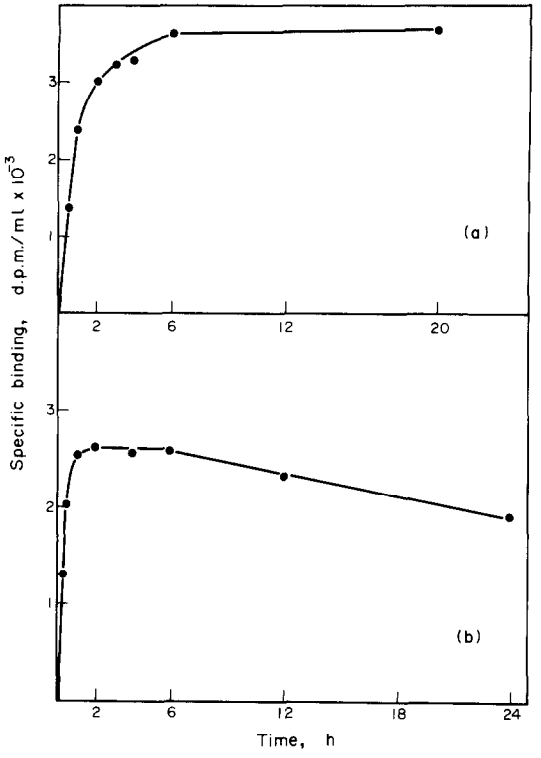

Fig. 5. Time studies of ligand binding: a; Specific binding of 5×10^{-10} M [^{3}H]-17β-E_2 in male calf anterior pituitary cytosol (protein conc. = 0.7 mg/ml) with time. b; Specific binding of 1×10^{-9} M [^{3}H]-testosterone in male calf anterior pituitary cytosol (protein conc. = 1.1 mg/ml) with time. Nonspecific binding was determined by the inclusion of a 100-fold molar excess of unlabeled steroid in reaction mixtures and was subtracted from total binding for determination of specific binding. Assays were performed using the protamine sulfate precipitation technique.

tissue specificity, heat lability, sensitivity to proteolytic enzymes and sulfhydryl group blocking agents and salt dependent changes in sedimentation coefficients [16].

Both 17β-E_2 and testosterone are bound to saturable, high affinity sites in calf anterior pituitary cytosols. The association constant for 17β-estradiol binding (1.2×10^{10} M^{-1}) was an order of magnitude greater than that for testosterone (0.8×10^9 M^{-1}). These values are similar to those reported for the association constants for 17β-estradiol receptors [1, 2] and testosterone receptors [8, 9, 10] in the rat anterior pituitary.

The concentration of 17β-estradiol receptor sites determined in the present studies was $1.6 \pm 0.2 \times 10^{-14}$ mol/mg of cytosol protein in the male calf pituitary and $3.2 \pm 0.3 \times 10^{-14}$ mol/mg protein in the female pituitary ($P < 0.01$). These values are an order of magnitude lower than those reported for the rat anterior pituitary. Korach and Muldoon[2] reported that the concentration of 17β-E_2 binding sites in the rat anterior pituitary was 2×10^{-13} mol/mg of cytosol protein. The different 17β-E_2 receptor levels in the bovine and rat anterior pituitary may reflect a difference in the control mechanisms for gonadotropin secretion in the two species. Korach and Muldoon[2] also found that the concentration of 17β-E_2 binding sites was the same in male and female rats and immature and adult animals of either sex. Other authors have also reported no statistically significant difference in the concentration of 17β-estradiol binding sites in male and female rat anterior pituitary [17, 18]. The reason for a sex related difference in the concentration of 17β-estradiol binding sites in the calf

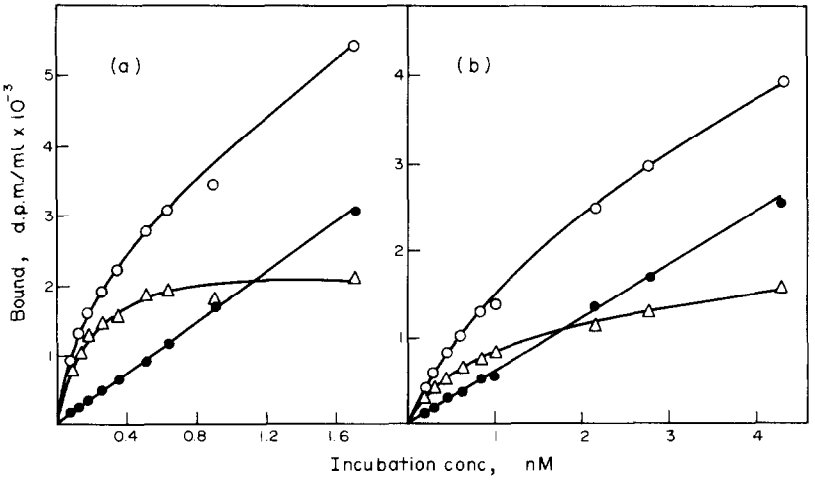

Fig. 6. Saturation analysis: a; Binding of various concentrations of [^{3}H]-17β-E_2 by male calf anterior pituitary cytosol (protein conc. = 0.9 mg/ml) following incubation at 4°C for 18 h. Specific binding (△——△) was determined as the difference between total binding (○——○) and binding in the presence of a 100-fold molar excess of unlabeled 17β-estradiol (●——●). b; Binding of various concentrations of [^{3}H]-testosterone by male calf anterior pituitary cytosol (protein conc. = 0.8 mg/ml) following incubation at 4°C for 4 h. Specific binding (△——△) was determined as the difference between total binding (○——○) and binding in the presence of a 100-fold molar excess of unlabeled testosterone (●——●). Assays were performed using the protamine sulfate precipitation technique.

Table 1. Comparison of estrogen and androgen binding in the cytosols of male and female calf anterior pituitaries

	17β-Estradiol			Testosterone		
	K_a ($M^{-1} \times 10^{10}$)	Receptor site conc. (mol/mg protein $\times 10^{-14}$)	Number of determinations	K_a ($M^{-1} \times 10^{8}$)	Receptor site conc. (mol/mg protein $\times 10^{-14}$)	Number of determinations
Male	1.1 ± 0.2*	1.6 ± 0.2	7	8.4 ± 0.6	1.7 ± 0.3	5
Female	1.2 ± 0.2	3.2 ± 0.3	5	7.7 ± 0.8	2.1 ± 0.5	7
Statistical significance	N.S.†	$P < 0.01$‡		N.S.	N.S.	

* Standard Error † Not statistically significant ‡ P value determined by two sample t test

but not the rat anterior pituitary is not readily apparent. It is conceivable that in cattle but not in the rat the greater concentration of 17β-estradiol binding sites in the anterior pituitary of the female plays a role in the development of cyclicity of gonadotropin secretion.

The present studies did not reveal any difference in the binding of testosterone by anterior pituitaries from male and female calves. The concentration of specific androgen binding sites in this tissue is quite similar to the values reported for the rat. The concentration of specific testosterone binding sites in rat anterior pituitary was reported as 1.2×10^{-14} mol/mg protein by Naess *et al.*[9] and as 2×10^{-14} mol/mg protein by Samperez *et al.*[8].

The discrepancy between the present report and that by Kahwanago *et al.*[4] with regard to the occurrence of specific androgen receptors in the bovine anterior pituitary may be related to the differences in the age and hormonal states of the animals used and/or the techniques employed. It is conceivable that the androgen receptor of calf anterior pituitary is no longer present in adult female or castrated male cattle. In the present study, but not that of Kahwanago *et al.*[4], 10% glycerol was included in cytosol preparations used for androgen binding assays.

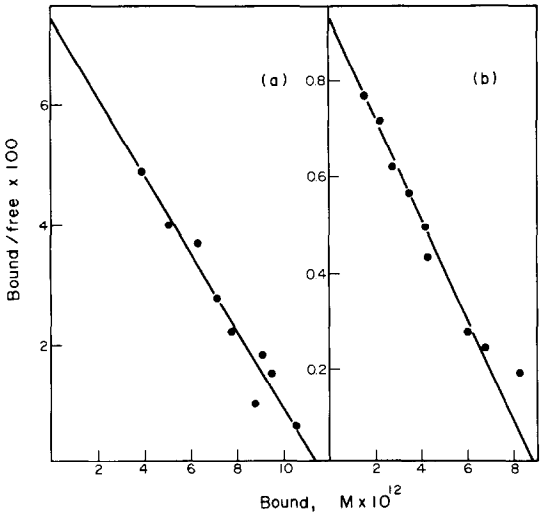

Fig. 7. Scatchard plots: a; Analysis of data for specific binding of 17β-estradiol presented in Fig. 6-a according to the method of Scatchard [14]. B; Analysis of data for specific binding of testosterone presented in Fig. 6-b.

Androgen receptors have been shown to be stabilized by glycerol [19] and this difference may be significant.

Competitive binding studies indicate that the androgen receptor of calf anterior has similar affinities for testosterone and 5α-dihydrotestosterone. The conversion of testosterone to 5α-DHT may not be an essential step in the mechanism of action of androgens in the anterior pituitary as in the prostate [20]. Naess *et al.*[10] reported that 5α-DHT and testosterone compete equally well with [^{3}H]-testosterone for binding in the rat anterior pituitary cytosol. However, those authors also found that 5α-DHT was extensively converted to 5α-androstane-3α,17β-diol in their incubation mixtures, which would cause the affinity of 5α-DHT for the receptor to be underestimated.

Other investigators have determined the equilibrium dissociation constants for both 5α-DHT [8] and testosterone [11] binding in rat anterior pituitary cytosol and have found that 5α-DHT is bound with a greater affinity than testosterone. However, it was not determined whether the rat anterior pituitary has two different receptors which bind 5α-DHT and testosterone respectively or a single androgen receptor which binds both but with different affinities. The 5α-reductase enzyme which converts testosterone to 5α-DHT has been demonstrated in rat anterior pituitary [21], but the enzyme in the anterior pituitary had relatively weak activity compared to that of the prostate. Further studies are required to determine whether the conversion of testosterone to 5α-DHT is important in the mechanism of action of androgens in the anterior pituitary as it is in the prostate.

These studies have demonstrated that androgen as well as estrogen binding proteins are present in the cytoplasm of calf anterior pituitary. By every criterion examined these binding proteins have properties that are characteristic of true steroid hormone receptors. However, the roles these androgens and estrogen receptors play in the control of anterior pituitary function, particularly in the feedback control of gonadotropin secretion by circulating sex hormones, have yet to be elucidated.

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