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SUCROSE DENSITY GRADIENT CHARACTERIZATION OF CYTOPLASMIC [1,2-H^3]-5α-DIHYDROTESTOSTERONE BINDING PROTEINS IN THE ACCESSORY SEXUAL GLANDS OF THE MALE SYRIAN HAMSTER

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SUMMARY

Cytoplasmic extracts (105,000 *g* supernatants) prepared from the accessory sexual glands of androgen stimulated male Syrian hamsters contained proteins which bound [1,2-H^3]5α-dihydrotestosterone and migrated with sedimentation coefficients of 8–9*S* on linear sucrose gradients. Although similar binding and components were demonstrated in cytosols prepared from liver and kidney tissue, they were absent in serum and cytosols prepared from lungs and autonomous prostatic adenocarcinomas. Like the classical androgen receptor, binding to this 8–9*S* component was abolished by incubation at 60°C or by preincubation with pronase or parahydroxymercuribenzoate. Unlike androgen receptors, however, the sedimentation rate of this 8–9*S* component was not altered at high ionic strength (0.6 M KCl). Competition studied indicated that a large excess of nonradioactive 5α-dihydrotestosterone only partially inhibited the 8–9*S* binding of the radioactive androgen. Although [1,2-H^3]5α-dihydrotestosterone and [1,2,6,7-H^3]progesterone were both bound to this 8–9*S* component, similar binding of [2,4,6,7-H^3]estradiol was not detected. The 8–9*S* binding component was precipitated with 33–50% saturated $(NH_4)_2SO_4$ and gel filtration of the redissolved proteins revealed only one major peak of [1,2-H^3]5α-dihydrotestosterone binding. These data suggest the presence of low affinity 8–9*S* binding proteins in androgen sensitive tissues of the male hamster which are distinct from 8*S* androgen receptors and may be involved in the intracellular accumulation of androgens.

INTRODUCTION

Once circulating testosterone has diffused into androgen target cells, it is reduced to 5α-dihydrotestosterone which may then bind to several proteins. This binding may be specific or nonspecific. Specific binding is characterized by a high affinity, saturation at physiological concentrations of the hormone and ligand specificity, while nonspecific binding is of low affinity, nonsaturable, and lacks ligand specificity. Receptor proteins which specifically bind 5α-dihydrotestosterone have been identified in cytoplasmic and nuclear extracts prepared from accessory sexual glands of several species [1]. Similar specific cytoplasmic receptors have also been identified in other androgen responsive tissues including rat testis [2], hamster flank organ [3], and Shionogi androgen-dependent mouse mammary tumors [4]. Mainwaring and Mangan [4] studied several species, excluding hamsters, and concluded that the accessory sexual glands contain receptors that are similar to the more widely studied and characterized receptors in rat ventral prostrate glands [5, 6].

Although in initially proved difficult to demonstrate these binding components to be different by physicochemical techniques, separation of high affinity from low affinity binding components was eventually achieved by using sucrose density gradient analyses of labeled cytosols. With this approach Mainwaring [7] found a region of nonspecific binding (3.5*S*) and a region of specific binding (8*S*) in rat ventral prostrate cytosols incubated with [1,2-H^3]-5α-dihydrotestosterone. Our laboratory has confirmed the presence of these two binding regions in rat ventral prostrate cytosols [8].

The present study was undertaken to investigate the physicochemical properties of the cytoplasmic 5α-dihydrotestosterone binding protein(s) in the accessory sexual glands of male Syrian hamsters using sucrose density gradient analyses. A preliminary report of this study has been published [9].

EXPERIMENTAL

Experimental animals. Outbred male Syrian hamsters were obtained from the Lakeview Hamster Colony (Newfield, NJ) and averaged 90–120 gm in body weight. All animals had *ad libitum* access to commercial rodent chow and tap water, and were exposed to 11 h of light and 13 h of darkness daily. All castrations were performed under ether anesthesia and via the scrotal route. A transplantable hamster prostrate adenocarcinoma originally described by Fortner *et al.* [10] was obtained from the Southern Research Institute in Birmingham, AL. Small pieces (2–3 mm^2) of nonnecrotic tissue were suspended in Ringer's solution and injected into the flanks of recipient hamsters.

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Based upon its ability to grow in castrated plus adrenalectomized hamsters we classified this tumor as androgen-independent.

Chemicals. [1,2-H^3]-5α-Dihydrotestosterone (17β-hydroxy-5α-androstan-3-one; 40 Ci/mmol), [2,4,6,7-H^3]-estradiol (110 Ci/mmol) and [1,2,6,7-H^3]-progesterone (82 Ci/mmol) were obtained from the New England Nuclear Corp. All radioactive steroids were stored in ethanol under refrigeration. The purity of [1,2-H^3]-5α-dihydrotestosterone was periodically verified with thin layer chromatography. Nonradioactive steroids including 5α-dihydrotestosterone, testosterone, and androstanediol (5α-androstan-3α, 17β-diol) as well as pronase, *p*-hydroxymercuribenzoate, bovine serum albumin and glyceraldehyde-3-phosphate dehydrogenase were obtained from the Sigma Chemical Co. Cyproterone acetate (6α-chloro- 17α-acetoxy-1α,2α-methylene-4,6-pregnadiene-3,20-dione) was a gift of Dr. Stanley Gould, Berlin Laboratories, New York, NY and 17α-methyl-B-nortestosterone (17α-methyl-17β-hydroxy-B-nor-4-androstene-3-one) was a gift from the Research Laboratories, Smith, Kline and French Laboratories, Philadelphia, PA.

Fractionation of tissues. Eight to ten castrated hamsters per experiment were decapitated in a cold room and tissues were excised and rinsed in 0.02 M sodium-phosphate buffer (pH 7.4 at 4°C) containing 1.5 mM EDTA and 2 mM mercaptoethanol. This buffer was selected because it gave optimal 8S binding of [1,2-H^3]-5α-dihydrotestosterone in cytosols prepared from rat ventral prostrate glands (Wakeling and Visek, unpublished data). After mincing, the tissues were homogenized in 4–5 ml of buffer with a motor-driven Teflon–glass homogenizer cooled in an ice bath. The homogenates were then centrifuged at 128,000 *g* for 1 h in the SW 50.1 rotor of a Spinco Model L2-65B ultracentrifuge. The resultant cytosols were frozen and stored at −30°C. Storage of the cytosols for several weeks had no apparent effect on the amount or the properties of the binding.

Labeling of cytosols. Cytosols were incubated at 0–4°C for 1 h. Longer incubations did not increase [1,2-H^3]-5α-dihydrotestosterone binding. Each incubation vial received the appropriate mass of radioactive or nonradioactive steroid dissolved in absolute ethanol. Following incubation the labeled cytosols were treated with a suspension of 0.5% dextran–5% charcoal (Norit A. Fisher Scientific Co.) prepared in sodium-phosphate buffer in order to absorb a portion of the unbound steroid [11, 12]. The cytosols were then agitated vigorously and placed on ice for approximately 10 min before centrifugation at 3,000 *g* for 5 min in a clinical centrifuge.

Sucrose density gradient analyses. Linear 5–15% sucrose gradients (prepared in 0.02 M phosphate buffer) containing 10% (v/v) glycerol [13] were prepared in 5 ml cellulose nitrate tubes with the aid of a two chambered gradient former and a Buchler gradient mixer were equilibrated for approximately 9 h. Aliquots (0.3 or 0.5 ml) of labeled cytosols were then layered on the gradients and the tubes were centrifuged at 149,000 *g* for 14 h in the SW 50.1 rotor. Fractionation of the gradients was accomplished by the upward displacement of the contents of each tube with a 70% sucrose solution using a Harvard infusion/withdrawal pump and an ISCO Density Gradient Fractionator (Model 184). Each of the 30 six-drop fractions was collected in a scintillation vial. The vials were advanced by a Model 328 Fraction Collector (Instrument Specialties Co., Lincoln, NE). After addition of 0.4 ml distilled H_2O and 10 ml Aquasol (New England Nuclear), the radioactivity of each vial was assayed in a Nuclear-Chicago Mark II Scintillation Counter. The counting efficiency (average efficiency = 33%) was determined by the channels ratio method using chloroform as the quenching agent. The amount of bound radiolabeled steroid was calculated from the area under the 8–9S peak. These values were then expressed as a percent of control or converted to the mass of steroid bound. The apparent sedimentation coefficient of the radioactive peaks as well as the approximate molecular weight of the binding proteins were calculated according to Martin and Ames [14] using bovine serum albumin and glyceraldehyde-3-phosphate dehydrogenase as markers.

Ammonium sulfate-fractionation and sephadex chromatography. Cytoplasmic proteins were precipitated by the gradual addition of saturated $(NH_4)_2SO_4$ (adjusted to pH 7.4 with aqueous NH_3) during constant stirring at 0–4°C [15]. The precipitated proteins were collected by centrifugation and redissolved in phosphate buffer. Aliquots were layered on linear 5–15% sucrose gradients and analyzed as previously described. In some cases unfractioned cytosols were incubated with [1,2-H^3]-5α-dihydrotestosterone prior to addition of $(NH_4)_2SO_4$ while in other experiments the radioactive androgen was added to the dissolved protein fractions. Gel chromatography was performed on a column (32 cm × 1 cm) of Sephadex G 200 (Pharmacia Fine Chemicals Inc.). The radioactive samples were eluted with sodium-phosphate buffer and dextran blue was used to determine the void vol.

RESULTS

Detection of cytoplasmic 5α-dihydrotestosterone binding proteins

Macromolecules which bound [1,2-H^3]-5α-dihydrotestosterone were detected through the use of sucrose density gradients, in cytosols prepared from the seminal vesicles (Fig. 1a) and coagulating glands of hamsters castrated 2–3 days earlier, but not in ventral prostrate or Cowper's gland cytosols. In order to induce synthesis of the binding macromolecule in these later tissues, hamsters were injected subcutaneously with 2 mg of 5α-dihydrotestosterone for 12 days and then castrated. Three days after castration all of the accessory sexual gland cytosols from these treated hamsters contained binding complexes having a sedimentation coefficient of 8–9S (Fig. 1a) and an

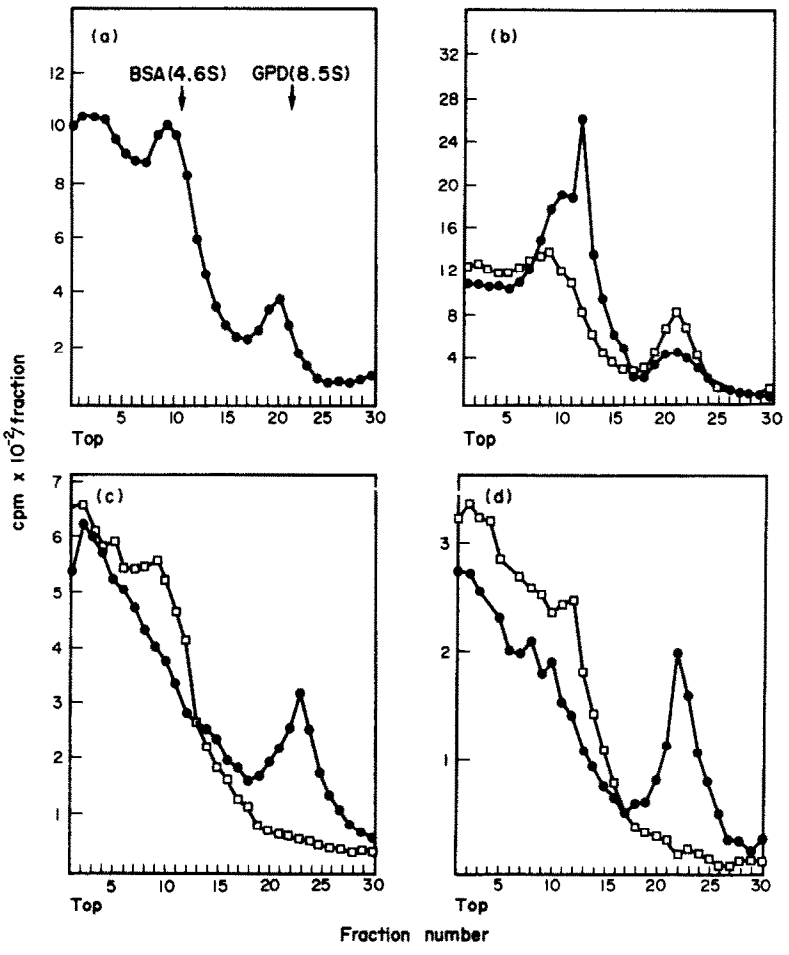

Fig. 1. Sucrose density gradient analyses of cytoplasmic [1,2-H^3]-5α-DHT binding. All cytosols prepared from pooled tissues of 8–10 hamsters. a. Determination of sedimentation coefficient of cytosol [1,2-H^3]-5α-DHT–receptor complex. Cytosol prepared from seminal vesicles of 2 day castrates. After incubation for 1 h at 0–4°C cytosol was centrifuged at 149,000 *g* for 14 h. Standard protein markers (BSA, 4.6*S*; GPD, 8.5*S*) were centrifuged simultaneously through identical 5–15% linear sucrose gradients. Positions of markers determined by measuring O.D. at 280 mμ. b. [1,2-H^3]-5α-DHT (7.4×10^{-8} M) binding by cytosols isolated from the livers (●——●) and kidneys (□——□) of hamsters castrated for 5 days. Incubation at 0–4°C for 4 h. c. [1,2-H^3]-5α-DHT (1.86×10^{-8} M) binding by cytosols isolated from ventral prostrates (●——●) and a prostatic adenocarcinoma (□——□). Incubation for 4 h at 0–4°C. d. Seminal vesicle cytosol incubated for 1 h at 0–4°C with either 0.1 N NaOH (control) (●——●) or 10^{-3} M *p*-hydroxymercuribenzoate (dissolved in 0.1 N NaOH) (□——□) prior to incubation for 4 h at 0–4°C with [1,2-H^3]-5α-DHT (4.6×10^{-9} M).

approximate molecular weight of 1.6×10^5. The 4–5*S* peaks were often indistinct or represented by small shoulders which may have arisen from serum contamination. Although treatment of the labeled cytosols with dextran charcoal had no effect on the localization or concentration of bound [1,2-H^3]-5α-dihydrotestosterone in the 8–9*S* peaks, it was effective in reducing the excess free androgen at the top of the gradients. Cytosols prepared from kidneys and livers of intact or castrated hamsters demonstrated similar 8–9*S* peaks as well as distinctive 4*S* peaks (Fig. 1b), but this binding was not as thoroughly characterized. Peaks sedimenting at 8–9*S* were not detected in serum or cytosols prepared from lung tissue. Although 8–9*S* binding peaks were detectable in prostatic cytosols they were absent in cytosols prepared from autonomous prostatic adenocarcinomas (Fig. 1c). Preincubation (1 h at 0–4°C) of the accessory sexual gland cytosols with pronase (1 mg/ml) or *p*-hydroxymercuribenzoate (10^{-3} M) dissolved in 0.1 N NaOH (Fig. 1d) abolished all binding of [1,2-H^3]-5α-dihydrotestosterone. The same vol. of 0.1 NaOH added to the control cytosol (Fig. 1d) had essentially no effect on the 8–9*S* binding.

Effect of temperature and ionic strength

Short incubations at 37°C increased [1,2-H^3]-5α-dihydrotestosterone binding but prolonged incubations at this temperature had variable effects. In some cases prolonged incubations at 37°C abolished all 8–9*S* binding while in the experiment shown in Fig. 2, incubation at 37°C for 2 h increased binding by

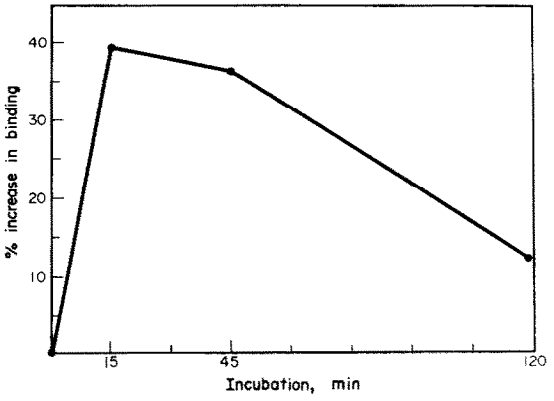

Fig. 2. Effect of incubation at 37°C on the association of [1,2,-H^3]-5α-dihydrotestosterone with cytoplasmic receptors. Cytosol prepared from coagulating glands of hamsters castrated for 2 days. Aliquots of the cytosol were incubated with [1,2-H^3]-5α-dihydrotestosterone (1×10^{-9} M) for a total of 2 h, a portion of which was at 37°C and the remaining at 0–4°C. Bound [1,2-H^3]-5α-dihydrotestosterone was determined by sucrose density gradient analysis. Percent increases in binding were determined by comparing the amount of androgen bound after incubation at 37°C for different time periods with a control incubated at 0–4°C for 2 h.

0–4°C for 2 h. Heating at 60°C for 15 min consistently abolished all 8–9*S* binding of [1,2-H^3]-5α-dihydrotestosterone. Cytosols frozen in the absence of steroid at −30°C for up to 3 wk showed no loss of 8–9*S* binding capacity. Although raising the ionic strength to 0.6 M KCl consistently increased the 8–9*S* binding of [1,2-H^3]-5α-dihydrotestosterone, lower concentrations of KCl had no effect (Fig. 3). In no case did KCl cause dissociation of the 8–9*S* complexes into 4*S* subunits, even when the sucrose gradients and the cytosols were both adjusted to 0.6 M KCl.

Binding of other radioactive steroids. The cytoplasmic binding of radioactive steroids other than [1,2-H^3]-5α-dihydrotestosterone was also investigated. Although [1,2,6,7-H^3]-progesterone was bound to an 8–9*S* as well as a 4–5*S* component, [2,4,6,7-H^3]-estradiol was bound only to a 4–5*S* component (Fig. 4). The 8–9*S* binding peaks for [1,2,6,7-H^3]-progesterone and [1,2-H^3]-5α-dihydrotestosterone appeared to coincide in all cytosols excluding those prepared from ventral prostrate glands. In prostate tissue the the cytoplasmic [1,2-H^3]-5α-dihydrotestosterone complexes were slightly heavier than the [1,2,6,7-H^3]-progesterone receptor complexes.

Competition by nonradioactive androgens and antiandrogens

The competitive effects of nonradioactive androgens on the cytoplasmic binding of [1,2-H^3]-5α-dihydrotestosterone in the hamster accessory sexual glands are summarized in Table 1. In cytosols prepared from the seminal vesicles, Cowper's glands, and 13% compared to the control cytosol, incubated at

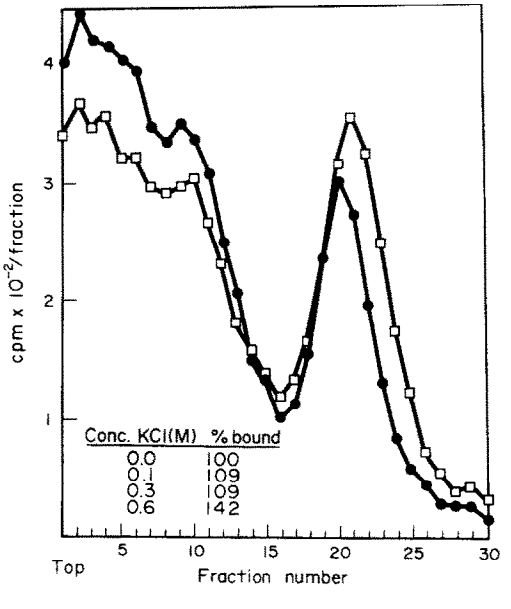

Fig. 3. Effect of 0.6 M KCl on binding of [1,2-H^3]-5α-dihydrotestosterone to cytoplasmic receptors. Control seminal vesicle cytosol (●——●) and cytosol adjusted to 0.6 M KCl (□——□) were incubated for 1 h at 0–4°C with [1,2-H^3]-5α-dihydrotestosterone (1.9×10^{-8} M). Inserted data summarizes effects of lower concentrations of KCl (0.1–0.6 M) on [1,2-H^3]-5α-dihydrotestosterone binding.

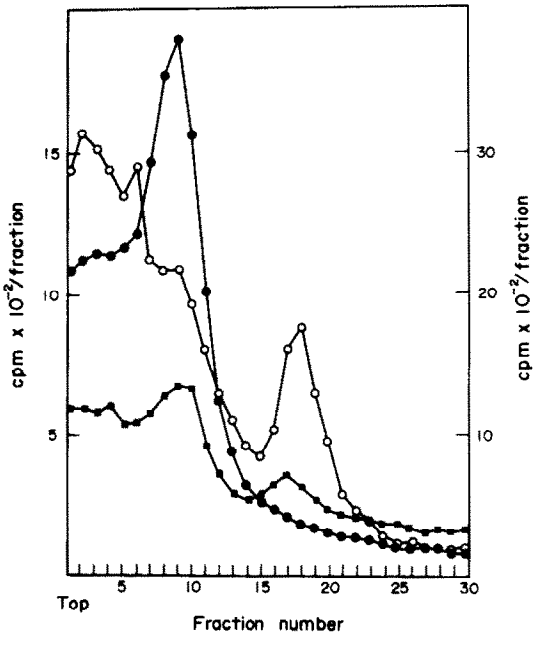

Fig. 4. Binding of other radioactive steroids by cytosol receptors. Cytosol, which was prepared from the coagulating glands of hamsters which had been castrated for 3 days was incubated for 1 h at 0–4°C with either [1,2-H^3]-5α-dihydrotestosterone (○——○), [1,2,6,7-H^3]-progesterone (■——■), or [2,4,6,7-H^3]-estradiol (●——●). The scale on the left refers to the counts of [1,2-H^3]-5α-dihydrotestosterone or [1,2,6,7-H^3]-progesterone, while the scale on the right refers to [2,4,6,7-H^3]-estradiol. Concentration of labeled steroids = 2×10^{-8} M.

Table 1. *In vitro* inhibition of cytoplasmic [1,2-H^3]-5α-dihydrotestosterone binding by nonradioactive androgens.

Nonradioactive androgen (10^{-4} M)	Percent bound in the 8–9S peak			
	Ventral prostrate	Seminal vesicles	Cowper's glands	Coagulating glands
None	100	100	100	100
5α-DHT	79†	41	53	48
Testosterone	75†	91	71	91
Androstanediol	84	142	102	106

The amount of [1,2-H^3]-5α-dihydrotestosterone* bound was determined by sucrose gradient analysis and expressed as a percent of control binding in the absence of a nonradioactive androgen.

* Concentration in incubated cytosols = 1×10^{-8} M; incubations were for 1 h at 0–4°C.

† Average of three separate experiments. All other values represent single determinations.

coagulating glands, nonradioactive 5α-dihydrotestosterone competed for binding sites more effectively than either testosterone or androstanediol. However, in the ventral prostate gland cytosols very little competition was detected with any of the androgens tested. Although the inhibitory effects of the antiandrogens (Table 2) varied depending on the tissues, progesterone appeared to be the most effective compound. 17α-Methyl-B-nortestosterone partially inhibited [1,2-H^3]-5α-dihydrotestosterone binding in all tissues, but cyproterone acetate had no effect on binding in ventral prostate cytosols.

Saturation analysis and partial purification of the [1,2-H^3]-5α-dihydrotestosterone complex

The binding of 5α-dihydrotestosterone by the accessory sexual gland cytosols was determined by sucrose gradient analyses after incubation with increasing concentrations of radioactive androgen (Fig. 5). Binding by all cytosols was linear in this concentration range and there was no indication of saturation at a [1,2-H^3]-5α-dihydrotestosterone concentration of 2×10^{-8} M. Fractionation of the labeled cytosols with saturated $(NH_4)_2SO_4$ and subsequent binding analyses on sucrose gradients indicated that the 8–9S binding peak for [1,2-H^3]-5α-dihydrotestosterone was precipitated by 33–50% $(NH_4)_2SO_4$. Serum contamination occasionally resulted in 4–5S binding in the 55–70% fraction. When both radioactive and nonradioactive 5α-dihydrotestosterone were incubated with the redissolved 33–50% fraction, subsequent analyses on sucrose gradients revealed an inhibition of [1,2-H^3]-5α-dihydrotestosterone binding equivalent to that reported in Table 1. When the redissolved 0–50% fraction was eluted from a column of Sephadex G 200, only one major peak of [1,2-H^3]-5α-dihydrotestosterone binding could be detected and this was eluted shortly after the void vol.

DISCUSSION

The results of these studies demonstrate the existence of low affinity, high capacity 5α-dihydrotestosterone binding proteins in the accessory sexual glands of male Syrian hamsters which were castrated for several days after *in vivo* exposure to androgens.

Table 2. *In vitro* inhibition of cytoplasmic [1,2-H^3]-5α-dihydrotestosterone binding by nonradioactive androgens.

Nonradioactive antiandrogen (10^{-4} M)	Percent bound in 8–9S peak			
	Ventral prostate	Seminal vesicles	Cowper's glands	Coagulating glands
None	100	100	100	100
Cyproterone acetate	119	68	61	49
17α-methyl-B-nortestosterone	65	77	70	73
Progesterone	58	51	31	0

The amount of [1,2-H^3]-5α-dihydrotestosterone* bound was determined by sucrose gradient analysis and expressed as a percent of control binding in the absence of nonradioactive antiandrogen.

* Concentration in incubated cytosols = 1.0×10^{-8} M; incubations were for 1 h at 0–4°C.

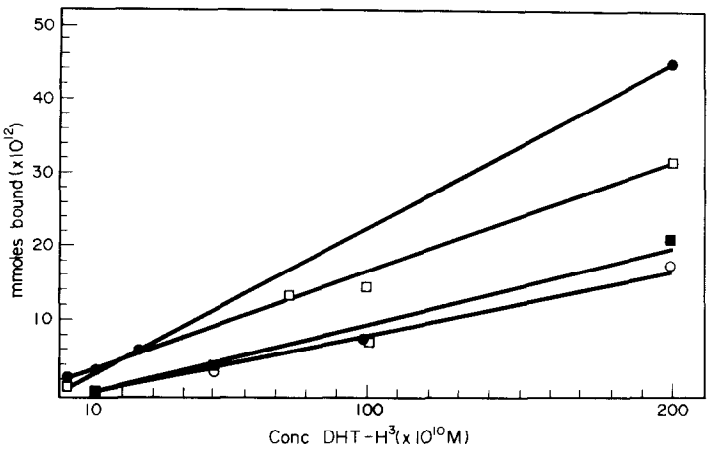

Fig. 5. The binding of [1,2-H^3]-5α-dihydrotestosterone by accessory sex gland cytosols after incubation with increasing concentrations of radioactive androgen. Seminal vesicle (●——●) and coagulating gland (□——□) cytosols were prepared from hamsters which had been castrated for 3 days. Prostrate (■——■) and Cowper's gland (○——○) cytosols were prepared from hamsters which had been injected subcutaneously with 2.0 mg of 5α-DHT for 12 days and were then castrated for 3 days. Cytosols were incubated for 1 h at 0–4°C with increasing concentrations of [1,2-H^3]-5α-dihydrotestosterone before binding analysis by sucrose density gradient ultracentrifugation.

These complexes migrated with a sedimentation coefficient of 8–9*S* on linear sucrose gradients (Fig. 1a). The binding components appeared to be tissue specific since they were not detected in cytosols prepared from lung tissue (not depicted) or autonomous prostatic adenocarcinomas (Fig. 1d). Although the operational term "cytosol" represents a fraction which may include extracellular components, lack of serum 8–9*S* binding peaks indicates that the 8–9*S* components detected in accessory sexual gland cytosols represent true intracellular binding proteins. Similar 8–9*S* complexes as well as distinctive 4*S* peaks were also detected in cytosols prepared from kidney and liver (Fig. 1b), classically considered as nontarget tissues. However, although more recent reports indicate that rat kidney [16] and liver [17] contain androgen receptors which may mediate the induction of specific proteins, the binding detected in these tissues in the present study appeared to be of low affinity.

Some of the properties of these 8–9*S* binding components closely resemble those described for intracellular androgen receptors. The abolition of all 8–9*S* binding after brief incubation at 60°C or with the proteolytic enzyme pronase indicates that these binding macromolecules are thermally denaturable proteins. The presence of cysteine sulfhydryl groups at the androgen binding site(s) or their involvement in the maintenance of the functional configuration of the binding protein is indicated by the absence of [1,2,-H^3]-5α-dihydrotestosterone–protein complexes after preincubation with *p*-hydroxymercuribenzoate (Fig. 1d). This agent also abolishes the high affinity specific 8*S* 5α-dihydrotestosterone-binding peak in rat ventral prostate cytosol prepared in sodium-phosphate buffer, without detectable changes in the nonspecific 3.5–4*S* peak (Wakeling and Visek, unpublished data). Wilson *et al.* [18] have recently reported that the low affinity 9S binding proteins in rat androgen-responsive tissues are also heat labile and sensitive to sulfhydryl reagents.

The increased 8–9*S* binding of [1,2-H^3]-5α-dihydrotestosterone detected after brief incubation at 37°C (Fig. 2) probably resulted from the increased binding to low affinity sites. One of the criteria often used in detecting low affinity binding is the insensitivity or increased binding seen after limited heating [18]. In contrast, the incubation of isolated rat prostatic complexes at 37°C causes complete loss of specific 8*S* androgen binding [7]. Although incubation at 37°C did not alter the sedimentation coefficient of the hamster cytoplasmic receptor complexes, published reports [19] indicate that thermal activation reduces the sedimentation coefficient of the rat 8*S* prostatic receptors to 3.6–3.9*S*.

In contrast to published reports concerning the reversible dissociation of the oligomeric 8*S* androgen–receptor complex into 4*S* subunits [4, 8], a similar ionic strength did not dissociate the low affinity cytoplasmic [1,2-H^3]-5α-dihydrotestosterone complexes obtained from the hamster accessory sexual glands (Fig. 3). A similar lack of dissociation in the presence of KCl has also been reported for receptors which bind estradiol [21] and corticosterone [22], as well as for low affinity 9*S* androgen binding proteins [18]. In the present study, increasing the ionic strength of the cytosols or the cytosols and the gradients to 0.6 M KCl actually increased the 8–9*S* binding of [1,2-H^3]-5α-dihydrotestosterone. This high salt concentration may have caused conformational changes in the binding protein to expose additional low affinity binding sites. The lack of dissociation at high ionic strength was probably unrelated to the buffer system since KCl (0.4 M) resulted in the dissociation of high affinity 8*S* rat prostatic complex prepared in the same

sodium-phosphate buffer (Wakeling and Visek, unpublished data).

[1,2,6,7-H^3]-progesterone was also bound to 8–9*S* as well as distinctive 4–5*S* cytoplasmic components in the accessory sexual glands (Fig. 4). The possibility that [1,2,6,7-H^3]-progesterone and [1,2-H^3]-5α-dihydrotestosterone bound to the same protein rather than to two proteins having the same approximate sedimentation coefficients is indicated by the competition of nonradioactive progesterone for [1,2-H^3]-5α-dihydrotestosterone binding (Table 2). Progestational agents are known to exert androgenic effects and inhibit 5α-dihydrotestosterone binding to specific receptors in several tissues [23, 24]. Despite the fact that high affinity receptors which specifically bind progesterone have been reported in the rat prostate [25], the physiological significance of progesterone binding to the low affinity androgen binding proteins in the present study is unknown. The distinctive binding of [2,4,6,7-H^3]-estradiol to cytoplasmic 4*S* components and not to the 8–9*S* component indicates that the latter components possess specificity in terms of the steroid which they bind.

The only competing androgen which consistently reduced the 8–9*S* binding of [1,2-H^3]-5α-dihydrotestosterone was nonradioactive 5α-dihydrotestosterone (Table 1). Although testosterone was slightly inhibitory, androstanediol did not compete for binding sites. These data suggest that although the 8–9*S* component is of low affinity, it is specific in terms of the androgen which it will bind. It is very possible that the large excess (10,000-fold) of non-radioactive 5α-dihydrotestosterone was capable of displacing some of the low affinity sites since the total androgen concentration exceeded the high capacity binding potential of these 8–9*S* components. The partial inhibition by excesses (10,000-fold) of cyproterone acetate and 17α-methyl-B-nortestosterone also suggests that the 8–9*S* binding is nonspecific. Both of these antiandrogens [26, 27] inhibit binding of [1,2-H^3]-5α-dihydrotestosterone to specific 8*S* cytoplasmic receptor proteins in the rat ventral prostrate while having no effect on nonspecific 4*S* binding. The significant nonspecific nature of the 8–9*S* binding was also reflected in the lack of saturation at a total ligand concentration of 2×10^{-8} M (Fig. 5). Using the same sodium-phosphate buffer, Wakeling and Visek [8] reported that the 8–9*S* receptor isolated from the rat ventral prostate was saturated at an androgen concentration of 2.4×10^{-8} M, while the 4*S* nonspecific peak was not saturated at a concentration of 4.3×10^{-8} M. Partial purification of the hamster cytoplasmic complexes by ammonium sulfate precipitation and gel filtration suggests that the low affinity 8–9*S* peaks detected on sucrose gradients represented a single binding protein. The 8–9*S* complexes were precipitated between 33–50% saturation with $(NH_4)_2SO_4$, which is the same percent saturation which was used by Wilson *et al.* [18] to precipitate low affinity binding proteins in supernatant fractions of rat epididymis. In contrast, Mainwaring and Peterken [15] recovered 8*S* androgen receptors in the 0–33% $(NH_4)_2SO_4$ fraction.

Although the 8–9*S* binding proteins detected in the accessory sexual glands of the hamster appear to be of relatively low affinity and high capacity, it is possible that a low concentration of high affinity sites, located either within the same or a separate protein, are also contained within these 8–9*S* peaks. Using precipitation with saturated $(NH_4)_2SO_4$ followed by gel filtration, Fang and Liao [28] demonstrated two specific 5α-dihydrotestosterone binding proteins in cytosol fractions of the rat ventral prostate. Although these two proteins exhibited different characteristics and appeared to perform different functions, they both sedimented as 3.5*S* complexes on sucrose density gradients. Zimmering and Kahn [29] reported two different binding entities for estradiol binding in the sheep endometrium, although sedimentation patterns in sucrose gradients indicated a single 8*S* peak. Heterogeneous binding sites within a single protein have also been reported for estradiol binding in the calf uterus [30], androgen binding in rat liver [17] and 5α-dihydrotestosterone binding in rat anterior pituitary [31].

The physiological function(s) of the low affinity androgen binding proteins described in this report is unknown. The detection of these 9*S* components in cytosols prepared from ventral prostate or Cowper's glands only after *in vivo* exposure to androgens suggests that the synthesis of these particular proteins may be under androgenic control. Although the detection of 9*S* components in cytosols prepared from the seminal vesicles and coagulating glands of nonandrogen primed animals may simply reflect the larger amounts of tissue available from these accessory glands; it may also be a reflection of higher androgen concentrations in these tissues. The presence of low affinity binding proteins in tissues of the male hamster and rat [18] suggests that they may be present in the androgen responsive tissues of rodent and perhaps other species as well. These binding proteins may serve to enhance the accumulation or intracellular partitioning of steroids and thus maintain a pool of androgens available for binding to receptors.

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