BINDING OF TESTOSTERONE TO CYTOPLASMIC COMPONENTS OF THE IMMATURE RAT UTERUS

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SUMMARY. The high speed supernatant of the immature rat uterus contains a macromolecule which binds ^{3}H-testosterone specifically and with high affinity (K_d ca 2 x 10^{-10} M at 4°). Mild heating (37°) or treatment with pronase results in release of ^{3}H-testosterone from the complex while nucleases have no effect. When examined in media of low ionic strength, the testosterone-protein complex aggregates with a predominant peak at ca 8S while in 0.4 M KCl buffered media a single component sedimenting at ca 4S is observed. Binding to this protein is specific for testosterone as 5α-dihydrotestosterone is a weak competitor for this binding site. The uterine cytosol contains at least one more type of binding site having a similar affinity for both testosterone and 5α-dihydrotestosterone. These binding sites are not saturable with hormone concentrations as high as 10^{-6} M.

INTRODUCTION. Recent investigations have shown that in the uterus estrogens are first bound to cytoplasmic receptor molecules and subsequently transported into the cell nucleus where they become associated with chromatin (1). Although the biological meaning of the interaction of estradiol with chromatin remains obscure, such an association may represent a fundamental event in the hormonal regulation of uterine growth.

Uterine growth can be stimulated by a number of steroids including estrogens, progestins and androgens (2). In addition to its uterotrophic activity, testosterone can also antagonize the

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uterotrophic response induced by estrogens (2). Although estradiol and testosterone induce distinctive alterations in the histology of the immature rat uterus (3), both steroids produce similar alterations in several biochemical end-points (3). The mechanism of the uterotrophic and anti-uterotrophic action of testosterone is unknown. It appears that testosterone cannot compete with estradiol for receptor sites, even when present in excessive amounts (4). In contrast, the anti-estrogen MER-25, competes effectively for the estradiol-binding sites (5) and it also inhibits the uterotrophic effect of estradiol (3). It is noteworthy that MER-25 is only slightly effective as an antagonist against the uterotrophic action of testosterone (3). These observations suggest that testosterone may act on the uterus by a mechanism other than direct competition for the estradiol-binding sites. In this communication, data will be presented to show that the uterine cytosol of the immature rat contains a macromolecule which has a high affinity and specificity for testosterone.

METHODS. Immature female hooded rats (20-22 days old) were decapitated and the uteri were removed and homogenized in 0.01 M Tris buffer, 0.0015 M EDTA, pH 7.2 (2.5 -5 uteri per ml). A soluble fraction (cytosol) was obtained by centrifuging the homogenate at 224,000 x g for 30 minutes. This high speed supernatant fraction contained 2 - 5 mg of protein per ml as determined by the method of Lowry (6). Aliquots of cytosol were incubated at 4^{o} with ^{3}H-testosterone (45 Ci per mmole) or ^{3}H-5α-dihydrotestosterone (44 Ci per mmole) for 2 hrs. Competition studies were performed by incubating the cytosol with the tritiated steroid and a ten-fold ex-

cess of non-labeled steroid. Separation of bound and free steroid was accomplished by gel filtration on Sephadex G-25 at 23°, Sephadex G-50 at 4° or by the dextran-coated charcoal technique of Korenman et al (7). With ^{3}H-testosterone concentration of 10^{-9} M or less all three methods gave similar results. When higher concentrations of ^{3}H-testosterone are used, filtration on Sephadex at 4°

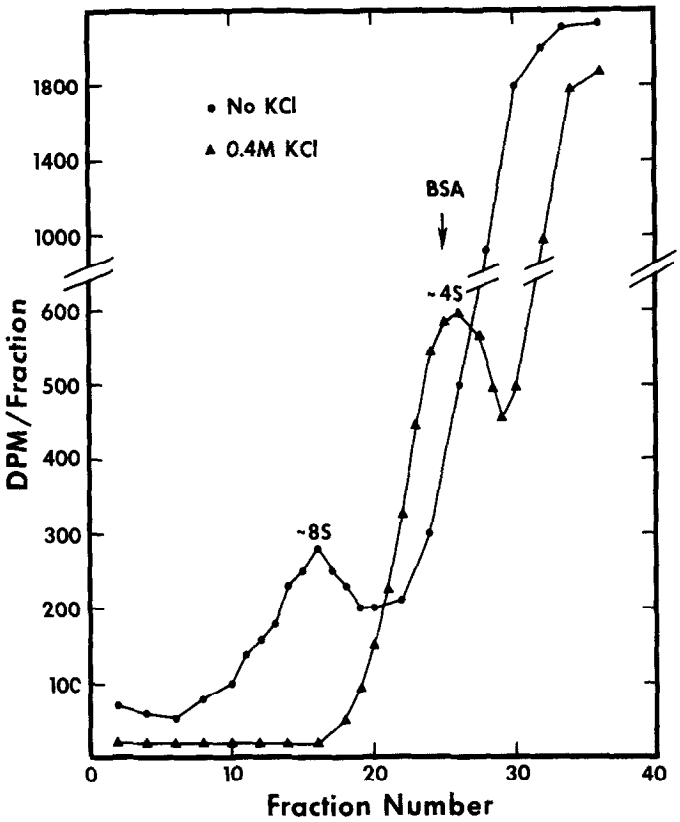

Fig. 1. Sucrose density gradient centrifugation of ^{3}H-testosterone binding components of uterine cytosol. Aliquots (0.2 ml) of cytosol pre-incubated at 4° with 1 x 10^{-9}M of ^{3}H-testosterone were applied on 3.6 ml of 5-20% sucrose gradients containing Tris-EDTA buffer, pH 7.2, with or without 0.4 M KCl. Centrifugation was carried out for 16 hrs. at 2° in a Spinco Model L_2-65B ultracentrifuge using the SW-56 rotor at 56,000 rpm (average force 308,000 x g). The tubes were pierced and 36 fractions were collected and counted after the addition of 3 ml of ethanol and 10 ml Omnifluor. Approximate sedimentation coefficients were determined using bovine serum albumin (BSA) as standard.

gives somewhat higher values due to incomplete dissociation of the testosterone-albumin complex. In this communication only the data obtained with the charcoal method will be presented. Approximate sedimentation coefficients of the testosterone-macromolecule complexes were obtained by sucrose density gradient centrifugation. The methods of gel filtration and sucrose density gradient sedimentation, as well as techniques of counting have been previously described (8, 9).

RESULTS AND DISCUSSION. The interaction of ^{3}H-testosterone with

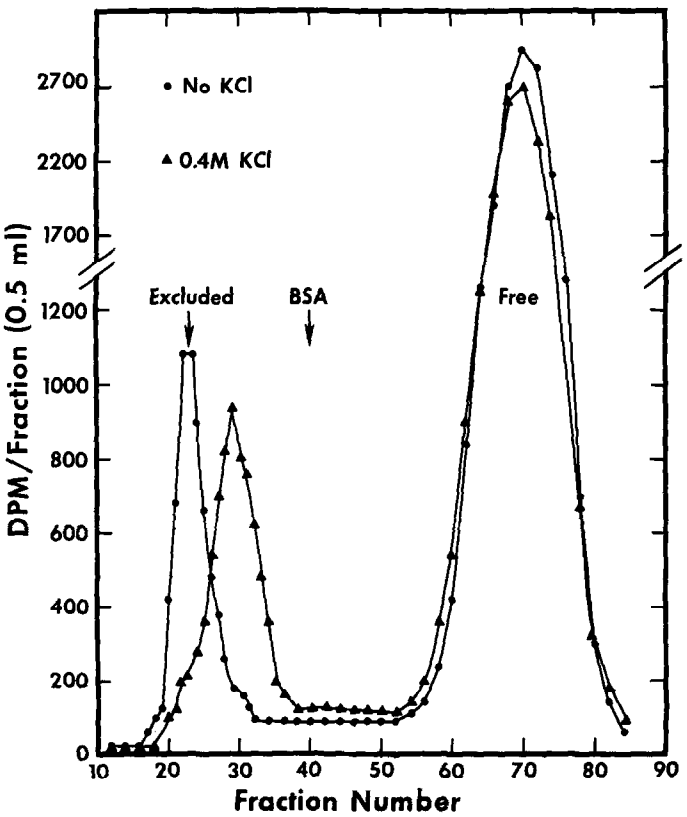

Fig. 2. Gel filtration on Sephadex G-200 of uterine cytosol preincubated with 1 x 10^{-9} M of ^{3}H-testosterone for 2 hrs. at 4°. Elution was carried out at 4° at a flow rate of 1.5 ml per hr. with Tris-EDTA buffer, pH 7.2, or with Tris-EDTA buffer, pH 7.2 containing 0.4 M KCl. Fractions of 0.5 ml were collected. Column, 1.2 x 52 cm; bed volume, 35 ml.

components of uterine cytosol using sucrose gradient centrifugation at 2° is shown in Fig. 1. When examined in low ionic strength medium bound ^{3}H-testosterone is associated predominantly with components sedimenting at ca 8S as well as with heavier components. In the presence of 0.4 M KCl, bound ^{3}H-testosterone sediments as a single peak at ca 4S. No 8S ^{3}H-testosterone-binding component is present in rat serum (not shown). The binding of testosterone to components of uterine cytosol is sufficiently tight to withstand chromatographic separation of the complex from free steroid on Sephadex G-200 columns (Fig. 2). In the absence of KCl the bound testosterone is eluted with the void volume of the column, while

Table 1. Binding of ^{3}H-testosterone by rat uterine cytosol, rat serum and bovine serum albumin and competition with ^{3}H-testosterone binding by non-labeled testosterone.

Hormone(s) Added	Amount Bound* (dpm/mg Protein)		
	Cytosol	Serum	Albumin
^{3}H-Testosterone (1×10^{-9}M)	4050	75	100
^{3}H-Testosterone (1×10^{-9}M) + Testosterone(1×10^{-8}M)	760	70	120

*Aliquots of cytosol (5 mg Protein per ml), albumin Solution (5 mg per ml) or rat serum (1:10 dilution), were incubated with the hormone indicated at 4° for 2 hrs. To 0.2 ml of incubation mixture, 1 ml of charcoal suspension was added and after thorough shaking the mixture was left for 15 minutes at 4°. The charcoal was then separated by centrifugation (2600 rpm for 10 min) and an aliquot of the supernatant was counted. Determinations were carried out in triplicate. The charcoal suspension consisted of 0.5% charcoal (Norit A) and 0.05% dextran (type 70T) in Tris-EDTA buffer, pH 7.2.

in the presence of 0.4 M KCl it is retained and has an elution pattern distinctly different from that of albumin. The data shown in Table 1 provide further proof that the binding of ^{3}H-testosterone to uterine cytosol is not due to serum contamination. Bound ^{3}H-testosterone in the uterine cytosol is released by heating or treatment with pronase but not with nucleases (Table 2) indicating the participation of protein in the steroid-binding site.

Table 2. Release of ^{3}H-testosterone from uterine cytosol binding component by heating, proteases and nucleases.

Treatment for 30 mins.	Amount Bound* dpm/mg Protein	% Released
No Enzyme (0^{o})	4340	
No Enzyme (23^{o})	2470	43
No Enzyme (37^{o})	480	89
No Enzyme (60^{o})	90	98
Pronase (0^{o})	230	95
RNase (0^{o})	3250	25
DNase (Mg^{++}) (0^{o})	3640	16

*Aliquots of cytosol (1 ml) were first incubated with 1 x 10^{-9}M ^{3}H-testosterone for 2 hrs. at 4^{o}. This was followed by an additional 30 min incubation at the temperatures indicated with or without the addition of 1 mg of enzyme. In the incubation mixture containing DNase, 2 µl of 1 M magnesium chloride was also added. Dextran-coated charcoal was used to separate free from bound steroid as described in Table 1.

As shown in Fig. 3, binding of ^{3}H-testosterone to uterine cytosol is specific since only testosterone and not estradiol, progesterone or cortisol, competes for the binding sites. Of particu-

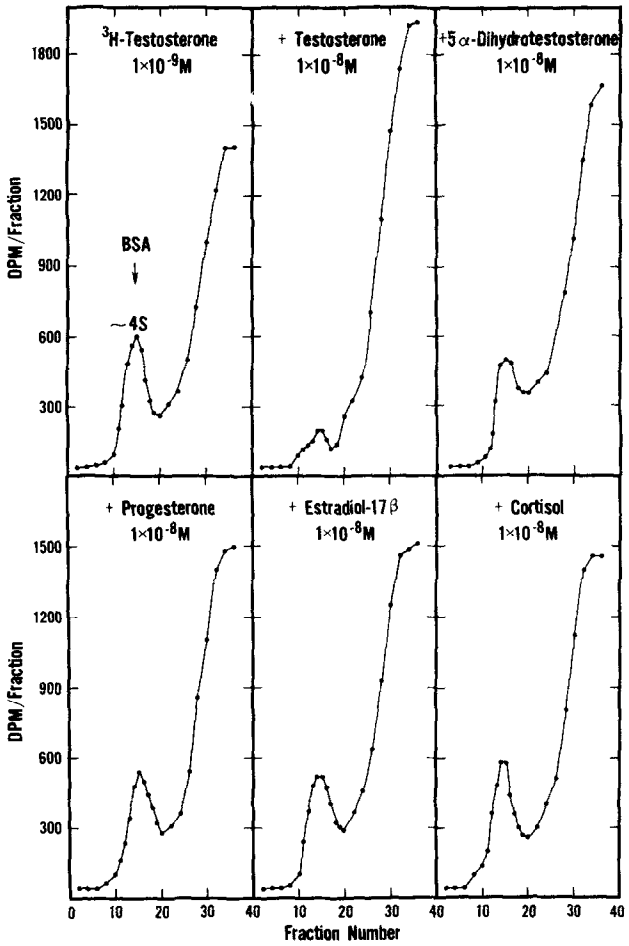

Fig. 3. Competition with ^{3}H-testosterone binding to uterine cytosol by non-labeled testosterone and other steroids. Sucrose density gradient centrifugation was performed as in Fig. 1. All gradients contained 0.4 M KCl.

lar interest is the observation that 5α-dihydrotestosterone is much weaker than testosterone in competing with ^{3}H-testosterone for the binding sites. The Scatchard plots shown in Fig. 4 indicate that uterine cytosol contains more than one type of binding sites for androgens. The first type of binding sites has a high affinity (K_d ca 2 x 10^{-10} M at 4°) and low capacity for testosterone but not for 5α-dihydrotestosterone. The second type of binding sites has

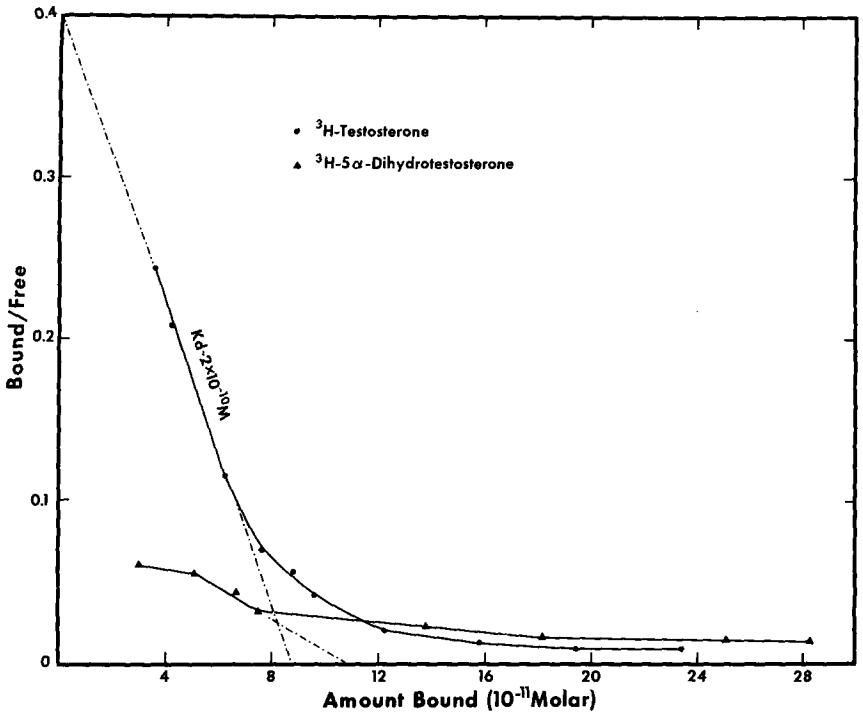

Fig. 4. Binding of ^{3}H-testosterone and ^{3}H-5α-dihydrotestosterone to uterine cytosol after incubation of cytosol with increasing amounts of labeled hormone at 4° for 2 hrs. Dextran-coated charcoal was used to separate free from bound steroid as described in Table 1. The data are presented in Scatchard Plots. The values are adjusted to correspond to 1 mg of protein per ml. The actual protein concentration was 4 mg protein per ml of cytosol.

lower but similar affinity and a large capacity for both testosterone and 5α-dihydrotestosterone.

The results presented in this report demonstrate that rat uterine cytosol contains a protein with high affinity and specificity for testosterone. The binding sites of the uterine component are different from the androgen-binding sites of the rat prostate which have a higher affinity for 5α-dihydrotestosterone rather than testosterone (10). It is interesting to note that 5α-dihydrotestosterone has much lower uterotrophic and anti-uterotro-

phic activity than testosterone (11, 12). The presence of a specific progesterone-binding component in the rat uterine cytosol has also been reported (13). Thus the uterine cell contains specific binding sites of high affinity for each of the three classes of sex steroids, estrogens, progestins and androgens. This suggests that each hormone may exert its uterotrophic effect by having different physiological functions at the cellular level. The synergism or antagonism of the hormones observed *in vivo* may likewise be the net result of the different effects (stimulating or inhibitory) of each hormone and not due to a direct competition for the same active sites.

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