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NUCLEAR BINDING OF [^{3}H]-ANDROGENS BY THE EPIDIDYMIS OF SEXUALLY MATURE CASTRATED RABBITS

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SUMMARY

We have demonstrated that when minces of epididymides from castrated rabbits are incubated with [^{3}H]-testosterone radioactivity bound to a macromolecular component, it enters the nuclei. This entry is a temperature dependent phenomenon which is greater upon incubation at 23°C than at 0°C. In contrast, no macromolecular bound hormone could be detected in leg muscle cytosol or nuclei. The amount of radioactivity extractable from epididymal nuclei was a function of the molar concentration of KCl in the extraction medium. However, even when 1.0 M KCl was used, greater than 30% of the radioactivity bound to nuclei remained unextractable. Of the naturally occurring steroids that we used (testosterone, estradiol-17β, and cortisol) in competition studies, unlabeled testosterone was the most effective in preventing the nuclear accumulation of labeled androgen. Estradiol-17β also was effective in this regard, but was much less potent than testosterone under the conditions employed. Unlabeled cortisol was an ineffective inhibitor. The antiandrogens, cyproterone and SKF 7690, both of which inhibit binding to the epididymal cytoplasmic androgen receptor, completely inhibited nuclear accumulation of radioactivity, implying that nuclear accumulation is dependent on an initial binding to the cytoplasmic receptor. We have also demonstrated that the rabbit epididymis is capable of converting testosterone to 5α-dihydrotestosterone (5α-DHT). Although the tissues were incubated with [^{3}H]-testosterone, 5α-DHT was selectively bound by the cytoplasmic receptor and concentrated in the nuclei.

INTRODUCTION

During our studies concerning androgen binding in the rabbit epididymis, we have characterized two distinct binding moieties. One of these binding proteins originates in the testis and is transported to the epididymis *via* testicular fluid [1–2]. This binding component, designated androgen binding protein (ABP), can be demonstrated in testicular supernatants [2] in rete testis fluid (RTF) [2–4], in supernatants prepared from the caput epididymis of intact sexually mature rabbits [5], and in fluid collected from the cauda of the epididymis [4]. While ABP is absent from supernatants prepared from the corpus and cauda of intact sexually mature rabbits, it can be demonstrated in these segments obtained from the epididymides of intact sexually immature rabbits during certain stages of sexual maturation [3, 6–7]. The following observations indicated that ABP was not the target tissue receptor responsible for mediating the action of androgens on the epididymis: (1) its testicular origin, (2) its intraluminal location within the epididymis, (3) the rapid rate at which the androgen-ABP complex dissociates, and (4) the inability of the antiandrogens cyproterone and cyproterone acetate, which inhibit androgen binding to androgen receptors in other tissues, to effectively compete for binding sites on ABP [4, 7].

In addition to ABP of testicular origin, castrated adult rabbit epididymal cytosol also contains an intracellular androgen binding moiety which exhibits all of the characteristics usually attributed to target cell receptors, namely: (1) high affinity, (2) low capacity, (3) steroid specificity, (4) slow rate of dissociation, and (5) ionic strength dependent alterations of sedimentation coefficient [8]. Cyproterone and cyproterone acetate both inhibit binding of [^{3}H]-5α-DHT to this epididymal cytoplasmic receptor [4, 8], whereas, at the concentrations used, they do not inhibit binding to rabbit ABP or rabbit testosterone binding globulin (TeBG) [4, 7, 9]. ABP is only detectable in supernatants from the caput epididymidis of intact adult rabbits [5]. However, the cytoplasmic receptor can be demonstrated in all three major anatomical segments (caput, corpus, cauda) of this organ in castrated adult rabbits [6, 8]. A distribution of the androgen receptor along the length of the epididymis is what one would anticipate, since the entire duct is dependent on androgens for the maintenance of its structural and functional integrity [10].

Since the current concepts regarding the mechanism of steroid action require that the hormone–receptor complex be transferred to a nuclear site to initiate at least a portion of the processes classically designated as the "action" of the hormone, we have commenced studies to investigate nuclear binding of androgens in the epididymis of adult castrated rabbits [4]. In this communication we present further data on this phenomenon as well as data on testosterone metabolism by the epididymis.

MATERIALS AND METHODS

Chemicals

[1,2,6,7-^{3}H]-testosterone (85 Ci/mmol) was purchased from New England Nuclear Corporation. Unlabeled steroids were purchased from Steraloids. Cyproterone (1,2α-methylene-6-chloro-17-hydroxy-4,6-pregnediene-3,20-dione) was donated by Schering-A. G., Berlin, and SKF 7690 (17α-methyl-17β-hydroxy-B-nor-4-androsten-3-one) was donated by Smith-Kline and French. Ultra Pure sucrose and enzyme and buffer grade Tris (base) were purchased from Schwarz–Mann. Spectrafluor was purchased from Amersham–Searle. All other chemicals were reagent or analytical grade.

Animals and surgical procedures

Sexually mature (at least six months old) New Zealand white rabbits were used in all the experiments reported here. Animals to be castrated were anesthetized with Sodium Nembutal (30 mg/kg). The scrotum was incised in the midline and a small incision was made in each tunica vaginalis testis through which the testis and epididymis were withdrawn. The epididymis was teased away from the testis and the testicular blood vessels were ligated. The testis was excised, the epididymis was returned to the scrotum, and the incisions were closed.

Preparation and incubation of tissue

Four days following castration, the animals were killed by administering a lethal dose of Nembutal. The epididymides were removed from 2–4 animals, trimmed of fat and connective tissue, rinsed in TES buffer [10 mM Tris–HCl (pH 7.5), 1.0 mM EDTA, 0.25 M sucrose], and weighed. The weighed tissues were pooled, thoroughly minced with iridectomy scissors, and divided into separate aliquots. The aliquots were then incubated with approximately 2.8×10^{-8} M [^{3}H]-testosterone alone or in combination with approximately 5.6×10^{-6} M unlabeled competitor in 2 ml of TES/g of tissue for 2 h at 0°C. At the conclusion of the 0°C incubation period certain aliquots were then incubated at 23°C for an additional 60 min.

Preparation of cytosol and nuclei

When the incubations were concluded, the tissues were gently homogenized in the incubation medium using a Polytron (Pt-10). The homogenates were then centrifuged at 800 *g* to sediment the nuclear fraction. The 800 *g* supernatant was decanted and centrifuged for 30 min at 229,000 *g* to obtain the cytosol fraction. The 800 *g* pellets were washed free of unbound hormone by resuspending them in 40 ml of TES and sedimenting the nuclei. The wash step was repeated twice. On occasion, the 800 *g* pellets were freed of connective tissue contamination and non-nuclear debris by filtration through cheesecloth and/or sedimentation through 1.75 M sucrose [11].

Extraction of nuclei

The washed nuclear pellets were extracted by resuspending them in a volume of TE (TES without sucrose) containing 0.4 or 1.0 M KCl equal to one-half the volume of the original incubation mixture using a glass–Teflon homogenizer. The homogenates were allowed to stand on ice for 60 min to extract the binding components. The samples were then centrifuged for 30 min at 16,300 *g* to sediment nuclear debris. The supernatants were analyzed for the presence of macromolecular bound steroid using sucrose gradient analysis.

Examination of radioactive steroids extracted from nuclei

Rabbits that had been castrated for four days were killed, their epididymides were removed, trimmed of fat and connective tissue, and minced. The tissue minces were preincubated at 0°C for 2 h and incubated at 23°C for 60 min with approx. 2.8×10^{-8} M [^{3}H]-testosterone alone or in combination with approx. 8.6×10^{-5} M unlabeled testosterone. The minces were then homogenized using a Polytron Pt-10. The homogenates were centrifuged at 1020 *g* for 15 min. The supernatants were decanted. The pellets were resuspended in 10 ml of TES using a glass–Teflon homogenizer. The resuspended pellets were then poured over cheesecloth. The cloth was washed with 30 ml of TES. The filtrates were then centrifuged at 4080 *g* to sediment the nuclei. The nuclei were resuspended in 40 ml of TES by vortexing and resedimented by centrifuging at 4080 *g*. This wash step was repeated twice. After the third wash, the nuclear pellets were resuspended in 1 ml of 1.0 M KCl and extracted as outlined above. The 16,300 *g* supernatants (nuclear extracts) were then applied to 6 mm × 17 cm. columns of Sephadex G-25 and eluted with TE buffer at 4°C. The flow rate of the columns was approx. 30 ml/h. Aliquots of the fractions were counted and the fractions constituting the peaks of bound and free label were pooled separately and extracted three times with anhydrous ether using 10 ml of ether for each extraction. Two ml of deionized water were added to the 16,300 *g* pellets. These were also extracted as above. The organic phases were combined and 50 μg each of testosterone, 5α-androstan-17β-hydroxy-3-one (5α-DHT), 5α-androstan-3α, 17β-diol, and 5α-androstan-3β, 17β-diol were added. The ether was then evaporated to dryness. The walls of each tube were washed with 2 ml of ether. This ether was evaporated and 100 μl of absolute ethanol was added to each tube.

An aliquot (50 μl) of each nuclear extract was then spotted on a silica gel plate as were unlabeled standards and labeled testosterone and 5α-DHT. The plates were chromatographed using a solvent system containing chloroform–acetone (93:7 v/v). Testosterone was visualized using U.V. light and other standards were detected after spraying the plates with a

10% solution of phosphomolybdic acid in acetone and heating at approximately 100°C for 5 min. The plates were then divided into columns perpendicular to the origin and then subdivided into 1/2 cm. sections horizontal to, and beginning 1/2 cm. below, the origin. The areas were then scraped and counted in 5 ml of a toluene-spectrafluor scintillation fluid. Testosterone, 5α-DHT, and the diols were well separated under these conditions. However, the 3α and 3β diols were usually not clearly separated from each other.

Examination of radioactive steroids extracted from cytosol

The 1020 *g* supernatants described in the previous section were centrifuged for 30 min at 229,000 *g* to prepare cytosols. Unlabeled testosterone was added to each of the cytosols to give a concentration of approximately 8.6×10^{-5} M. This was done to dissociate any labeled hormone that might have been loosely bound to androgen binding protein or to TeBG. After the cytosols had been incubated with unlabeled testosterone for at least 30 min, a 1 ml aliquot of each cytosol was applied to the Sephadex G-25 columns, described above, to separate free from bound hormone. The bound and free peaks of radioactivity were pooled separately, extracted with ether, evaporated, and analyzed by thin-layer chromatography as described above for the nuclei.

Sucrose gradient analysis

Linear 5–20% sucrose gradients were prepared using a Beckman gradient former. The sucrose was dissolved in 10 mM Tris–HCl (pH 7.5), 1.0 mM EDTA, 12 mM thioglycerol, 0.01, 0.4, or 1.0 M KCl, and 10% glycerol. Aliquots (0.2 ml) of labeled cytosol or nuclear extract were layered on the gradients and centrifuged for 18 h at 40,000 rev./min (average force 149,000 *g*) using a Beckman SW 50.1 rotor. Fifteen-drop fractions were collected from the bottom of tubes and counted in a scintillation fluid containing Triton X-100–toluene–spectrafluor (1230:2366:100 by vol.) or toluene–spectrafluor (2366:100 v/v). The counting efficiency for [^{3}H] was 24% and 39%, respectively.

RESULTS

Androgen binding to epididymal cytosol and nuclei

When cytosol prepared from minced epididymides from castrated rabbits which had been incubated with [^{3}H]-testosterone at 0°C was analyzed for macromolecular binding on low ionic-strength (0.01 M KCl) gradients, patterns similar to that illustrated in Fig. 1 were obtained. Two peaks of binding were usually present, one in the 4–5*S* region of the gradient and one in the 8*S* region of the gradient. As we have shown previously [8], binding to the 4–5*S* peak is rapidly dissociable and likely represents binding to ABP and/or TeBG. However, the 8*S* peak dissociates slowly and shows salt dependent transformation to

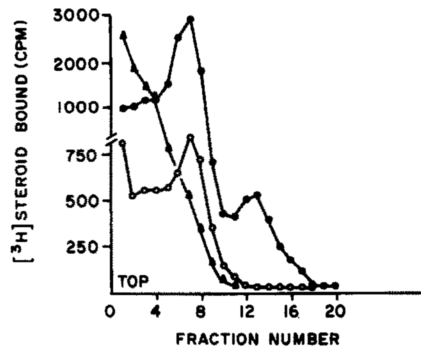

Fig. 1. Androgen binding to epididymal cytosol and nuclei. cytosol (●——●) prepared from epididymal minces which had been incubated with [^{3}H]-testosterone at 0°C was analyzed on sucrose gradients containing 0.01 M KCl. KCl (1.0 M) extracts of nuclei, purified through 1.75 M sucrose, were prepared from epididymal minces preincubated with [^{3}H]-testosterone at 0°C and subsequently incubated at 23°C (○——○) or at 0°C (▲——▲) were analyzed on sucrose gradients containing 1.0 M KCl.

a more slowly sedimenting moiety [8]. The 8*S* binder is likely to be the epididymal androgen receptor.

It has been demonstrated [12] that the transfer of cytoplasmic steroid receptors into nuclei is a time and temperature dependent phenomenon. To ascertain if the androgen binding protein in cytosol from the epididymides of castrated adult rabbits was capable of being transferred to nuclei, the following experiments were performed. Aliquots of epididymal minces were preincubated at 0°C with [^{3}H]-testosterone and then either incubated at 0°C or 23°C. When nuclei were prepared, extracted with 1.0 M KCl, and analyzed for macromolecular bound radioactivity, the results shown in Fig. 1 were obtained. As can be noted, no detectable peak of bound hormone was extractable from nuclei that had been incubated at 0°C. In contrast, a distinct peak of bound radioactivity was extractable from nuclei which had been incubated at 23°C. Similar results were obtained with purified nuclei and the crude washed 800 *g* "nuclear pellet." These experiments indicated that the transfer of the epididymal cytoplasmic androgen binder to nuclei is temperature dependent and provided additional data as to the receptor nature of the binding moiety.

When leg muscle minces from a castrated rabbit were preincubated with [^{3}H]-testosterone at 0°C and subsequently incubated at 23°C, there was no detectable androgen binding to either cytosol or nuclear extracts. These data implied that nuclear transfer of macromolecular bound radioactivity was not only a function of incubation temperature but also of the nature of the tissue with which the [^{3}H]-testosterone had been incubated.

The effect of KCl concentration on extraction of androgen from epididymal nuclei

In our initial studies on androgen binding to epididymal nuclei, we noted that the amount of radioactivity extractable by a 60 min incubation with 0.4 M

Table 1. The effect of KCl concentration on extraction of intranuclear radioactivity

KCl concentration	Percentage* of radioactivity extracted
0.4 M	37.8
1.0 M	66.2

* Purified nuclei from epididymal minces that had been preincubated with [^{3}H]-testosterone for 2 h at 0°C and 1 h at 23°C were prepared as described in Materials and Methods. The nuclei were resuspended in TES and divided into three aliquots. The suspensions were centrifuged at 1020 *g* to sediment the nuclei and the supernatants were decanted. Two of the nuclear pellets were extracted with 0.4 M or 1.0 M KCl as described above. The third pellet was extracted with ethanol. The amount of radioactivity extracted with salt was compared to that extracted by ethanol. The results presented are the mean of two such assays.

KCl represented only 30–50% of that which could be extracted with absolute ethanol (Table 1). To determine if a larger amount of radioactivity could be extracted, we increased the KCl concentration to 1.0 M. As can be noted in Table 1, the increased salt concentration yielded approx. a 60% increase in the amount of extractable radioactivity, yet more than 30% of the radioactivity bound to nuclei still resisted extraction. We have not yet determined if the residual radioactivity can be released from nuclear binding sites with further increases in KCl concentration, longer extraction periods, additional extractions, enzymatic digestion, or other procedures. These data suggest that one or more classes of salt resistant steroid binding sites are associated with nuclei.

Examination of labeled steroids extracted from the epididymis

Since the tissue minces had been incubated with [^{3}H]-testosterone, it was of interest to ascertain if any metabolism of testosterone had occurred during the incubation, and if there were any differential distribution of the metabolites among the compartments that we were analyzing. As can be noted (Table 2) when epididymal minces were incubated with [^{3}H]-testosterone alone (Experiment A), three labeled steroids co-chromatographing with authentic unlabeled and/or tritiated testosterone, 5α-androstandiols (diols), and 5α-DHT were detected. Small amounts of radioactivity were also present at the origin of the t.l.c. plate and/or migrating ahead of DHT.

When the distribution of testosterone and its metabolites amount the compartments was analyzed, we observed that virtually all of the unbound radioactivity in the cytosol was intact testosterone (Table 2, A_{CF}). In contrast, 22% of the label bound to the cytoplasmic receptor was DHT (Table 2, A_{CB}). Approx. equal amounts of bound and free radioactivity were extracted from nuclei with 1.0 M KCl. However, 14% of the bound radioactivity (Table 2, A_{NB}) was DHT, but only 5% of the free radioactivity (Table 2, A_{NF}) was DHT. These data indicate that binding to the epididymal cytoplasmic receptor and to nuclei favors DHT. It is of interest to note that the amount of residual radioactivity remaining in the nuclei (Table 2, A_{NP}) after the KCl extraction is approximately 60% of that present in the nuclear bound fraction (Table 2, A_{NB}) and that the percentages of testosterone and DHT in both fractions are similar. This observation suggests that the ligand specificity of the salt solubilized and residual binding components are similar, if not identical. The presence of unlabeled testosterone in the incubation mixture caused a dramatic reduction in the amount of radioactive hormone bound in the cytosol and recoverable from nuclei (Table 2, Experiment B), demonstrating the presence of a limited number of binding sites. All of the label recovered in these experiments co-chromatographed with authentic testosterone, suggesting saturation of the 5α-reductase.

Steroid specificity of nuclear binding of androgens

To further ascertain if unlabeled steroids would inhibit the binding of labeled androgen to nuclei, we incubated epididymal minces with 2.8×10^{-8} M [^{3}H]-testosterone alone or in combination with a 200-fold excess of unlabeled steroids. Figure 2 illustrates that while unlabeled cortisol had little or no effect on preventing the accumulation of macromolecular bound radioactivity in nuclei, unlabeled testosterone completely inhibited such accumulation. Unlabeled estradiol-17β also inhibited androgen binding

Table 2. Identification of radioactivity extracted from cytosol and nuclei following incubation with [^{3}H]-testosterone

	Total counts recovered	% Radioactivity as: T	DHT	Diols	*Other
A_{CB}	3761	78	22	0	0
A_{CF}	51333	93	3	1	3
A_{NB}	612	86	14	0	0
A_{NF}	578	95	5	0	0
A_{NP}	397	83	15	0	0
B_{CB}	401	100	0	0	0
B_{CF}	31843	89	0	0	11
B_{NB}	35	100	0	0	0
B_{NF}	87	100	0	0	0
B_{NP}	40	100	0	0	0

Epididymal minces from rabbits which had been castrated for four days were preincubated at 0°C with [^{3}H]-testosterone alone (A) or with [^{3}H]-testosterone and unlabeled testosterone (B) for two h and then incubated at 23°C for 1 h. Distribution of labeled hormone in subcellular compartments was then analyzed as described in Materials and Methods. A background of 60 c.p.m. was subtracted from each t.l.c. fraction. The results presented here are the mean of two separate experiments. CB = radioactivity bound to cytosol, CF = free radioactivity in cytosol, NB = radioactivity bound in 1.0 M KCl extract of nuclei, NF = free radioactivity in 1.0 M KCl extract of nuclei, NP = radioactivity extracted from the 16,300 *g* sediment from the nuclear extract.

* Other represents radioactivity remaining at the origin or migrating in front of DHT.

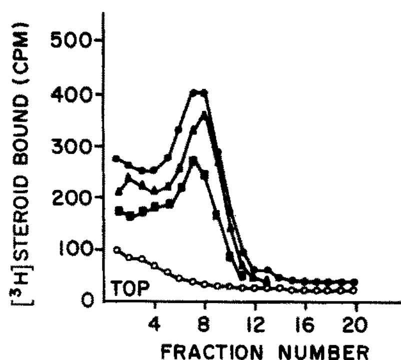

Fig. 2. The effect of competitors on androgen binding to epididymal nuclei. Epididymal minces were preincubated at 0°C with [^{3}H]-testosterone alone (●——●) or in combination with unlabeled testosterone (○——○), unlabeled estradiol-17β (■——■), or unlabeled cortisol (▲——▲) and then incubated at 23°C. Crude nuclear pellets were prepared, extracted with 1.0 M KCl and analyzed for binding on sucrose gradients containing 1.0 M KCl.

to nuclei; however, its inhibitory effect was much less than that exhibited by unlabeled testosterone (Fig. 2).

The effect of antiandrogens on androgen binding to epididymal nuclei

Since the antiandrogens, cyproterone, cyproterone acetate, and SKF 7690, inhibit androgen binding to the epididymal cytoplasmic androgen receptor [4, 8], they should also be capable of inhibiting nuclear androgen binding if that process were receptor mediated. To determine the effect of these antiandrogens on nuclear binding, we incubated minces of epididymides from castrated rabbits with 2.8×10^{-8} M [^{3}H]-testosterone alone or in combination with a 200-fold excess of antiandrogen. Figure 3 illustrates that in the presence of either cyproterone or SKF 7690 no macromolecular bound radioactivity was extractable from epididymal nuclei, suggesting that binding to the cytoplasmic receptor is necessary for nuclear transfer.

DISCUSSION

It has long been established [10] that the epididymis is an organ that is dependent upon androgens for the regulation of its structural and functional integrity. It has been recently demonstrated that sperm maturation in the epididymis, i.e., the development of their ability to fertilize ova, and sperm survival are also androgen-dependent phenomena [6, 13–14]. In recent years it has been clearly established that in order for steroid hormones to initiate at least some of the processes that are associated with their action, they must bind to cytoplasmic receptors in target tissues. This aspect of androgen action on the epididymis has now been demonstrated in the rat [15–16], rabbit [8, 17], guinea-pig [18], and rhesus monkey [19]. The second step in the current theory on the mechanism of steroid hormone action requires that the receptor–hormone complex be transferred to target tissue nuclei. The transfer of androgens to epididymal nuclei has also been demonstrated in some of the above species [4, 19–21].

In the studies presented here, we have demonstrated the nuclear binding of androgens in epididymides from castrated sexually mature rabbits. The transfer of the hormone–receptor complex into the nucleus is temperature dependent, in that the complex could be readily demonstrated in extracts of nuclei that had been incubated at 23°C, but not in extracts of nuclei that have been incubated at 0°C. Such a finding does not preclude the possibility that a small amount of complex, which was not detectable by sucrose gradient ultracentrifugation, may enter nuclei following incubation at 0°C.

We have noted that when nuclei were extracted with 0.4 M KCl, the soluble fraction contained no more than 40% of the radioactivity that could be extracted from whole nuclei with ethanol. When the KCl concentration was raised to 1.0 M, an additional 20% of the label was extracted. However, approx. 40% of the radioactivity remained associated with nuclear components. An error is introduced into these calculations by the fact that we did not determine the proportion of bound and free hormone in the soluble extracts and the fact that the ethanol extracts would have contained hormone that was formerly bound to specific intranuclear sites, that which was non-specifically associated with nuclear membranes and other contaminating material, and free radioactivity. When bound label in the nuclear extracts was separated from free label by Sephadex chromatography, it was observed that the radioactivity was similarly distributed between the two fractions. One can observe from the study shown in Table 2 that, when this is taken into account, the residual binding in the nuclear pellet still represents 25% of the total radioactivity and approximately 40% of the salt solubilized bound radioactivity. These data suggest that a number of salt resistant binding sites for androgens and/or the androgen–receptor complex exists in epididymal nuclei. These sites are in addition to one or more classes

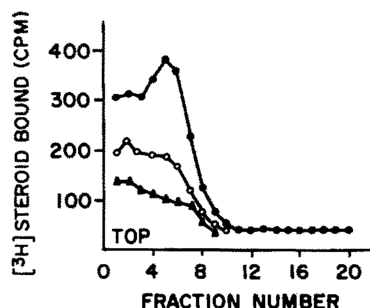

Fig. 3. The effect of antiandrogens on androgen binding to epididymal nuclei. The procedure outlined in Fig. 2 was utilized, except that unlabeled cyproterone (○——○) or unlabeled SKF 7690 (▲——▲) was used as a competitor and compared with a sample which had been incubated with [^{3}H]-testosterone alone (●——●).

of sites which are differentially solubilized by KCl. Our data do not, however, indicate whether these sites are of a hormone (or receptor) specific nature.

The observation that a portion of the radioactivity associated with nuclei is resistant to salt extraction has been noted by other investigators. Pasqualini *et al.*[22–23] have demonstrated that when the fetal guinea-pig kidney was exposed to [^{3}H]-aldosterone *in vivo* or *in vitro* 50–70% of the total radioactivity in the nuclei of renal cells was extracted by 1 M and 3 M NaCl–Tris solutions. They have also shown [24] that a portion of the [^{3}H]-estradiol associated with fetal guinea pig brain nuclei is extracted with 1 M NaCl. Lebeau *et al.*[25] observed that 80% of the [^{3}H]-estradiol bound to chick liver nuclei was recoverable in a protein fraction which could not be extracted by high salt or detergent solutions. They demonstrated that this tightly bound radioactivity could be "solubilized" by treatment of the nuclei with a dilute trypsin solution. This solubilized receptor retains many of the properties observed in intact nuclei. Middlebrook *et al.*[26], in their studies on glucocorticoid receptors in mouse fibroblasts, were able to detect residual binding to nuclear particles following a 0.3 M KCl extract. They have shown that, depending on conditions of incubation, 6% to greater than 50% of nuclear bound triamcinolone acetonide is resistant to KCl extraction. It was further demonstrated that treatment of the KCl extracted nuclear pellet with DNase or pronase alone or in combination extracted additional radioactivity from the pellet. However, only DNase treatment resulted in the extraction of macromolecular bound radioactivity. Our studies, taken in conjunction with those discussed above [22–26], show that salt resistant binding sites exist in nuclei of several different tissues for several steroid hormones. These observations may add an additional complication in the theory of steroid hormone action, since it becomes of interest to know which type of binding represents the physiologically meaningful interaction of the hormone or receptor–hormone complex with nuclei. It is possible that the two (or more) orders of nuclear binding sites may each be involved in a different aspect of hormone action.

We have determined that both [^{3}H]-testosterone and [^{3}H]-5α-DHT can be isolated from the epididymis following incubation of tissue minces with [^{3}H]-testosterone alone. This finding indicates that the rabbit epididymis is capable of reducing testosterone to 5α-DHT. In addition, we have shown that testosterone can also be metabolized to androstanediols and other metabolites by this system. Since the tissues were incubated with a large amount of [^{3}H]-testosterone, the observation that approx. 15–20% of the label bound to the cytoplasmic receptor and bound to nuclei was 5α-DHT, while only 3% of the free hormone in the cytosol was DHT, indicates that the cytoplasmic receptor and nuclei have a much greater affinity for the 5α-reduced compound than for testosterone. Two reports in the literature deal with androgen binding to rat epididymal nuclei [20, 21]. Tindall *et al.*[20] report that 1 h following an i.v. injection of [^{3}H]-testosterone approximately 70% of the radioactivity recovered from epididymal nuclei was dihydrotestosterone. The *in vivo* route of administration presents the probability that the kinetics of testosterone metabolism are different from those occurring in our system. Blaquier and Calandra [21], using minces of rat epididymides incubated with [^{3}H]-testosterone showed that the amount of bound radioactivity extracted from nuclei which corresponded to DHT varied according to the concentration of [^{3}H]-testosterone in the incubation medium from 92% to 55%. Although 5α-DHT may be the predominant androgen present in the nuclei of androgen dependent tissues under physiological conditions, it is clear that the ratio of testosterone/5α-DHT can vary according to experimental conditions. Variations in this ratio may also be due to species differences in 5α-reductase concentration or activity.

Estradiol-17β is capable of causing some slight inhibition of androgen binding to nuclei. We observed a similar phenomenon regarding inhibition of androgen binding to the epididymal cytoplasmic androgen receptor [4]. Korach and Muldoon[27] have noted that, although both testosterone and 5α-DHT are very poor competitors for estrogen binding sites in rat anterior pituitary glands as compared to estrogenic compounds, 5α-DHT was able to inhibit the initial rate of formation of the estradiol–receptor complex in a concentration dependent manner. They proposed that this phenomenon may be involved in modulating the effects of estrogens on the pituitary. We have shown that the epididymis, of at least immature rabbits, contains both cytoplasmic [28] and nuclear [4] binding components for estradiol-17β. This receptor appears to be distinct from androgen receptors, in that its affinity for estrogenic compounds is far greater than that for androgens. It is possible that low affinity binding by these receptors for the opposite ligand may be of some consequence as a mechanism for regulating epididymal function.

As we have demonstrated previously [4, 7, 9], neither cyproterone nor its 17-acetate is able to inhibit androgen binding to rabbit ABP or to rabbit TeBG. However, these compounds do inhibit androgen binding to the epididymal cytoplasmic androgen receptor [4, 8] and do prevent nuclear transfer of [^{3}H]-androgen–receptor complex. SKF 7690 is capable of inhibiting androgen binding to ABP [4, 7] and TeBG [9] as well as to the epididymal cytoplasmic androgen receptor [8]. These observations are important since they suggest that the active binding site on ABP and TeBG is in some manner different from that on the androgen receptor. This latter fact suggests that antiandrogens could be synthesized to specifically prevent binding to a given protein.

Although no direct evidence has been provided to

date that the receptor systems present in the epididymis are of direct importance in the regulation of epididymal function or sperm maturation, two of the steps currently required by the theory of steroid hormone action have been demonstrated in the epididymis, namely: (1) steroid binding to specific cytoplasmic receptors and (2) interaction of the receptor-hormone complex with the nucleus. In addition, Blaquier has shown that androgens stimulate protein [29] and RNA [30] synthesis in rat epididymal tissue. It is thus likely that in the epididymis, as in other tissues, steroid hormones promote gene activation. It is our current hypothesis that a product of such activation of the epididymal genome may be involved in initiating the functional maturation of spermatozoa.

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