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ANDROGEN INTERACTIONS WITH INTACT NUCLEAR ENVELOPES FROM THE RAT VENTRAL PROSTATE

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Summary—Intact nuclear envelopes containing nuclear pore complexes have been prepared from the rat ventral prostate. The polypeptide profile of the nuclear envelopes from the rat prostate resembled that of nuclear envelopes prepared from the male rat liver. Isolation of the nuclear envelopes after incubation of purified nuclei with radioactive dihydrotestosterone results in labelling of the membrane. More dihydrotestosterone is bound after incubations at 22°C for 18 h than at 2°C for 18 h or 22°C for 2 h. Scatchard analysis revealed a class of binding sites with an apparent K_d of 46 nM. Dihydrotestosterone, testosterone, cyproterone acetate and methyltrienolone were effective as competitors of labelled dihydrotestosterone binding to the nuclear envelopes, while estradiol did not compete. Castration of the rats 24, 48 and 96 h prior to preparation of nuclei resulted in loss of androgen binding to the membranes. Extraction with 0.6 M NaCl resulted in the loss of 72% of the androgen binding.

INTRODUCTION

Most steroid hormones act by the same intracellular mechanism [1, 2]. After binding to a cytosolic receptor protein present in target tissues, the steroid–receptor complex translocates to the nucleus, where its interaction at the genome results in altered cellular function. For continued hormone action, the steroid and the receptor are recycled into the cytoplasm, or degradation of the receptor occurs and more receptor is synthesized. Clearly the steroid–receptor complex must interact with the nuclear envelope during translocation into the nucleus and possibly during its recycling into the cytoplasm. This interaction may be a process during which modulation of steroid hormone action occurs, yet few studies have been directed toward the elucidation of the mechanism by which steroids traverse the nuclear envelope.

As a step toward understanding the process, we have characterized steroid interactions with a nuclear envelope fraction isolated after heparin-treatment of purified nuclei from the rat ventral prostate [3]. We identified a class of saturable high affinity binding sites which displays specificity for androgens and which is not present in nuclear envelopes prepared from 24 h-castrated animals. This class of sites may be involved in transport of the steroid across the nuclear envelope. However, electron microscopy revealed that the membrane fraction did not contain any nuclear pore complexes [3]. Since nucleocytoplasmic exchange has been suggested to occur via passage through nuclear pores [4, 5], we wished to determine whether a preparation containing nuclear pore complexes possessed an additional class of steroid binding sites or more of the same class of binding sites. These additional binding sites located on nuclear pores might then be more readily implicated in a transport process.

This paper describes the characterization of dihydrotestosterone binding to such a preparation. The same methodology as that used previously in the characterization of dihydrotestosterone binding to nuclear envelopes which did not contain nuclear pore complexes was used.

EXPERIMENTAL

Materials

5α-[1, 2, 4, 5, 6, 7, 16, 17-^{3}H]Dihydrotestosterone (208 Ci/mmol) was purchased from New England Nuclear Corporation, Boston, MA, U.S.A.; unlabelled steroid from Steraloids Inc., Wilton, NH, U.S.A.; 1,4-*bis*[2,5-phenyloxazolyl]benzene (POPOP) from Nuclear-Chicago, Des Plaines, IL, U.S.A.; 2,5-diphenyloxazole (POP) from Fisher Scientific Company, Fair Lawn, NJ, U.S.A.

Methods

Animals. Adult male Sprague–Dawley rats (250–350 g) were purchased from Charles River Canada Inc., Montreal, Canada and maintained on a diet of Wayne Lab Blox (Allied Mills, Chicago, IL, U.S.A.) and water *ad libitum*. The rats were killed by decapitation and the ventral prostates quickly removed, rinsed in 0.32 M sucrose containing 3 mM $MgCl_2$ (Buffer A), stripped of connective tissue, weighed and placed on ice.

Castration, where indicated, was performed via the scrotal route under Halothane anaesthesia.

Preparation of nuclei. All procedures were carried out at 2°C. Prostatic nuclei were prepared by slight modifications of the method of Widnell and Tata[6]. The prostatic tissue was minced and resuspended in

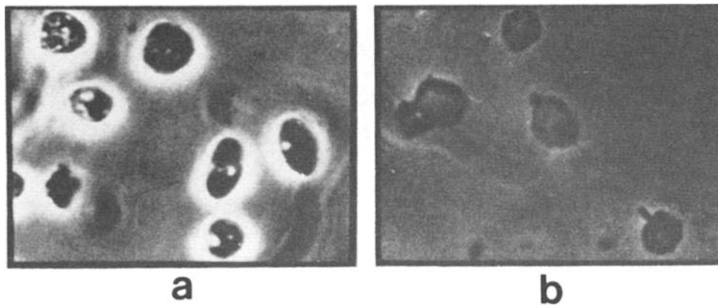

Fig. 1. Phase contrast micrographs of (a) nuclei, and (b) nuclear envelope "ghosts" isolated from the rat ventral prostate. Magnification: × 1300.

Buffer A (0.10 g/ml). The tissue was homogenized in a Polytron (Brinkman Instruments Inc.) at setting 4 for 10 s, then mashed through a wire screen (400 mesh) rinsing with three 2.5 ml aliquots of Buffer A. The homogenate was centrifuged at 3000 *g* for 10 min and the pellet was resuspended in 2.4 M sucrose, containing 1 mM $MgCl_2$, which had been brought to pH 7.4 with $NaHCO_3$. The suspension was centrifuged at 72,000 *g* for 60 min. The pellet was resuspended in 0.25 M sucrose, containing 1 mM $MgCl_2$ and this suspension was centrifuged at 800 *g* for 10 min. This pellet of purified nuclei was then used to prepare nuclear envelopes.

Preparation of nuclear envelopes. Purified nuclei were used to prepared nuclear envelopes by modifications of the procedure of Kay *et al.*[7]. The nuclei were resuspended in 0.25 M sucrose containing 1 mM $MgCl_2$, which had been brought to pH 7.4 with $NaHCO_3$, and subjected to centrifugation at 750 *g* for 5 min. The pellet was resuspended in digestion buffer (10 mM Tris–HCl, 0.30 M sucrose, 0.1 mM $MgCl_2$, 5 mM 2-mercaptoethanol, pH 8.5) to which freshly dissolved DNAse I was added to a concentration of 10 μg/ml. The suspension was incubated at 20°C for 20 min. Following centrifugation at 12,000 *g* for 5 min the crude nuclear envelopes were resuspended in 10 mM Tris–HCl, pH 7.4 and 1 ml was layered onto a discontinuous gradient of 2 ml 0.25 M sucrose and 3 ml each of 1.5, 1.8 and 2.0 M sucrose (all sucrose solutions made up in 10 mM Tris–HCl, pH 7.4). The gradients were centrifuged at 100,000 *g* for 90 min.

Incubations of nuclei with steroid. Nuclei from rat ventral prostate were prepared as described above. 100–250 μg (2–4×10^7 nuclei) nuclear protein was suspended in 10 mM Tris–HCl, pH 7.4. The nuclei were then incubated in a final volume of 1 ml in the presence of radioactive steroid for specified times and at specified temperatures. To a duplicate tube, 100-fold excess unlabelled dihydrotestosterone was also added to determine non-specific binding. The suspensions were then centrifuged for 1 min in a Beckman Microfuge B. Supernatants were removed with a Pasteur pipette. The pellet was washed twice by resuspension in 10 mM Tris–HCl, pH 7.4 followed by centrifugation and removal of the supernatant. Nuclear envelopes were then prepared as described above.

Protein determinations. Subcellular fractions of the rat prostate were measured for protein content by the method of Lowry *et al.*[8] using bovine serum albumin as standard.

Electron microscopy of the nuclear envelope. Nuclear membranes were prepared for electron microscopy by fixation of the nuclear membrane pellets (after centrifugation at 100,000 *g* for 90 min) in glutaraldehyde; followed by OsO_4 and embedded in Spurr (Electron Microscopy Sciences, Fort Washington, PA, U.S.A.). Thin sections were stained with uranyl acetate and lead citrate [9] and viewed on a Philips 400 Electron Microscope.

SDS–Polyacrylamide Gel Electrophoresis. SDS–PAGE was performed on slab gels according to the Laemmli system [10] on 12% polyacrylamide gels. One-hundred μg protein was applied to the gel and gels were stained with Coomassie blue nitrate.

RESULTS

Characterization of the nuclear envelope preparation

Nuclei from the rat ventral prostate, prepared as described in the Experimental section, were free of cytoplasmic contaminants and cellular debris, as monitored by phase contrast microscopy (Fig. 1a). A nuclear envelope fraction was then prepared essentially as described by Kay *et al.*[7] and further purified on a discontinuous sucrose density gradient. Figure 1(b) shows a phase contrast micrograph of intact nuclear envelopes or "ghosts" obtained before application to the sucrose density gradient. The nuclear envelopes are observed to be hollow spheres, relatively devoid of intranuclear material. After the sucrose density centrifugation, nuclear envelope material was found at the 0.25 M/1.5 M, 1.5 M/1.8 M and 1.8 M/2.0 M sucrose interfaces. Figure 2 is an electron micrograph of purified nuclear envelopes obtained after the sucrose density ultracentrifugation. Considerable vesicularization of the membranes is

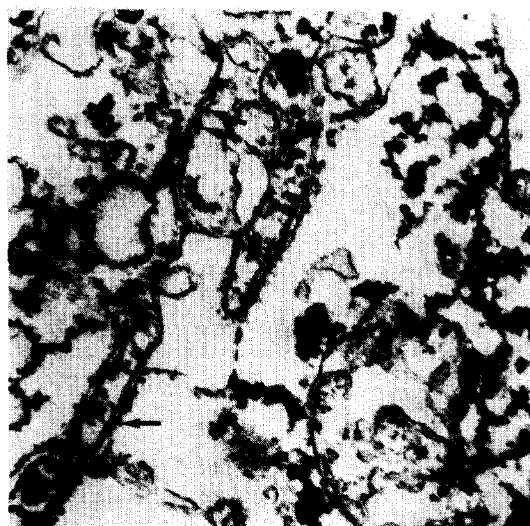

Fig. 2. Electron micrograph of a thin section from nuclear envelopes. Pellets of nuclear envelopes were prepared for electron microscopic examination as described in the Experimental section (× 55,350). Arrows indicate nuclear pore complexes.

apparent as well as the presence of large torn sheets. The two layered nuclear envelope membrane system is readily apparent although it is difficult to identify inner and outer nuclear membranes. Nuclear pore complexes are present in this preparation. There also appears to be chromatin attached to the membranes.

The peptide profile of the purified nuclear envelope preparation is shown in Fig. 3. Peptide profiles of purified prostatic nuclei and nuclear envelopes from the male rat liver, isolated by the same method as the prostate nuclear envelopes, are included. Comparison of the three profiles reveals that both nuclear envelope preparations are enriched in peptides having mobilities which correspond to mol. wt of 45,000–68,000, relative to the prostatic nuclei. These peptides are the lamins, the major peptides of the nuclear envelope [11, 12]. In addition, both nuclear envelope preparations are enriched relative to the prostatic nuclei, in one peptide having a molecular weight greater than 100,000. The liver and prostatic nuclear envelope peptide profiles are similar.

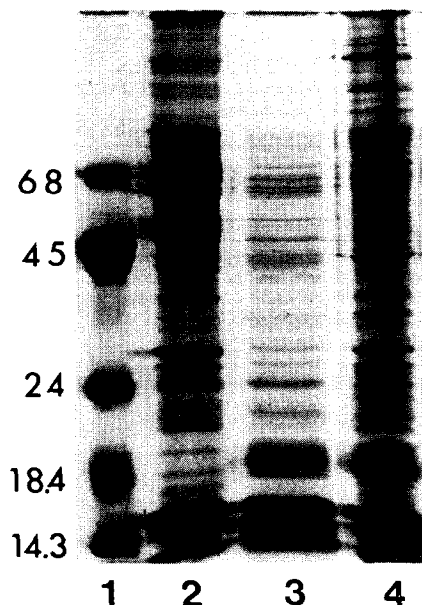

Fig. 3. Electrophotrophoretic analysis of polypeptide components of nuclear envelopes from the rat ventral prostate. Nuclei and nuclear envelopes (100 μg) were applied to the gel as described in the Experimental section. Lane 1: molecular weight markers. Lane 2: male rat liver nuclear envelope peptides. Lane 3: rat prostate nuclear envelope peptides. Lane 4: rat prostate nuclei peptides.

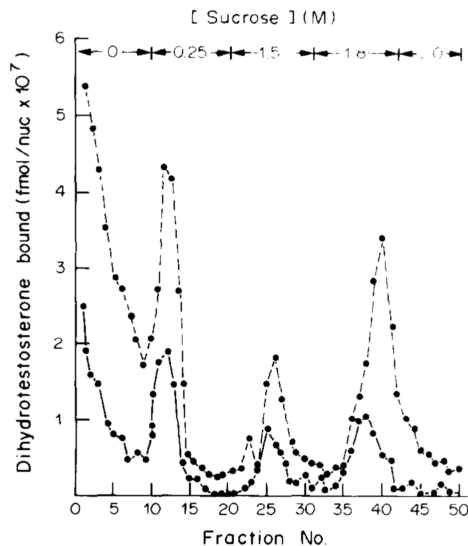

Fig. 4. Fractionation of a crude nuclear envelope preparation from the rat ventral prostate after incubation of nuclei with [^{3}H]dihydrotestosterone. Rat ventral prostate nuclei (2×10^7/tube) were suspended in 1.0 ml of buffer A containing 30 nM [^{3}H]dihydrotestosterone. To a duplicate tube, 100-fold excess unlabelled dihydrotestosterone was added. These tubes were incubated at 22°C for 18 h. The suspensions were centrifuged and supernatants were discarded. Crude nuclear envelopes were prepared and placed on the discontinuous sucrose density gradient. Fractions were collected from the top of the gradient and measured for radioactivity. Specific binding was calculated by subtracting non-specific binding (——; that binding remaining in the presence of excess unlabelled dihydrotestosterone) from total binding (.——.; that binding in the presence of labelled dihydrotestosterone alone).

Binding of dihydrotestosterone to nuclear envelopes

Binding of steroids to the purified nuclear envelopes was then investigated. After nuclei had been incubated with radioactive dihydrotestosterone, nuclear envelopes were isolated as described in the Experimental section. Fractions from the sucrose density gradient were obtained and the radioactivity in each was determined. In each case a duplicate tube containing in addition 100-fold excess unlabelled steroid was carried through the procedure to determine non-specific binding. Figure 4 shows the radioactivity profile of one such preparation in which 30 nM [^{3}H]dihydrotestosterone had been incubated

Table 1. Effect of time and temperature of incubation of nuclei with [^{3}H]dihydrotestosterone on binding of dihydrotestosterone to nuclear envelopes

Time of incubation (hours)	Temperature of incubation (°C)	Dihydrotestosterone specifically bound (fmol/10^7 nuclei)
2	22	3.15
18	2	2.37
18	22	8.32

Nuclei (2.1×10^7/tube) were incubated with 30 nM dihydrotestosterone in the presence and absence of 100-fold unlabelled dihydrotestosterone for the times and at the temperatures indicated. The incubation was terminated by centrifugation and nuclear envelopes were prepared. Specific binding to the nuclear envelopes was then determined.

Table 2. Competition for binding of [^{3}H]dihydrotestosterone to nuclear envelopes from the rat ventral prostate

Unlabelled hormone added	Concn (nM)	Hormone bound: fmol/nuc. $\times 10^7$	Hormone bound: % Control
None (control)		17.4	100.0
Dihydrotestosterone	3000	12.2	70.1
Testosterone	3000	11.3	64.9
Cyproterone acetate	3000	13.4	76.8
Methyltrienolone	3000	8.9	51.1
Estradiol	3000	18.6	107.0

Nuclei (2×10^7/tube) were prepared as usual and incubated with 30 nM [^{3}H]dihydrotestosterone for 18 h at 22°C in the presence of the non-radioactive steroids listed. The incubations were terminated and [^{3}H]dihydrotestosterone bound to the purified nuclear envelopes was determined.

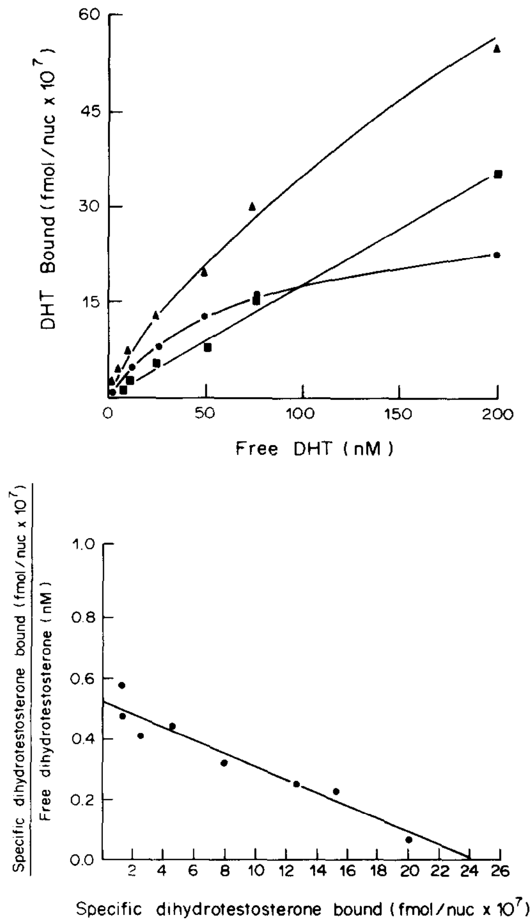

Fig. 5. Saturation analysis of [^{3}H]dihydrotestosterone binding to rat ventral prostate. Nuclei (3.1×10^7/tube) were incubated for 18 h at 22°C with increasing amounts of [^{3}H]dihydrotestosterone (1–65 nM) to measure total [^{3}H]dihydrotestosterone bound (▲). Another set of incubation mixtures was run simultaneously, but with the addition of 100-fold excess non-radioactive dihydrotestosterone to measure non-specific [^{3}H]dihydrotestosterone (■). The nuclear envelopes were prepared and fractions from the sucrose density gradient were collected and assayed for radioactivity. Specifically bound [^{3}H]dihydrotestosterone (●) was calculated from the difference in total and non-specific [^{3}H]dihydrotestosterone bound, after corrections for background (Fig. 5a) and plotted according to Scatchard (Fig. 5b). The line of best fit was determined by linear regression analysis and the correlation coefficient was 0.86 ($P < 0.05$).

with nuclei for 18 h at 22°C. Three peaks of radioactivity were observed. These corresponded to the 0.25 M/1.5 M, 1.5 M/1.8 M and 1.8/2.0 M sucrose interfaces, where we had collected purified nuclear envelopes as identified by both phase contrast microscopy and electron microscopy. In this experiment, 37% of the total radioactivity was bound specifically, as determined by subtraction of the non-specific radioactivity bound (binding in the presence of excess unlabelled steroid) from the total binding (binding in the presence of labelled steroid alone). This binding to the purified nuclear envelopes represented 55% of the radioactivity placed on the gradient, i.e. in the crude nuclear envelope fraction. The other radioactivity was recovered as free steroid which was recovered at the front of the gradient, and in the pellet. Approximately 14% of the radioactivity bound to the nuclei after the incubation was bound to the purified nuclear envelopes.

Table 1 shows the effect of incubation temperature and duration of incubation on the binding of 30 nM [^{3}H]dihydrotestosterone to the nuclear envelope fraction. From these results, it was decided to allow the nuclei to incubate with dihydrotestosterone for 18 h at 22°C in the next series of experiments.

A saturation analysis of the binding of dihydrotestosterone to nuclear envelopes from the rat ventral prostate was then carried out. Figure 5(a) shows that there was considerable non-specific binding but Scatchard analysis [13] revealed the presence of a class of binding sites with an apparent K_d of 46.2 nM and a maximum capacity of 24 fmol/10^7 nuclei or approx 168 fmol/g tissue. As yet more than one class of binding sites cannot be ruled out, especially in view of the fact that the non-specific binding was 50–67% of the total binding.

Steroid specificity of the binding was examined by competition experiments in which 100-fold unlabelled competitor steroid was added to nuclear incubations with 30 nM [^{3}H]dihydrotestosterone (Table 2). Dihydrotestosterone, the biologically active androgen in the rat ventral prostate [14, 15] and testosterone, another androgen, which requires conversion into dihydrotestosterone by the action of the

Table 3. The effect of castration on the binding of [^{3}H]dihydrotestosterone to nuclear envelopes from the rat ventral prostate

Time after Castration (hours)	Hormone bound (fmol/nuc × 10^7)		% Control	
	Non-specific	Specific	Non-specific	Specific
Control	26.5	13.4	100	100
24	32.4	5.1	122.2	38.1
48	27.9	0.0	105.3	0.0
96	17.2	2.5	64.9	18.7

Rats were castrated 24, 48 or 96 h before preparation of nuclei. Control animals were not castrated, but were subjected to anaesthesia. Rat ventral prostate nuclei (2.75×10^7/tube) were incubated with 30 nM [^{3}H] dihydrotestosterone in the presence (to determine non-specific binding) and in the absence (to determine total binding) of 100-fold excess unlabelled steroid. Nuclear envelopes were prepared as described above and specific binding of [^{3}H]dihydrotestosterone was determined by subtracting non-specific from total binding. These results are the means from two experiments.

5α-ketosteroid reductase, before being active in the prostate, are equally effective in competing for the binding of [^{3}H]dihydrotestosterone to nuclear envelopes. Cyproterone acetate, an anti-androgen, and methyltrienolone, a synthetic androgen, also competed effectively for binding of [^{3}H]dihydrotestosterone. Estradiol, on the other hand, was not able to compete for dihydrotestosterone binding sites.

As castration of the rat 24 h prior to isolation of nuclei had resulted in depletion of dihydrotestosterone binding to nuclear envelopes prepared after heparin treatment [3], we wished to see whether the site on this more intact preparation of nuclear envelopes was also affected by castration (Table 3). Castration 24 and 48 h prior to sacrifice of the animal resulted in loss of 62 and 100% of the specific sites respectively. Non-specific binding was not affected by castration after these two lengths of time. At 96 h after castration, non-specific binding was reduced to 65%, but specific binding appeared to increase to 20% of its original value.

Finally we wished to determine whether the specific dihydrotestosterone binding sites were salt extractable. Table 4 shows that incubation of nuclear envelopes in Tris–HCl alone for 30 min at 22°C prior to application on the discontinuous sucrose density gradient resulted in loss of 72% of the specific binding sites. Incubation in 0.6 M NaCl resulted in a further loss of 20% of the specific dihydrotestosterone binding sites. If dihydrotestosterone binding to nuclear envelopes after incubation in Tris–HCl for 30 min at 22°C is used as the control, 72% of these sites are extracted with 0.6 M NaCl.

DISCUSSION

We have shown that androgens interact specifically with nuclear envelopes from the androgen-responsive tissue, the rat ventral prostate [3] and an androgen-dependent cell line of the Shionogi mouse mammary carcinoma [16]. On the other hand, two androgen-independent cell lines of the Shionogi mouse mammary carcinoma did not display specific androgen binding. Liver nuclear envelopes also possess binding sites for androgens, but these sites differ from those on nuclear envelopes from more classical androgen target tissues, in that glucocorticoids compete as effectively as androgens for binding to these sites [17]. These studies have convinced us that binding sites for steroids exist on the nuclear envelopes of steroid target tissues. We now wish to further investigate the role of these sites and the possibility that they are involved in the modulation of the steroid response.

A possible role of the steroid binding sites on the nuclear envelope is in the transport of steroids to their site of action at the gene. Nuclear pores have been suggested as conduits for nucleocytoplasmic exchange [4, 5]. Our previous identification of dihy-

Table 4. The effect of NaCl on the specific binding of [^{3}H]dihydrotestosterone to nuclear envelopes from the rat ventral prostate

	Specific hormone bound fmol/nuc × 10^7	% Control
Control nuclei—not subjected to incubation	9.41	100.0
Nuclei subjected to a incubation in 10 mM Tris–HCl	2.67	28.4
Nuclei subjected to incubation in 0.6 M NaCl in 10 mM Tris–HCl, pH 7.4	0.75	8.0

Nuclei (2.5×10^7/tube) were incubated with 30 nM [^{3}H]dihydrotestosterone in the presence (to determine non-specific binding) and in the absence (to determine total binding) of 100-fold excess unlabelled dihydrotestosterone for 18 h at 22°C. Nuclear envelopes were prepared as usual but before application to the discontinuous sucrose density gradient they were subjected to a 30 min incubation at 22°C with 10 mM Tris–HCl, pH 7.4 or 0.6 M NaCl in 10 mM Tris–HCl, pH 7.4.

drotestosterone binding sites on the rat ventral prostate nuclear envelope made use of nuclear envelopes isolated after heparin-treatment of purified nuclei. This preparation did not contain nuclear pore complexes, as determined by electron microscopy. Furthermore, in studies of dihydrotestosterone binding to intact and detergent-treated nuclei [18] we were unable to detect differences in the capacity of the binding sites on the two nuclear preparations, indicating that the removal of the outer nuclear membrane and some inner nuclear membrane proteins by detergent did not result in loss of dihydrotestosterone binding sites. We deduced that nuclear envelope binding sites were localized to the inner nuclear membrane and perhaps to the nuclear pore-lamina fraction. In the present study we have prepared intact nuclear envelopes containing nuclear pore complexes in order to compare dihydrotestosterone binding to this preparation with our previous studies and determine whether there are interactions of steroids with nuclear pore complexes.

The preparation of rat ventral prostate nuclear envelopes used in this study [7] resulted in a preparation of relatively intact nuclear ghosts as monitored by phase contrast microscopy. Electron microscopy revealed some vesicularization of the envelopes, but the presence of the two-layered nuclear membrane system observed around most eukaryotic nuclei was readily apparent. The two-layered nuclear membrane system is not readily apparent in thin sections of purified nuclear envelope that has undergone a significant degree of fragmentation during the isolation and purification procedure [11]. Intact nuclear pore complexes could also be detected in the electron micrographs. Some chromatin can be observed in association with the membrane preparation. It has been suggested that low amounts of DNA are required for the structural integrity of the nuclear envelope [19]. It was of interest that nuclear envelopes were found at three interfaces of the sucrose density gradient. No obvious differences in the morphology of the nuclear envelopes at the three sucrose densities were noted but this is being investigated further. Agutter and Gleed [20] have reported that the density of sheep liver nuclear envelope preparations varied according to the concentrations of nuclei during the lysis stage.

We determined that optimal binding conditions were obtained after incubations of dihydrotestosterone with nuclear envelopes for 18 h at 22°C. Incubations for the same period at 2°C resulted in only 28.5% the specific binding observed at 22°C. It appears therefore that endogenous steroid is bound to some of the binding sites and that higher temperatures are required for the exchange of the endogenous ligand with exogenous dihydrotestosterone.

Scatchard analysis revealed a class of binding sites which was indistinguishable from the class of binding sites we had previously described on the haparin-obtained nuclear envelopes from the rat ventral prostate. This preparation resulted in dihydrotestosterone binding with an apparent K_d of 46 nM and a maximum binding capacity of 168 fmol/g tissue while the latter possessed a class of binding sites having an apparent K_d of 8.4 nM and a binding capacity of 152 fmol/g tissue. The affinity of the binding site is clearly an approximation, as some of the dihydrotestosterone bound to the nuclear envelopes before application to the sucrose density gradient dissociates during the ultracentrifugation.

The steroid specificity of the nuclear envelope binding sites also resembles that of the ventral prostate nuclear envelope binding site previously described. Furthermore, it resembles that of the dihydrotestosterone receptor of the rat ventral prostate [21, 22]. As we are measuring binding of exogenous steroid to nuclear envelopes from intact male rats, it is possible that the binding is mediated by the dihydrotestosterone receptor. It has been shown that 24 h after castration the concentration of androgen receptor in the prostatic cytoplasm is increased [23, 24]. Bruchovsky and co-workers suggested that this receptor originates in the nucleus [24, 25]. Castration resulted in the loss of nuclear envelope binding sites. The nuclear envelope may therefore represent a pool from which receptor is lost to the cytoplasm. On the other hand, it is possible that endogenous steroid is necessary for the binding site to be accessible to exogenous steroid. As nuclear receptor is often solubilized by extraction in 0.6 M NaCl, we investigated whether the nuclear envelope localized steroid binding sites were extractable in 0.6 M NaCl. We found that most sites were extractable by incubation in Tris–HCl alone at 22°C for 30 min. However, of those sites which were not, 72% were extractable in 0.6 M NaCl, while 28% did not appear to be extractable. Others have reported that not all bound steroid of androgen-sensitive cells can be extracted with salt [24, 26, 27] and Davies *et al.*[27] report that material refractory to extraction with 0.6 M KCl comprised between 16–25% specifically retained ^{3}H-labelled steroid. Finally, it should be noted that the androgen binding may be associated with the residual chromatin on the nuclear envelope preparation and this possibility is under investigation.

In summary, the class of dihydrotestosterone binding sites present on nuclear envelopes from the rat ventral prostate which contain intact nuclear pore complexes are similar to those which did not contain visible nuclear pores with respect to: (a) affinity and numbers of binding sites, (b) steroid specificity and (c) response to castration. We conclude that either a component(s) of the nuclear pore complex is not involved in dihydrotestosterone interactions with the nuclear envelopes or that the binding component of the nuclear pore complex was present in the previously investigated nuclear envelope fraction in an indistinguishable form. In addition the similarity of the response of the nuclear envelope binding sites to castration and salt-extraction with that of nuclear

dihydrotestosterone receptors indicates that the binding of dihydrotestosterone to the nuclear envelope may be mediated by the receptor.

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