Binding of Androgens to a Nuclear-Envelope Fraction from the Rat Ventral Prostate

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A nuclear-envelope fraction was isolated from the rat ventral prostate which is virtually free of DNA and contains little RNA or plasma membrane. Isolation of this nuclear-envelope fraction after incubation of purified nuclei with radioactive dihydrotestosterone results in labelling of the membrane. More binding of dihydrotestosterone is observed after incubations at 22°C for 17 h than at 4°C for 17 h or at 22°C for 60 min. Scatchard analysis revealed a class of binding sites with K_D 8.4 nM. Dihydrotestosterone and testosterone were almost equally effective as competitors of labelled dihydrotestosterone binding on the purified nuclear-envelope fraction, whereas diethylstilboestrol was less effective and dexamethasone did not compete well. When the outer membrane of the nuclei was removed with Triton X-100, a 24% decrease in specific binding of androgens was observed. Castration 24 h before preparation of nuclei resulted in loss of the androgen binding to the membrane.

It is generally accepted that most, if not all, steroid hormones act by the same intracellular mechanism (Gorski & Gannon, 1976; Chan & O'Malley, 1976*a,b*). After binding to a cytosolic receptor protein present only 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 membrane during translocation into the nucleus and possibly during its recycling into the cytoplasm. This interaction may be a point where control of steroid-hormone action is exerted, yet few studies have been directed toward the elucidation of the mechanism by which steroids traverse the nuclear membrane.

As a step toward the understanding of this process we have characterized a nuclear-membrane fraction isolated after heparin treatment of purified nuclei from the rat ventral prostate (Bornens, 1973, 1977; Bornens & Courvalin, 1978) and have investigated the binding of androgens to this nuclear-membrane fraction.

Materials and Methods

Animals

Adult male Wistar rats (250–350 g) were purchased from Woodlyn Laboratories, Guelph, Ontario, 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 0.01 M-Tris/HCl, pH 7.0, containing 0.05 mM-EDTA, 5 mM-MgCl₂ and 0.5 mM-mercaptoethanol (buffer A), stripped of connective tissue, placed on ice and weighed.

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

Preparation of nuclei

Nuclei were routinely prepared by the method of Bruchovsky *et al.* (1975). Detergent-treated nuclei were prepared by a modification of the method of Lesser & Bruchovsky (1974). Prostatic tissue was processed to yield the first crude nuclear pellet (Bruchovsky *et al.*, 1975). This was suspended in phosphate-buffered saline (Dulbecco & Vogt, 1954) containing Triton X-100 [octylphenoxypoly-(ethoxyethanol); Sigma Chemical Co.; 0.5%, v/v]

with five strokes of the loose-fitting plunger in a Dounce homogenizer and the resultant suspension was centrifuged at 700g for 10 min. The pellet was then resuspended in phosphate-buffered saline containing bovine serum albumin (0.3%, w/v) and Triton X-100 (0.22%, v/v). This suspension was centrifuged at 40g for 2 min to remove debris. The supernatant was centrifuged at 1000g for 20 min, the pellet resuspended in 0.88 M-sucrose and 1.5 mM-CaCl₂ in buffer A and layered on sucrose gradients consisting of 5 ml of 2.2 M-sucrose in buffer A containing 0.5 mM-CaCl₂ and 5 ml of 1.8 M-sucrose in buffer A containing 0.5 mM-CaCl₂.

Preparation of nuclear membranes

Nuclear membranes were prepared from nuclei by modifications of the procedures of Bornens (1973, 1977), Bornens & Courvalin (1978) and Hildebrand & Okinaka (1976). Nuclei were resuspended in 10 mM-Tris/HCl, pH 8.0, containing 10 mM-Na₂HPO₄, by gentle vortex mixing, to a concentration of 200 µg of DNA/ml. Heparin (Sigma Chemical Co.) was added so that the weight ratio of DNA concentration to heparin concentration was 1:2. This suspension was left on ice for 5 min, after which the suspension was diluted 10–30-fold with 10 mM-Tris/HCl, pH 9.0, and stirred in the cold for 40 min. Crude nuclear membranes were pelleted by centrifugation at 70 000g for 40 min (type 65 rotor, r_{av} 5.7 cm) in a Beckman L2-75B ultracentrifuge. Further purification was achieved by resuspending the pellet in 0.01 M-Tris/HCl (pH 7.4)/0.003 M-CaCl₂ (buffer B) containing 2.2 M-sucrose so that the final density of this suspension was 1.22 g/cm³. This suspension (5.5 ml) was layered in an SW27 cellulose nitrate tube containing 5.5 ml of sucrose in buffer B (density 1.24 g/cm³) and overlaid successively with 5.5 ml of sucrose solutions (in buffer B) of densities 1.20, 1.18 and 1.16 g/cm³. Then 9.0 ml of sucrose in buffer B (density 1.10 g/cm³) was added. Samples were centrifuged in a Beckman L2-65B ultracentrifuge for 14 h at 70 000g (SW27 rotor, r_{av} 11.6 cm). Gradients were monitored for protein with an LKB 8300 Uvicord II instrument at 280 nm and fractionated (0.5 ml fractions) from the top by pumping 2.2 M-sucrose into the bottom of the tube. Fractions were either measured for radioactivity after adding 0.5 ml of water and 10 ml of Ready-Solv (Beckman Instruments), or pooled and centrifuged at 70 000g for 90 min. This final pellet was the purified nuclear membrane. The yield of membrane was routinely 0.05 mg of protein/g fresh wt. of tissue or approx. 0.02 mg of protein/animal. Membranes were stored at –15°C.

Incubations of intact nuclei with steroid

Nuclei from rat ventral prostate were prepared as described above; 100–250 µg of nuclear protein was

suspended in 0.8 ml of buffer A containing 2% (v/v) ethanol to keep the steroid in solution. This concentration of ethanol did not alter the nuclei, as monitored by light-microscopy. The nuclei were then incubated for specified times and at specified temperatures in a final volume of 1 ml in the presence of radioactive steroid. To a duplicate tube, 100-fold excess of unlabelled steroid was also added to determine non-specific binding. The suspensions were then centrifuged at 24 000g for 10 min (SS-34 rotor, r_{av} 4.25) in a Sorvall RC-5B centrifuge. Supernatants were decanted and nuclear membranes were prepared as described above.

Biochemical analyses

Subcellular fractions of the rat prostate were measured for protein content by using the method of Lowry *et al.* (1951), with bovine serum albumin as standard. DNA was measured by the method of Thomas & Farquhar (1978), with calf thymus DNA as standard. RNA was measured by the Schneider (1957) method, with yeast RNA as standard. Phospholipid content was measured after extraction of nuclear-membrane lipids by the method of Garbus *et al.* (1963), and measurement of organic phosphate was by the method of Bartlett (1959). (Na⁺ + K⁺)-stimulated ATPase was determined by the method of Wilson & Vilee (1975).

Electron microscopy of nuclear membrane

Nuclear membranes were prepared for electron microscopy by fixation of the nuclear-membrane pellet (after centrifugation at 50 000g for 60 min) in glutaraldehyde, followed by OsO₄, and imbedded in Vestapol W (Polysciences Inc., Warrington, PA, U.S.A.). Thin sections were stained with uranyl acetate and lead citrate (Parr, 1973).

Chemicals

5 α -[1,2,4,5,6,7,16,17-³H]dihydrotestosterone (190 Ci/mmol) was purchased from New England Nuclear, and unlabelled steroids were from Steraloids, Wilton, NH, U.S.A.

Results

Characterization of the nuclear-envelope fraction

Nuclei from the rat ventral prostate, prepared as described above, were free of cytoplasmic contaminants and cellular debris, as monitored by light-microscopy. A nuclear-envelope fraction was then prepared essentially as described by Bornens (1973) and further purified by ultracentrifugation on a discontinuous sucrose density gradient (Hildebrand & Okinaka, 1976). The nuclear-envelope fraction before application to the discontinuous density gradient was called crude nuclear membranes, and that collected after centrifugation on the gradient

was called purified nuclear membranes. Fractionation and analysis of the sucrose density gradient after ultracentrifugation revealed (Fig. 1) two major and two minor protein peaks. The two major peaks banded at the 1.18/1.20g/cm³ interface and at the

Fig. 1. Fractionation of crude nuclear-membrane preparation by discontinuous-sucrose-gradient equilibrium sedimentation

Nuclei (3.5×10^5 /ml final concn. in incubation mixture) were incubated with heparin (400 µg/ml) at 0–4°C for 5 min. The incubation mixture was diluted 20-fold and stirred at 0–4°C for 40 min before centrifugation at 70 000g for 35 min. The pellet was layered on a discontinuous sucrose density gradient as described in the Materials and Methods section. Protein was determined by monitoring A_{280} as fractions (0.5 ml) from the top of the gradient were collected.

1.20/1.22g/cm³ interface. The fractions under these two peaks were either pooled and called purified nuclear membranes or collected separately, in which case those at the lighter density were called purified nuclear membrane I and those at the heavier density were called purified nuclear membrane II.

Chemical analyses of the nuclear-membrane fractions are shown in Table 1. Only 0.07% of the homogenate protein (2% of the nuclear-membrane protein) was recovered in the purified nuclear membranes. The crude nuclear-membrane fraction contained 69.8% of the homogenate DNA or 77% of the nuclear DNA, but ultracentrifugation of this fraction on the sucrose density gradient resulted in removal of 98% of this DNA, so that 1.2% of the nuclear DNA remained in the purified nuclear-membrane pellet. The DNA content of the purified nuclear membranes was 0.12 mg/mg of protein, and the phospholipid content was 0.88 mg/mg of protein. When the two major protein peaks from the sucrose density gradient were analysed separately, it was observed that the lighter band contained less DNA than the heavier band and that the phospholipid in the lighter band represented 70% of the protein weight present.

RNA analysis of the crude nuclear membranes revealed that the RNA content was 0.05 mg/mg of protein. Since most nuclear-membrane preparations contain more than this amount of RNA (Harris, 1978), it was decided that any further consideration of RNA as a contaminant was unnecessary.

Wilson & Vilee (1975) suggested that 5'-nucleotidase is unsuitable as a plasma-membrane marker in the rat ventral prostate, as this enzyme may be one of the phosphatases present in seminal fluid secreted by the rat. Therefore (Na⁺ + K⁺)-ATPase was used as a plasma-membrane marker. Only 0.2% of the

Table 1. Biochemical analyses of rat ventral-prostate subcellular fractions

The subcellular fractions were prepared and protein, DNA and phospholipid were determined. Recoveries, based on homogenate content as 100%, were: protein, 96.8%; DNA, 99.3%; phospholipid, 86.2%. The results represent the average of results from two preparations. The phospholipid content of the purified nuclear-membrane II fraction was not determined.

Fraction	Protein		DNA		Phospholipid	
	(mg/g of rat ventral prostate)	(% of total)	(mg/g of rat ventral prostate)	(% of total)	(mg/g of rat ventral prostate)	(% of total)
Homogenate	70.89	100	0.53	100	13.11	100
Nuclei	2.45	3.5	0.48	90.6	0.47	3.5
Crude nuclear membranes	0.57	0.8	0.37	69.8	0.18	1.37
Purified nuclear membranes	0.05	0.07	0.006	1.1	0.044	0.33
Purified nuclear membrane I	0.02	0.02	0.002	0.37	0.014	0.10
Purified nuclear membrane II	0.03	0.05	0.004	0.75	—	—

bulk-homogenate ($\text{Na}^+ + \text{K}^+$)-ATPase was recovered in the crude nuclear-membrane fraction, with a specific activity which was 29% that of the homogenate. It appeared therefore that the nuclear-membrane fraction probably did not contain significant amounts of plasma membrane.

Because of the abnormally high values obtained for phospholipid in the purified nuclear-membrane fractions (see Harris, 1978), it was decided to compare electron micrographs of the crude nuclear membranes and of the purified nuclear membrane I. Plate 1(a) shows that the crude nuclear-membrane preparation contained little evidence of nuclear pores, though there is some evidence that inner and outer membranes are present. There is limited vesicular formation. Plate 1(b) shows that the purified nuclear-membrane I preparation consisted of membranes with less vesicular formation and no evidence of inner and outer membranes. The band at the $1.18/1.20\text{g}/\text{cm}^3$ interface, then, is clearly composed of membranes, and the high phospholipid content is at present not understood.

Binding of steroids to the purified nuclear-membrane fraction

Binding of steroids to the purified nuclear-membrane fractions was investigated. After nuclei had been incubated with radioactive steroid, nuclear membranes were isolated as described in the Materials and Methods 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. Fig. 2 shows the radioactivity profile of one such preparation in which 10 nM - $[^3\text{H}]$ dihydrotestosterone had been incubated with nuclei for 17 h at 22°C . Comparison with Fig. 1 shows that the peak of radioactivity coincides with the lighter of the two most prominent protein bands, that band designated as purified nuclear membrane I. In this experiment, 41% 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 specific binding represented 63% of the radioactivity recovered in the crude nuclear-membranes fraction. The other radioactivity was recovered in a diffuse pellet. Approx. 9% of the radioactivity bound to nuclei after the

Fig. 2. Fractionation of a crude nuclear-membrane preparation from rat ventral prostate after incubation of nuclei with $[^3\text{H}]$ dihydrotestosterone

Rat ventral-prostate nuclei ($1.3 \times 10^7/\text{tube}$) were suspended in 0.8 ml of buffer A containing 2% (v/v) ethanol, and 0.2 ml of $[^3\text{H}]$ dihydrotestosterone was added to a final concentration of 10 nM . To a duplicate tube 100-fold excess of unlabelled dihydrotestosterone was added. These tubes were incubated for 17 h at 22°C . The suspensions were then centrifuged at 24000 g for 10 min. Supernatants were decanted and crude nuclear membranes were prepared and placed on the discontinuous sucrose density gradient as described in the Materials and Methods section. Fractions were collected from the top of the gradient and measured for radioactivity. Specific binding (----) was determined 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).

Table 2. Effect of time and temperature of incubation of nuclei with $[^3\text{H}]$ dihydrotestosterone on binding of dihydrotestosterone to the purified nuclear-membrane fraction

Nuclei ($1.7 \times 10^7/\text{tube}$) were incubated with 10 nM - $[^3\text{H}]$ dihydrotestosterone in the presence and absence of 100-fold non-radioactive dihydrotestosterone for the times indicated and at the temperatures indicated. The incubation was terminated by centrifugation at 24000 g for 10 min. The nuclear pellet was resuspended and purified nuclear membranes were prepared. Specific binding to the purified nuclear-membrane I fraction was determined.

Time of incubation	Temperature of incubation ($^\circ\text{C}$)	Dihydrotestosterone bound (fmol/g of tissue)
60 min	22	16.8
17 h	2	21.2
17 h	22	126.1

EXPLANATION OF PLATE 1

Electron micrographs of thin sections from nuclear membranes

Pellets of nuclear membranes were prepared for electron-microscopic examination as described in the Materials and Methods section (bar = 0.1 μm). (a) Crude nuclear-membranes preparation; (b) purified nuclear-membrane I preparation.

incubation was recovered in the purified nuclear membrane I.

Table 2 shows the effect of incubation temperature and duration of incubation on the binding of 10nM-[³H]dihydrotestosterone to the purified nuclear-membrane fraction. From these results, it was decided to allow the nuclei to incubate for 17 h at 22°C in the next series of experiments.

A Scatchard analysis of the binding of dihydrotestosterone was then carried out. Fig. 3 shows that there was considerable non-specific binding, but that the K_D for specific binding could be calculated as 8.4nM, with an apparent number of binding sites of 152fmol/g of tissue. As yet, more than one class of binding sites cannot be ruled out, especially in view of the fact that there is considerable non-specific binding. In addition, the amount of endogenously bound androgens is unknown at present.

Steroid specificity of the binding was examined by competition experiments in which 100nM and 1000nM unlabelled competitor steroid were added to

nuclear incubations with 10nM-[³H]dihydrotestosterone (Table 3). Dihydrotestosterone, the biologically active androgen in the rat ventral prostate (Wilson & Gloyne, 1970; Liao & Fang, 1969), was a slightly more effective competitor than testosterone, which requires conversion into dihydrotestosterone before being biologically active. Diethylstilboestrol, which would compete for oestradiol-binding sites, did compete for the dihydrotestosterone-binding sites, though it was about 50% as effective at 100nM. Dexamethasone, which would bind to glucocorticoid-binding sites, was a much less effective competitor.

Previous reports indicate that only the inner nuclear membrane remains after treatment of nuclei with non-ionic detergent (Blobel & Potter, 1966), although these conclusions have been questioned (Aronson & Blobel, 1975). We investigated the binding of androgens to nuclear membranes prepared after treatment of nuclei with Triton X-100 (Table 3). The same radioactivity profile on the discontinuous gradient was observed in each case, and results recorded in Table 3 show that 77.3% of the binding was obtained in the presence of 10nM-dihydrotestosterone, whereas competition with 100-fold excess of unlabelled dihydrotestosterone and testosterone resulted in binding almost identical with that to the nuclei prepared in the usual manner. It seems therefore that some of the specific binding sites may have been removed by Triton treatment, but the number of non-specific binding sites remained the same.

To substantiate the physiological significance of these binding sites, we investigated the effect of castration of the animals on these binding sites. We found that castration resulted in essentially complete depletion of binding to the purified nuclear membrane. Results are shown in Table 3.

Fig. 3. Specific and non-specific binding of [³H]dihydrotestosterone by purified nuclear membrane I

Various concentrations of [³H]dihydrotestosterone (1.5–40nM) were incubated with 1.4×10^7 nuclei for 17 h at 22°C to give total [³H]dihydrotestosterone bound. Another set of incubation mixtures was run simultaneously, but with the addition of 100-fold excess of non-radioactive dihydrotestosterone. The purified nuclear membranes were prepared and fractions from the discontinuous sucrose density gradient were collected and assayed for radioactivity. Those fractions corresponding to purified nuclear membrane I were pooled. The differences in radioactivities between the total [³H]dihydrotestosterone bound (■) and the non-specifically bound (○), after corrections for background, are plotted as the specifically bound [³H]dihydrotestosterone (▲). Each point represents the average value of two separate determinations. Inset: Scatchard plot of the [³H]dihydrotestosterone-binding data. Bound steroid is expressed as fmol/g of tissue.

Discussion

An understanding of steroid-hormone action necessitates consideration of the process by which the steroid-receptor complex crosses the nuclear membrane before it interacts with the genome and possibly afterward as it is recycled into the cytoplasm during continued action of the hormone. The translocation of some proteins across the nuclear membrane appears to occur via the nuclear pores (Gurdon, 1970; Paine & Feldherr, 1972), but there is a size limitation (Gurdon, 1970). Whether translocation of the steroid-receptor complex occurs via the pores or not, the transport of such a complex across the nuclear membranes will probably require a specific series of interactions with components of the nuclear envelope. We therefore decided to investigate this aspect of steroid-hormone action by characterizing interactions of steroids with nuclear-

Table 3. *Effect of various procedures on binding of [3 H]dihydrotestosterone to the purified nuclear-membrane fraction*
 In Expt. 1, nuclei (1.5×10^7 /tube) were prepared as usual and incubated with 10nM-[3 H]dihydrotestosterone for 17h 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 membranes was determined. In expt. 2, nuclei (1.5×10^7 /tube) that had been treated with Triton X-100 to remove the outer nuclear membrane were incubated with 10nM-[3 H]dihydrotestosterone for 17h 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 membrane was determined. In Expt. 3, the rats were castrated 24h before death. Rat ventral-prostate nuclei (1.5×10^7 /tube) were incubated with 10nM-[3 H]dihydrotestosterone in the presence of the non-radioactive steroids listed. The incubations were terminated and [3 H]dihydrotestosterone bound to the purified nuclear membrane was determined.

Experimental condition	Unlabelled hormone added	Concn. (nM)	Hormone bound	
			(fmol/g of tissue)	(% of control)
1. Nuclei prepared as usual	None		183.2	100.0
	Dihydrotestosterone	100	91.8	50.1
		1000	72.4	39.5
	Testosterone	100	103.9	56.7
		1000	79.2	43.2
	Diethylstilboestrol	100	176.1	96.1
		1000	97.7	53.3
	Dexamethasone	100	175.8	96.0
		1000	146.7	80.1
2. Triton-treated nuclei	None		141.7	77.3
	Dihydrotestosterone	1000	72.3	39.5
	Testosterone	1000	84.1	45.9
3. Nuclei prepared 24 h after castration	None		3.8	2.1
	Dihydrotestosterone	1000	3.7	2.0

envelope fractions prepared from the androgen target tissue, the rat ventral prostate.

The crude nuclear-membrane fraction prepared from rat ventral prostate by the method of Bornens (1973) did not contain many nuclear-pore complexes when observed by electron microscopy, but appeared to be a clean preparation of inner and outer nuclear membranes. However, biochemical analysis revealed that this membrane fraction contained much DNA, and further purification was achieved by sucrose-density-gradient centrifugation. Two major peaks sedimenting at the 1.18/1.20g/cm³ and 1.20/1.22g/cm³ sucrose interfaces resulted. The lighter peak, which proved to be the peak to which androgen bound, had a high phospholipid content, low content of RNA and DNA, and little plasma-membrane contamination. An electron micrograph of this band indicated the presence of membranes, but no longer showed clear evidence of both inner and outer membranes, nor were any nuclear-pore complexes apparent.

Preparation of purified membranes after incubation of purified rat ventral-prostate nuclei with radioactive androgens resulted in labelling of the lighter band. As this fraction had been subjected to an overnight centrifugation, the dissociation rate of the steroid from the binding sites on this membrane fraction at 0–4°C appears to be slow, although a direct measurement has not as yet been done.

The binding site is saturable and is of high affinity (K_D 8.4nM). Androgens that are biologically active in the rat ventral prostate compete for the dihydrotestosterone-binding sites. Diethylstilboestrol also competed for these sites, though less effectively than the androgens. Dexamethasone was not a good competitor. Thus the binding sites on this nuclear-membrane fraction display specificity for androgens.

Hildebrand & Okinaka (1976) found that heparin-mediated disruption of nuclei prepared by homogenization of hypo-osmotically swollen Chinese-hamster cells could be separated into three lipid-containing bands on sucrose density gradients, at buoyant densities of 1.10, 1.18 and 1.22g/cm³. On the other hand, when they used nuclei purified by detergent treatment, the second lipid-containing band disappeared, indicating that this band might represent the outer membrane. The use of Triton-treated nuclei from the rat ventral prostate resulted in 24.4% loss in specific binding of dihydrotestosterone to the purified nuclear membrane I. Therefore either this fraction, at the 1.18/1.20g/cm³ sucrose interface, unlike the Chinese-hamster fraction at the 1.18/1.20g/cm³ sucrose interface, may be composed of both inner and outer nuclear membranes, or the detergent may have succeeded in removing some binding sites from the inner nuclear membrane.

Barrack *et al.* (1977) reported saturable high-affinity salt-resistant binding sites for [3 H]oestradiol

associated with the uterine nuclear matrix, a residual structure prepared by extracting detergent-treated nuclei with 2.0M-NaCl and treating with ribonuclease and deoxyribonuclease. These sites may be similar to the binding sites on this nuclear-membrane fraction of the rat ventral prostate.

The assay used for androgen binding to nuclear membrane in this series of experiments involves incubation of nuclei with [³H]dihydrotestosterone for 17 h at 22°C. Although not confirmed, it is probable that labelled steroid has exchanged with all of the endogenous steroid under these conditions. The endogenous steroid may be bound to receptor, which is in turn bound to a membrane component. Nuclear membranes, prepared from animals that had been castrated 24 h previously, no longer retained the capacity to bind androgens. It has been shown that, 24 h after castration, the concentration of androgen receptor in the prostatic cytoplasm is increased (Grover & Odell, 1975; Van Doorn *et al.*, 1976). Bruchovsky and co-workers suggested that this receptor originates in the nucleus (Van Doorn *et al.*, 1976; Rennie *et al.*, 1977). The nuclear-membrane-bound receptor-steroid complex may represent a pool from which receptor is quickly lost to the cytoplasm during the recycling process. It is also possible that endogenous steroid is necessary for the binding site to be accessible to exogenous steroid. The effect of castration on binding requires further investigation.

In summary, we have reported herein a saturable high-affinity binding site on a purified nuclear-membrane fraction from the rat ventral prostate, which displays specificity for androgens and which is not present in nuclei prepared from 24 h-castrated animals. This binding site may be involved in translocation of steroids either into or out of the nucleus.

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