Nuclear acceptor sites for androgen–receptor complexes in seminal-vesicle epithelium

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(Received 30 January 1980/Accepted 18 June 1980)

An assay method *in vitro* was developed and applied to quantify acceptor binding of steroid–receptor complexes in nuclei from isolated epithelium of guinea-pig seminal vesicle. Steroid–receptor complex prepared from 1-day-castrated animals was incubated with purified nuclei from 1–28-day-castrated animals in a medium containing 0.15 M-KCl. Free and bound steroid–receptor complexes were measured and the data were submitted to Scatchard analysis. With nuclei from 1-day-castrated animals the K_d for binding of cytosolic [^3H]dihydrotestosterone–receptor complexes was found to be 0.83×10^{-10} M and the capacity for binding was 0.35 pmol/mg of nuclear DNA. Scatchard analysis consistently disclosed only a single line of constant slope and gave the same kinetic constants for nuclei obtained from animals castrated up to 28 days before assay. Administration of 2 mg of dihydrotestosterone, 3α -androstenediol or androsterone or 100 μg of oestradiol- 17β 1 h before killing of the 1-day-castrated animals that provided the nuclei resulted in a significant decrease in nuclear acceptor binding of the steroid–receptor complex compared with untreated animals. Thus our assay method disclosed nuclear acceptor sites that may be involved in responses to androgens (and oestrogens) *in vivo*. We conclude that there is a class of nuclear acceptor sites of high affinity and limited capacity that may be occupied by steroid–receptor complexes *in vivo*.

The general concept has evolved that classes of ‘acceptor sites’ exist in nuclei of steroid-hormone-responsive cells and that these ‘acceptor sites’ are presumed to be responsible for determining the specificity of interaction between steroid hormone–receptor complexes and DNA. The precise structure and function of these acceptor sites remain undocumented, but some studies performed in a variety of systems suggest that some subfraction of non-histone nuclear proteins is responsible for the ‘acceptor’ activity (Buller & O’Malley, 1976). Substantial progress in characterizing nuclear acceptors has been made by Spelsberg and his co-workers (Spelsberg *et al.*, 1977) using the chick oviduct progesterone receptor system as a model. Others have studied, in less detail, nuclear acceptors for rat uterine oestrogen receptor (Higgins *et al.*, 1973; Puca *et al.*, 1975) and for prostatic androgen receptors (Mainwaring *et al.*, 1976; Klyzsejko-Stefanowicz *et al.*, 1976). In the study of the foregoing model systems nuclei were purified from tissues that were composed of heterogeneous cell types. We have used, in contrast, an isolated epithelial preparation from the guinea-pig seminal

vesicle that is apparently cellularly homogeneous (Veneziale *et al.*, 1974). Thus our nuclear preparations derive from a single cell type. The advantages in using such a tissue as a model system for the study of steroid-hormone action have been outlined (Veneziale, 1977; Veneziale *et al.*, 1977b).

In the present paper we report the development of an assay method *in vitro* for nuclear acceptor sites that appear to interact with androgen–receptor complexes *in vivo*. Regulation by androgen of nuclear acceptor capacity per mg of DNA in the seminal-vesicle epithelium was also studied.

Materials and methods

Materials

[1,2,4,5,6,7- ^3H]Dihydrotestosterone (100–130 Ci/mmol) and [1,2,6,7- ^3H]testosterone (80–100 Ci/mmol) were obtained from Amersham Corp., Arlington Heights, IL, U.S.A. Aquasol was obtained from New England Nuclear Corp., Boston, MA, U.S.A. Non-radioactive steroids were obtained from Steraloids, Wilton, NH, U.S.A. Bio-Gel P-10 (50–100 mesh) was purchased from Bio-Rad Labor-

atories, Richmond, CA, U.S.A. Bovine serum albumin and calf thymus DNA were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. All reagents were of analytical grade.

Animals

Sexually active male guinea pigs (body wt. 600–800 g) of the Mayo inbred strain were utilized in this study. The animals were separated from their harems and within several days scrotal castrations were performed under diethyl ether anaesthesia.

Preparation of cytosolic fractions

Cytosolic fractions used in all experiments were prepared from the tissues of 1-day-castrated animals. Methods for the isolation of tissues from castrated guinea pigs have been previously described (Burns *et al.*, 1979). Tissues were homogenized by using a Potter–Elvehjem homogenizer at 0°C (12 strokes) in 2 vol. (w/v) of TEDG buffer, pH 7.4 [10 mM-Tris/HCl/1 mM-EDTA (disodium salt)/1 mM-dithiothreitol/10% (v/v) glycerol]. The homogenate was centrifuged at 105 000 g for 1 h at 2°C in an SW 50.1 rotor in a Beckman L5-50 ultracentrifuge. The clear supernatant fraction was transferred to a clean tube and was retained in ice for subsequent use in nuclear acceptor studies (see below) or was fractionated by precipitation with 40%-satd. $(\text{NH}_4)_2\text{SO}_4$ as described elsewhere (Burns *et al.*, 1979). The pellet was resuspended in TEDG buffer with or without KCl in the original volume of cytosol. For experiments requiring cytosolic receptors labelled with [^3H]dihydrotestosterone, the initial homogenization of tissues was performed in TEDG buffer containing a saturating concentration of [^3H]dihydrotestosterone (20 nM). [^3H]Testosterone–receptor complex could not be prepared in this manner because of substantial metabolism to [^3H]dihydrotestosterone. This was overcome by addition of a saturating concentration of [^3H]testosterone (30 nM) to the 105 000 g supernatant fraction. After incubation for 1 h at 0°C the [^3H]steroid–receptor complex was precipitated by addition of $(\text{NH}_4)_2\text{SO}_4$ as described above. Dichloromethane/ethanol (7:3, v/v) extracts of bound radioactivity (see below) in the cytosolic and nuclear fractions were analysed by t.l.c. on Baker Flex 1B silica-gel sheets (Steer & Veneziale, 1977). The solvent system used was chloroform/ethyl acetate (4:1, v/v). In each case the [^3H]steroid initially added to the cytosol remained unmetabolized.

Purified nuclear fractions

Nuclei from tissues of animals castrated for varying periods of time were purified by centrifugation through 2.2 M-sucrose (Buchi & Veneziale, 1977). The purified nuclear fraction was resuspended in 10 vol. (v/v) of TSKM buffer, pH 7.4

(0.25 M-sucrose/25 mM-KCl/10 mM-MgCl₂/50 mM-Tris/HCl), and collected by centrifugation at 1500 g for 5 min at 4°C in the TH-4 rotor in a Beckman TJ-6 centrifuge. The washed nuclear material was resuspended in fresh TSKM buffer at a concentration of 5×10^7 – 6×10^7 nuclei/ml.

Nuclear acceptor assay

Portions (50 μl) of the purified nuclear suspension containing 25–50 μg of DNA and portions (12.5–500 μl) of the cytosolic fractions were co-incubated in 3.0 ml conical glass centrifuge tubes and were adjusted to a final volume of 1.0 ml by the addition of TEDG buffer with or without 0.15 M-KCl. Incubation conditions were varied as described in the Results section to optimize the assay. Non-specific binding of free radioactive hormone to nuclei was determined by incubating nuclei with [^3H]dihydrotestosterone at the same concentration as in the cytosolic fraction. At the end of each incubation, the nuclei were pelleted by centrifugation at 1500 g for 5 min at 4°C in a TH-4 rotor in the Beckman TJ-6 centrifuge. The supernatant fraction was recovered and saved for analysis of unbound hormone–receptor complex (see below). The nuclear pellet was washed once with 2 ml of TEDG buffer and once with TE buffer [10 mM-Tris/HCl/1 mM-EDTA (disodium salt), pH 7.4] with or without 0.15 M-KCl. Bound radioactivity in the final washed nuclear pellet was extracted overnight with 1 ml of ethanol. The radioactivity of the ethanol extract was counted in 10.0 ml of Aquasol at 30% efficiency in a Nuclear–Chicago mark II liquid-scintillation spectrometer.

Unbound steroid–receptor complexes in supernatant fractions of each assay mixture were separated from free steroid by gel filtration essentially as described by Eisenfeld *et al.* (1976) on columns (1 cm $\times$ 10 cm) of Bio-Gel P-10 (equilibrated with TE buffer containing 0.15 M-KCl) as follows: 100 μl of 1% (w/v) Blue Dextran 2000 in TE buffer containing 0.15 M-KCl was added to each sample (300 μl) and the resultant mixture was chromatographed. Columns were eluted with TE buffer containing 0.15 M-KCl. The Blue Dextran-containing fractions, which also contained the steroid–receptor complex, were collected in glass scintillation vials and their radioactivities counted in 10 ml of Aquasol.

Sucrose-density-gradient analysis

In some experiments the supernatant fractions containing unbound hormone–receptor complexes were subjected to sucrose-density-gradient analysis with linear 5–20% (w/v) sucrose gradients prepared in TEDG buffer (Burns *et al.*, 1979). Nuclear-bound hormone–receptor complexes were also analysed on sucrose density gradients as follows:

after the nuclear pellets were washed with TE buffer (as above), radioactivity was extracted with TEDG buffer containing 0.4M-KCl for 1 h at 0°C. A portion of this salt extract was applied to linear 5–20% (w/v) sucrose gradients prepared in TEDG buffer containing 0.4M-KCl (Burns *et al.*, 1979).

Injections *in vivo*

In certain experiments animals were injected intraperitoneally with various steroids dissolved in propylene glycol (0.4 ml). Injections were given 1 h before removal of tissues.

Chemical analysis

Protein was quantified by the Lowry method as modified by Ross & Schatz (1973) for samples containing thiol reagents. Crystalline bovine serum albumin was used as standard. The 105 000 g cytosol contained about 20 mg of protein/ml, and the resuspended $(\text{NH}_4)_2\text{SO}_4$ precipitate contained about 4 mg of protein/ml. DNA was determined by the Dische (1930) diphenylamine assay with calf thymus DNA as standard. Purified nuclear preparations (1-day-, 2-day- and 8-day-castrated animals) from 1 g of tissue contained about 2–3 mg of DNA, which represented recovery of 40–50% of the DNA present in the tissue homogenate. Purified nuclear preparations from 28-day-castrated animals contained about 1 mg of DNA/g of tissue, representing a recovery of only 20–25% of the DNA present in the tissue homogenate.

Results

Binding of cytosolic [^3H]dihydrotestosterone–receptor complexes to nuclei from the tissues of

1-day-castrated animals as a function of time at two different temperatures is shown in Fig. 1. At 0°C the binding was characterized by a gradual increase throughout the 2 h of incubation. At 25°C, the binding increased rapidly and then decreased gradually to that observed after 2 h of incubation at 0°C. At 25°C cytosol [^3H]dihydrotestosterone–receptor complexes undergo rapid dissociation. After 1 h at 25°C only 40% of the initial cytosol binding remained (results not shown). In contrast, dissociation at 0°C is negligible during this time. The instability of [^3H]dihydrotestosterone–receptor complexes at 25°C may explain the rapid fluctuations in nuclear binding observed in Fig. 1.

Sucrose-density-gradient analysis of hormone–receptor complexes not bound after incubation with nuclei at 0°C for 2 h demonstrated a peak of radioactivity with a sedimentation coefficient of 7S in the absence of salt. This is similar to a previously reported value for cytosol [^3H]dihydrotestosterone–receptor complexes in seminal-vesicle epithelium (Burns *et al.*, 1979), and suggests that the cytosol receptor was maintained in its native form under the conditions of incubation (0°C) used in this assay.

KCl (0.4M) extracted only 60% of the radioactivity that could be extracted by ethanol from nuclei previously incubated with cytosol [^3H]dihydrotestosterone–receptor. Sucrose-density-gradient analysis of the 0.4M-KCl nuclear extract (in gradients containing 0.4M-KCl) demonstrated a peak of radioactivity sedimenting at 3–4S. Similar results have been observed in previous studies in which [^3H]dihydrotestosterone was incubated with intact seminal-vesicle epithelium preparations *in*

Fig. 1. Nuclear binding of cytosol [^3H]dihydrotestosterone–receptor

Portions (200 μl) of cytosol in TEDG buffer and purified nuclear fractions (50 μl) from the tissues of 1-day-castrated guinea pigs were incubated in duplicate for various times at 0 and 25°C in TEDG buffer without added KCl. Nuclear binding was determined as described in the Materials and methods section. O, Incubations at 0°C; ●, incubations at 25°C.

Fig. 2. Nuclear binding of partially purified [^3H]dihydrotestosterone–receptor complex

Portions (250 μl) of crude or partially purified cytosol in TEDG buffer and nuclear fractions (50 μl) from the tissues of 1-day-castrated guinea pigs were incubated in duplicate for various times at 0°C in TEDG buffer without added KCl. Nuclear binding was determined as described in the Materials and methods section. ●, Cytosol [^3H]dihydrotestosterone–receptor complexes; O, partially purified [^3H]dihydrotestosterone–receptor complexes.

vitro (Burns *et al.*, 1979). Thus the sedimentation properties of [3 H]dihydrotestosterone–receptor complexes bound to purified nuclei in both cell-free and whole-tissue incubations were similar.

The results shown in Fig. 1 suggested that there may have been a temperature-dependent activation of hormone–receptor complexes resulting in a more rapid rate of nuclear binding at 25°C than at 0°C. Since incubation at 25°C appeared to be deleterious to the hormone–receptor complex and possibly to nuclei as well (Pikler *et al.*, 1976), other ways of effecting receptor activation were considered. It had been shown that $(\text{NH}_4)_2\text{SO}_4$ precipitation was an effective means of promoting activation of a variety of steroid-hormone–receptor complexes (Buller & O'Malley, 1976). Most (60%) of the initial cytosol [3 H]dihydrotestosterone–receptor complexes are recovered after $(\text{NH}_4)_2\text{SO}_4$ precipitation, but only 20% of the initial cytosol protein was recovered. Thus, in addition to activation, partial purification of receptor was achieved. In Fig. 2 the binding of cytosol and partially purified [3 H]dihydrotestosterone–receptor complexes to nuclei with time is

Fig. 3. *Receptor requirement for nuclear binding* Partially purified cytosol in TEDG buffer containing 0.15 M-KCl was heat-treated at 50°C for 10 min. Insoluble material was removed by low-speed centrifugation. Guinea-pig serum was diluted (1:9, v/v) with TEDG buffer containing 0.15 M-KCl, and [3 H]dihydrotestosterone was added to give the same final concentration as in the partially purified cytosol fractions. Various amounts of the labelled soluble fractions were incubated in duplicate at 0°C with nuclei (50 μ l) purified from the tissues of 1-day-castrated guinea pigs in TEDG buffer containing 0.15 M-KCl. Nuclear binding was determined as described in the Materials and methods section. ●, Partially purified [3 H]dihydrotestosterone–receptor complexes; ○, heat-treated [3 H]dihydrotestosterone–receptor complexes; ■, guinea-pig serum.

presented. The rate of binding to nuclei at 0°C was greater when partially purified receptor was used. After 1 h of incubation, binding was nearly maximal.

The essential nature of the receptor for promoting hormone binding to nuclei is demonstrated in Fig. 3. Substantial binding of radioactivity to nuclei was observed only after incubation with an active receptor preparation. Partially purified receptor that had been heat-treated (10 min at 50°C) and guinea-pig plasma, each containing the same amount of [3 H]dihydrotestosterone as the active cytosolic fraction, were unable to promote very much binding of radioactivity to nuclei.

The effect of increasing the concentrations of partially purified [3 H]dihydrotestosterone–receptor complex on binding to nuclei in the absence and presence of 0.15 M-KCl is shown in Fig. 4. In the absence of KCl, binding to nuclei was strictly proportional to the concentration of partially purified receptor. When 0.15 M-KCl was included in the incubation mixture, saturation of nuclear acceptor sites became apparent. We found that the binding of [3 H]dihydrotestosterone to the receptor and the sedimentation behaviour of the complex were unaffected by 0.15 M-KCl. Therefore the appearance of saturable nuclear binding was probably not due to quantitative or qualitative changes in the hormone–receptor complex induced by KCl.

Fig. 4. *Effect of KCl on nuclear binding of partially purified [3 H]dihydrotestosterone–receptor complex* Partially purified cytosol in TEDG buffer with or without 0.15 M-KCl was incubated in duplicate for 1 h at 0°C with nuclei (50 μ l) from tissues of 1-day-castrated guinea pigs in TEDG buffer with or without 0.15 M-KCl. Nuclear binding was determined as described in the Materials and methods section. ●, No KCl; ○, + 0.15 M-KCl.

A representative saturation curve and its corresponding Scatchard (1949) plot are shown in Fig. 5. The results, as did those from numerous experiments, indicated the presence of one class of binding sites with a K_d of $0.4 \times 10^{-10} \text{ M}$ and a nuclear acceptor capacity (n_s) of 0.29 pmol/mg of DNA.

Fig. 5. Saturation curve (a) and Scatchard analysis (b) of binding of partially purified [³H]dihydrotestosterone-receptor complex to nuclei

Partially purified cytosol in TEDG buffer containing 0.15 M-KCl was incubated in triplicate with nuclei (50 μl) prepared from the tissues of 1-day-castrated guinea pigs for 1 h at 0°C in TEDG buffer containing 0.15 M-KCl. Non-specific binding was determined as described in the Materials and methods section. Unbound [³H]dihydrotestosterone-receptor complexes were isolated by gel filtration on Bio-Gel P-10, and bound hormone was determined as described in the Materials and methods section. ●, Total binding; ○, specific binding (difference between total and non-specific binding); ■, non-specific binding. (b) The K_d determined from the slope of the line obtained by linear regression analysis was $0.4 \times 10^{-10} \text{ M}$ and the concentration of binding sites from the x-intercept was 7.8 pM. The correlation coefficient was -0.882.

When the data were plotted in the form of a Lineweaver-Burk plot (not shown), the kinetic parameters were found to be: K_d , $0.38 \times 10^{-10} \text{ M}$; n_s , 0.27 pmol/mg of DNA. Thus the two graphical methods gave excellent agreement. The K_d observed for binding of the [³H]dihydrotestosterone-receptor complex to nuclear acceptor sites is about 10-fold lower than that observed for [³H]dihydrotestosterone binding to the receptor (Burns *et al.*, 1979).

To assess the physiological significance of nuclear binding as observed by our assay, we determined the acceptor capacity for [³H]dihydrotestosterone-receptor complexes in nuclei after administration *in vivo* of different hormones. As shown in Table 1,

Table 1. Effect of steroid administration on nuclear acceptor capacity

Nuclear acceptor assays were carried out as described in the Materials and methods section in the presence of 0.15 M-KCl for 1 h at 0°C. Nuclear and partially purified receptor fractions were prepared from the tissues of animals castrated 24 h before they were killed. Animals used for the preparation of nuclei were injected with the indicated steroids as described in the Materials and methods section. Controls received the vehicle alone. Binding data were analysed by the method of Scatchard (1949), and results are expressed as the means ± s.d. for three independent experiments. Data were analysed by using Student's *t* test. Significance: **P* < 0.001 compared with the control.

Steroid injected	Dose	Nuclear acceptor capacity (pmol/mg of DNA)
None	—	0.35 ± 0.03
Dihydrotestosterone	2 mg	0.11 ± 0.03*
3 α-Androstanediol	2 mg	0.10 ± 0.03*
Androsterone	2 mg	0.07 ± 0.03*
Oestradiol	100 μg	0.12 ± 0.06*

Table 2. Influence of castration on nuclear acceptor capacity

Purified nuclei were prepared from the tissues of animals castrated for the times indicated. Partially purified receptor was prepared from the tissues of animals castrated 24 h previously. Assays were carried out as described in the Materials and methods section in the presence of 0.15 M-KCl for 1 h at 0°C. See the legend to Table 1 for the remaining details of data analysis.

Time after castration (days)	Nuclear acceptor capacity (pmol/mg of DNA)	K_d (pM)	Range of K_d (pM)
1	0.35 ± 0.03	83	50–130
2	0.30 ± 0.12	70	40–110
8	0.34 ± 0.16	51	20–70
28	0.51 ± 0.05	43	10–100

administration *in vivo* of dihydrotestosterone, 3 α -androstenediol, androsterone and oestradiol-17 β resulted in a substantial decrease in nuclear acceptor capacity. The binding affinity in each case was not significantly different from the uninjected controls (results not shown).

By using the nuclear acceptor assay we have examined the acceptor capacity of nuclei isolated from the tissues of animals castrated for various lengths of time (Table 2). When expressed per mg of nuclear DNA, there was no significant change in nuclear acceptor capacity with increasing time after castration.

In a preliminary study nuclear acceptor capacity for [3 H]testosterone-receptor complexes was evaluated. Although the K_d is the same as that for [3 H]dihydrotestosterone-receptor complexes, the number of sites was only 0.18 pmol/mg of DNA. Attempts to study the possibility that [3 H]testosterone-receptor complexes and [3 H]dihydrotestosterone-receptor complexes are binding to unique acceptor sites have been unsuccessful. We have been unable to show competition between radioactive and non-radioactive hormone-receptor complexes when incubated with nuclei in a sequential fashion.

Discussion

In the present study, assay conditions have been defined that are appropriate for the measurement of nuclear acceptor sites for androgen-receptor complexes. We have chosen the isolated seminal-vesicle epithelium as a model system for these studies, since it is cellularly homogeneous by electron microscopy (Veneziale *et al.*, 1974) and is dependent on androgens for maintenance of its structure and function (Veneziale, 1977; Veneziale *et al.*, 1977a,b). Nuclei purified from a homogeneous population of cells may exhibit a simplified profile of nuclear proteins when compared with a heterogeneous nuclear preparation, and thus permit a more precise identification of acceptors for androgen-receptor complexes.

Assays were conducted at 0°C with partially purified receptor preparations under conditions of moderate ionic strength (0.15 M-KCl). Low temperatures and partially purified receptors were utilized to minimize dissociation and degradation of steroid-receptor complexes and to limit changes in chromatin structure. These are important considerations, since proteolysis of receptors may result in decreased ability to bind DNA or chromatin without concomitant loss of steroid binding (Sala-Trepat & Vallet-Strouve, 1974). On the other hand, degradation of chromatin may expose additional acceptor sites not routinely expressed *in vivo* (Spelsberg *et al.*, 1977), thus resulting in overestimation of nuclear acceptor site capacity. Use of partially purified

receptors further decreased the possibility of chromatin damage (Pikler *et al.*, 1976), and, by activating the receptor, provided rapid binding kinetics at 0°C. Non-specific protein binding that might mask acceptor sites was also limited by using a partially purified receptor preparation in nuclear acceptor assays. In the presence of 0.15 M-KCl, binding of progesterone-receptor complexes was limited to the highest affinity class of acceptors in chick oviduct chromatin (Pikler *et al.*, 1976). We have also observed that binding of [3 H]dihydrotestosterone-receptor complexes to nuclei in the presence of 0.15 M-KCl was saturable and limited to a single class of acceptor sites with high affinity and limited capacity. Binding was dependent on active receptor, since neither guinea-pig plasma nor inactive preparations of [3 H]dihydrotestosterone-receptor complexes promoted binding of [3 H]dihydrotestosterone to nuclei. By using values of 0.35 pmol of sites/mg of nuclear DNA in 1-day-castrated animals (Table 2) and 6 pg of DNA/nucleus there were about 1300 binding sites/nucleus. In our system the K_d for binding [3 H]dihydrotestosterone-receptor complexes (0.8×10^{-10} M) is 10-fold lower than that reported for rat prostate nuclei, and the number of binding sites is less than the 2000–6000 sites/nucleus reported by the same investigator (Liao, 1977). The reasons for these discrepancies are not obvious, but they might be somehow related to our use of a nuclear preparation purified from a homogeneous cell population.

The relevance of cell-free binding of steroid-receptor complexes to nuclei has been questioned in several studies that were unsuccessful in attempts to show decreased binding of steroid-receptor complexes *in vitro* after the administration *in vivo* of physiologically maximal doses of hormone (Chamness *et al.*, 1974). There are several reasons for such a result that have been outlined, which include dissociation, reassociation, or both, of the complex during nuclear isolation and the possibility that the initial binding to acceptor sites *in vivo* is quickly followed by a shifting to other nuclear sites (nuclear 'processing') (Spelsberg *et al.*, 1977). In our studies, administration *in vivo* of various hormones did decrease steroid-receptor complex binding to nuclei. Three different androgens, dihydrotestosterone, 3 α -androstenediol and androsterone, were equally effective in this respect. From previous studies (Burns *et al.*, 1979) we know that dihydrotestosterone and 3 α -androstenediol both positively influence the seminal-vesicle epithelium *in vivo* and that the different patterns of response may suggest that each is active in its own right. Androsterone was found to be only marginally effective when used at a dose 3-fold higher than that used in the present experiments. The ability of androsterone to decrease nuclear acceptor binding to

the same extent as dihydrotestosterone and 3α -androstanediol is therefore difficult to explain. Perhaps it behaves in our system as oestriol behaves in the rat uterus (Anderson *et al.*, 1975), i.e. it (androsterone) is capable of inducing initial receptor translocation as effectively as more potent androgens but fails to promote long-term retention of receptor in the nucleus. Long-term nuclear retention of oestrogen-receptor complexes is thought to be necessary for eliciting uterine growth (Anderson *et al.*, 1975). The oestradiol- 17β -induced decrease in nuclear acceptor binding may be related to the potent inhibitory effect on seminal-vesicle epithelium observed after administration *in vivo* (Burns *et al.*, 1979) to intact animals. We had demonstrated, in the same study, that oestradiol- 17β decreased the binding of dihydrotestosterone to the androgen receptor, and it may be suggested by the present results that oestradiol- 17β translocates the androgen receptor into the nucleus. The translocation of the rat uterine oestrogen receptor into the nucleus after incubation *in vitro* with high concentrations of dihydrotestosterone ($1\mu\text{M}$) (Ruh *et al.*, 1975) and after administration *in vivo* of high doses (3–15 mg) of dihydrotestosterone (Rocheffort & Garcia, 1976) have already been described. It is interesting to speculate that in pharmacological doses oestrogen may act as an anti-androgen at the target-tissue level by the formation of a less than fully active androgen receptor-hormone complex.

Regulation of target-cell sensitivity to steroid hormones may be at two levels. On one level, changes in cytoplasmic receptor content may make a tissue more or less responsive to the hormone. On another level, changes in nuclear acceptors may alter responses both quantitatively and qualitatively. Cytoplasmic receptor concentration (binding/mg of protein) in rat prostate gland is regulated by androgens shortly after castration, but becomes independent of androgens after prolonged periods after castration (Sullivan & Strott, 1973). In the seminal-vesicle epithelium, which undergoes substantial changes in morphology and function after castration (Veneziale, 1977; Veneziale *et al.*, 1977a; Barham *et al.*, 1979), we evaluated the nuclear acceptor capacity from 1 to 28 days after castration to check whether similar patterns of regulation occurred. We observed that the nuclear acceptor capacity (per mg of DNA) and K_d remained unchanged. This is not entirely unexpected, and is really consistent with the fact that the tissue maintains its ability to respond to androgens despite long-term castration (Burns *et al.*, 1979). However, qualitative changes in nuclear acceptors that may have occurred would not have been detected in this assay.

Further studies in this area must await a detailed characterization of nuclear acceptor components, which can be most easily accomplished in a cellularly homogeneous target tissue like the guinea-pig seminal-vesicle epithelium.

This work was supported by N.I.H. grant HD12657 and by the Mayo Foundation.

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