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Correlation of androgen-responsiveness of Shionogi mouse mammary carcinoma cell lines with binding of dihydrotestosterone to nuclear envelopes

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Purified nuclear envelopes have been isolated from an androgen-responsive and two androgen-unresponsive cell lines of the Shionogi mouse mammary carcinoma. The binding of dihydrotestosterone to nuclear envelope fractions isolated from the three variant cell lines is correlated with the androgen-responsiveness of the cell line. Nuclear envelopes prepared from the two androgen-unresponsive cell lines did not bind dihydrotestosterone specifically following incubation with radioactive dihydrotestosterone from 2.5 to 45.0 nM at 20°C for 18 h. Under the same binding conditions, nuclear envelopes prepared from the androgen-responsive cell line demonstrated saturable, specific binding of dihydrotestosterone. Scatchard analysis revealed a class of binding sites with an apparent K_d of 14.2 nM and a maximum binding capacity of 28.7 fmol/mg protein. Proteinase and heat treatments resulted in the complete loss of androgen-binding activity, whereas DNAase treatment resulted in the loss of 38% of the binding activity. The binding sites were specific for dihydrotestosterone. Testosterone was only a weak competitor and estradiol did not compete. Extraction with concentrations of KCl up to 1.0 M did not result in loss of androgen binding.

Introduction

The mechanism of steroid action involves the binding of the steroid hormone to a specific cytoplasmic receptor. Following activation, the steroid-receptor complex is translocated to the nucleus where its interaction with the genome results in altered cellular function [1–3]. 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 and/or steroid-receptor complex must interact with the nuclear envelope during the uptake process into and exit out of the nucleus.

In breast cancer, steroid receptor measurements have been used to predict whether a tissue will respond to steroid manipulations [4]. However, certain estrogen receptor-containing tumors fail to respond as predicted [5]. One explanation for this failure to respond to steroid even when receptors are present is that a step distal to the interaction of the steroid with the receptor may be defective. A defect at the level of the nuclear envelope could account for the lack of response in certain steroid-unresponsive tumors.

To investigate whether translocation of steroid into or out of the nucleus may be defective in certain steroid-unresponsive tumors, we are using androgen-dependent and androgen-independent cell lines of the Shionogi mouse mammary carcinoma. Differences in the binding of dihydrotestosterone to nuclei isolated from an

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Abbreviation: DHT, dihydrotestosterone.

androgen-responsive cell line (TD-2) and two androgen-unresponsive cell lines (S-42 and TAOC-1) of the Shionogi mouse mammary carcinoma have been demonstrated [6]. Nuclei purified from the TD-2 cell line have 4.5-times the number of dihydrotestosterone-binding sites as nuclei from the S-42 cell line and 6.3-times the number of sites as nuclei from the TAOC-1 cell line. However, the nucleus is composed of many potential binding sites including chromatin [7–9], basic nuclear proteins [10,11], ribonucleoprotein particles [12] and the nuclear envelope [13–15]. Determination of the contribution of the nuclear envelope to the nuclear binding requires studies of steroid binding to isolated nuclear envelopes.

In this paper, we characterize binding of dihydrotestosterone to purified nuclear envelopes from an androgen-responsive and two androgen-unresponsive cell lines of the Shionogi mouse mammary carcinoma and report that androgen binding to the nuclear envelopes is correlated with the androgen-responsiveness of the tissue.

Materials and Methods

Chemicals. 5α-[1,2,4,5,6,7,16,17-^{3}H]Dihydrotestosterone (208 Ci/mmol) was purchased from New England Nuclear, Boston, MA; unlabelled steroids from Steraloids Inc., Wilton, NH; 1,4-bis-(5-phenyloxazol-2-yl)benzene from Nuclear Chicago, Des Plaines, IL; 2,5-diphenyloxazole from Fisher Scientific Company, Fair Lawn, NJ; DNAase I Type I and proteinase Type VI from Sigma, St. Louis, MO.

Experimental tumors. Dr. Nicholas Bruchovsky, British Columbia Cancer Agency, Vancouver, British Columbia, Canada, supplied our laboratory with sufficient animals to establish a colony of DD/S strain mice carrying the androgen-responsive Shionogi mouse mammary carcinoma, TD-2, and the two autonomous cell lines, S-42 and TAOC-1. Passage of the cell lines was accomplished by injection of single cell suspensions.

The androgen-responsive cell line grows only in male mice of the DD/S strain. Castration results in tumor regression which is reversible on testosterone injection. The autonomous cell lines grow equally well in female, male and castrated male mice.

The mice were killed by cervical dislocation and the tumors quickly removed, rinsed in 0.32 M sucrose containing 3 mM $MgCl_2$ (homogenization medium), stripped of connective tissue, placed on ice and weighed.

Preparation of nuclear envelopes. Purified nuclei were obtained by modifications of the method of Widnell and Tata [16] as described by Kay et al. [17]. All operations were performed at 0–4°C. The tumors were mashed through a stainless-steel wire screen (400 mesh) rinsing with homogenization medium (40 ml/10 g starting tissue). The cells were recovered by centrifugation at $25 \times g$ for 5 min. The pellet was resuspended in 40 ml of homogenization medium and centrifuged at $25 \times g$ for 5 min. Following resuspension, the cells were disrupted using a Polytron homogenizer (Brinkmann Instruments, Inc.) set at 4.5 for 30 s and then centrifuged at $700 \times g$ for 10 min. The pellet was resuspended in 400 ml of homogenization medium and homogenized, using a Dounce apparatus, with ten strokes each of the loose-fitting pestle A and tight-fitting pestle B. The suspension was centrifuged at $750 \times g$ for 10 min. The crude nuclear pellet was resuspended in 40 ml 2.4 M sucrose containing 1 mM $MgCl_2$, which had been brought to pH 7.4 with $NaHCO_3$ solution. The nuclear suspension was centrifuged at $45\,000 \times g$ for 60 min to yield a pellet of purified nuclei. The nuclear pellet was washed by resuspension in 40 ml 0.25 M sucrose containing 1 mM $MgCl_2$, which had been brought to pH 7.4 with $NaHCO_3$ solution, and centrifuged at $750 \times g$ for 5 min. This pellet of nuclei was then used to prepare nuclear envelopes.

The nuclei were suspended in 10 mM Tris-HCl containing 0.29 M sucrose, 0.1 mM $MgCl_2$ and 5 mM 2-mercaptoethanol (pH 8.5) (40 ml/10 g starting tissue) to which DNAase I Type I was added to a final concentration of 10 μg/ml. This suspension was incubated at 20°C for 15–20 min. Following centrifugation at $12\,000 \times g$ for 5 min, the crude nuclear envelopes were washed by resuspension in 40 ml 10 mM Tris-HCl (pH 7.4), followed by centrifugation at $12\,000 \times g$ for 5 min. The pellets were resuspended in 2 ml 10 mM Tris-HCl (pH 7.4) and layered on discontinuous gradients made up of 5 ml 0.25 M sucrose and 10 ml each of 1.5 M, 1.8 M 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 to yield a band of purified nuclear envelopes at the 1.5/1.8 M sucrose interface. The band was removed and washed twice by resuspension in 40 ml 10 mM Tris-HCl (pH 7.4) and centrifugation at 12 000 × *g* for 5 min.

Electron microscopy. Nuclear envelopes were fixed for 1–2 h with 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) and post-fixed in 2% osmium tetraoxide in 0.1 M phosphate buffer (pH 7.2). Dehydration of the samples was performed in ascending concentrations of ethanol and finally propylene oxide. The samples were then embedded in Spurr. Thin sections were stained with uranyl acetate and lead citrate [18] and examined in a Philips 400 electron microscope.

Analytical methods. Protein was determined by the method of Lowry et al. [19], with bovine serum albumin as standard. DNA was measured by the method of Thomas and Farquhar [20], with calf thymus DNA as standard.

Enzyme assays. 5'-Nucleotidase was determined by the method of Goldfine et al. [21], and the inorganic phosphate was measured by the method of Ames [22]. Arylesterase was assayed according to Shephard and Hubscher [23]. Succinate dehydrogenase was determined according to Kun and Abood [24], except that *p*-iodonitrotetrazolium violet was used as substrate. Acid phosphatase was assayed in the same manner as alkaline phosphatase as described by Kinne and Kinne-Saffran [25], except that 50 mM citrate buffer (pH 5.0) was used as the incubation medium. Each assay was performed in duplicate and under conditions such that product formation was linearly related to time and protein concentration.

Incubation of nuclear envelopes with steroid. Nuclear envelopes from the three tumor cell lines (200–400 μg protein/assay tube) were incubated at 20°C for the times specified in a final volume of 0.25 ml 10 mM Tris-HCl (pH 7.4) containing radioactive dihydrotestosterone. To a duplicate set of tubes, 100-fold excess unlabelled steroid was also added to determine nonspecific binding. The incubation was stopped by addition of 1.0 ml cold 10 mM Tris-HCl (pH 7.4) and centrifugation in a Beckman Microfuge B for 1 min. The supernatant was removed. The pellets were resuspended in 1.0 ml 10 mM Tris-HCl (pH 7.4) and the suspension was centrifuged for 1 min. The supernatant was removed and the wash procedure was repeated. The tips of the microfuge tubes, containing the nuclear envelope pellets, were removed and placed in scintillation vials. 10 ml toluene containing 0.3% (w/v) 2,5-diphenyloxazole and 0.1% (w/v) 1,4-bis-(5-phenyloxazol-2-yl)benzene was added to the vials which were then incubated at 30°C for 20 min. After cooling to 20°C, the samples were assayed for radioactivity in a liquid scintillation spectrophotometer.

Results

The method of Kay et al. [17] was modified for the isolation of purified nuclear envelopes from three tumor cell lines. The second DNAase digestion suggested by Kay et al. [17] was omitted as it resulted in the disruption of nuclear envelopes formed by the first digestion without the formation of additional nuclear envelopes. Since only one DNAase digestion was used, some intact nuclei remained, as monitored by phase-contrast microscopy. Therefore, it was necessary to purify further the nuclear envelope fractions. A discontinuous sucrose gradient centrifugation succeeded in separating nuclear envelopes from the intact nuclei. Yields of nuclear envelope were 0.104 ± 0.06, 0.050 ± 0.03 and 0.065 ± 0.04 mg protein/g tissue (average of three experiments) for the TD-2, S-42 and TAOC-1 tumor cell lines, respectively. Phase-contrast microscopy, used as the routine screen, revealed that the nuclear envelopes were isolated as ghosts and membrane sheets.

The nuclear envelope fractions from the three tumor cell lines were examined by electron microscopy in order to visualize the double-membrane system of the nuclear envelope. Fig. 1 shows a representative electron micrograph of a nuclear envelope 'ghost' from the TAOC-1 cell line which demonstrates the presence of the double-membrane system. The arrows indicate possible nuclear pore complexes. Since there are no detectable differences in the appearance of nuclear envelopes from the three cell lines, only one electron micrograph is included to represent all three cell lines.

The nuclear envelope fractions contained low amounts of DNA. The DNA content was $0.059 \pm$

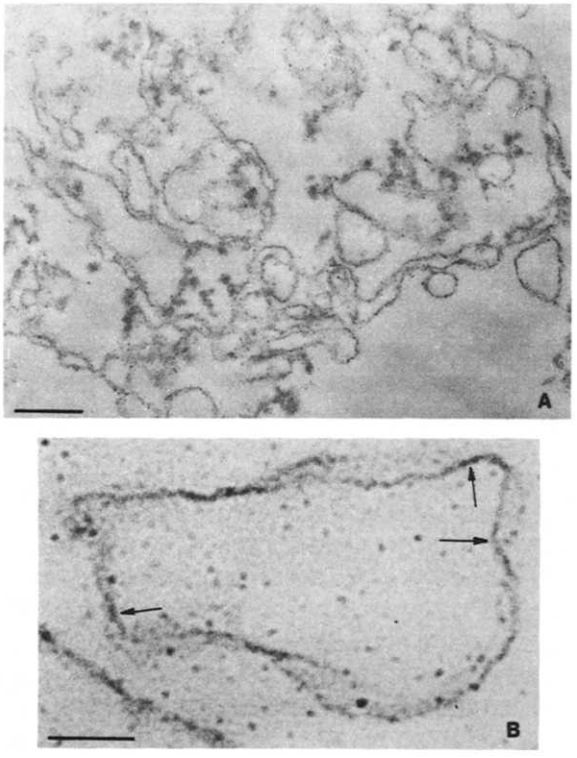

Fig. 1. Electron micrographs of purified nuclear envelopes. (A) Nuclear envelopes from the S-42 cell line prepared by the modified method of Kay et al. [17]. (scale bar = 1 μm). (B) Nuclear envelope ghost from the TAOC-1 cell line prepared by the modified method of Kay et al. [17]. The arrows indicate possible nuclear pore complexes. (scale bar = 0.5 μm).

0.041, 0.080 ± 0.014 and 0.061 ± 0.017 mg/mg protein for the TD-2, S-42 and TAOC-1 cell lines, respectively. This accounted for 0.05–0.12% of the DNA content of the homogenate fractions.

Marker enzymes were used to monitor the isolation and purity of the nuclear envelope fractions from each of the three tumor cell lines. Arylesterase, the endoplasmic reticulum marker, was detectable in the nuclear envelope fractions from the three cell lines, but accounted for only 0.08–0.13% of the enzyme activity present in the homogenate fractions. Acid phosphatase, the lysosomal marker enzyme, was also detectable in the nuclear envelope fractions of all three cell lines, but only in amounts ranging from 0.01 to 0.06% of the activity measured in the homogenate fractions. Succinate dehydrogenase, the mitochondrial marker enzyme, and 5′-nucleoti-

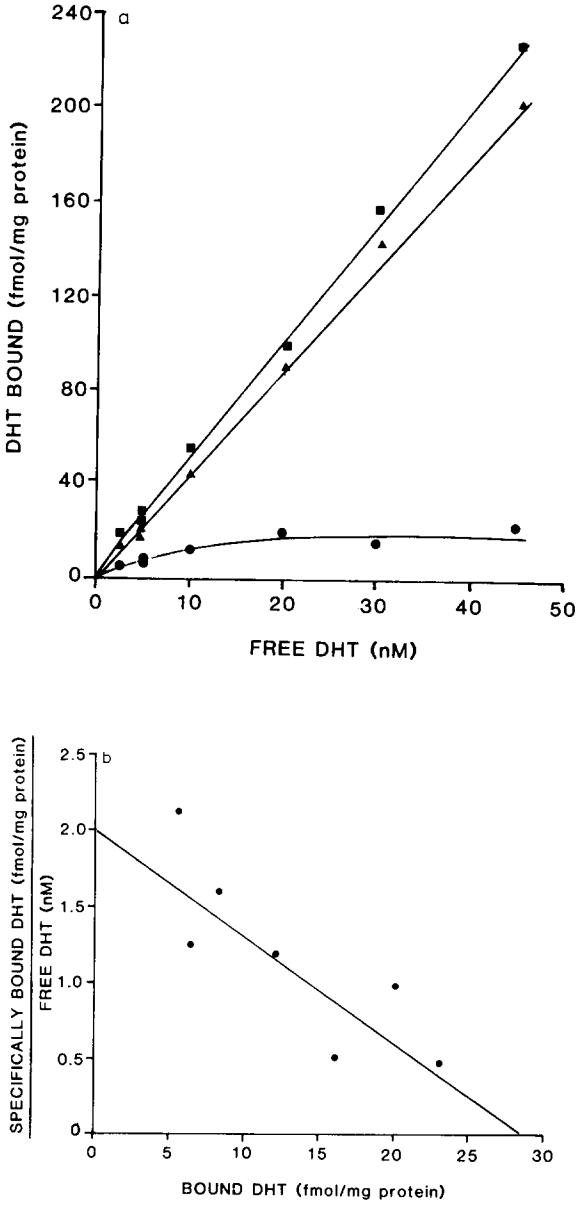

Fig. 2. Saturation analysis of [^{3}H]dihydrotestosterone binding to nuclear envelopes from the TD-2 cell line. Nuclear envelopes (200–400 μg protein per assay tube) were incubated for 18 h at 20°C with increasing concentrations of [^{3}H]dihydrotestosterone (DHT) (2.5–45.0 nM) with (▲, to measure non-specific binding) and without (■, to measure total binding) 100-fold excess unlabelled dihydrotestosterone. Specific binding (●) was calculated from the difference in retained radioactivity in the presence and absence of unlabelled dihydrotestosterone (a) and plotted according to Scatchard (b). The line of best fit was determined by linear-regression analysis and the correlation coefficient was −0.79 ($P < 0.025$). Each value is the mean of three to six determinations.

TABLE I

EFFECT OF VARIOUS PROCEDURES ON BINDING OF [^{3}H]DIHYDROTESTOSTERONE TO NUCLEAR ENVELOPES FROM THE TD-2 CELL LINE

In experiment 1, after incubation of nuclear envelopes (200 μg protein/assay tube) with 7.3 nM [^{3}H]dihydrotestosterone in the presence and absence of 100-fold excess unlabelled dihydrotestosterone, the bound and free steroid were separated by centrifugation and the pellets washed twice with 1 ml 10 mM Tris-HCl (pH 7.4). The pellets were then incubated for 30 min at 20°C in 1 ml 10 mM Tris-HCl (pH 7.4) in the presence or absence 1 mg/ml proteinase or 10 μg/ml DNAase I Type I (the DNAase was shown to contain 4% of the proteolytic activity displayed by 10 μg/ml proteinase, as determined by Kunitz [33]), or incubated for 30 min at 95°C in 1 ml 10 mM Tris-HCl (pH 7.4). The incubation was stopped as described in Materials and Methods and the radioactivity retained by the pellet was determined. In experiment 2, nuclear envelopes (200 μg protein per assay tube) were incubated for 18 h at 20°C with 7.3 nM [^{3}H]dihydrotestosterone in the presence and absence of 100-fold excess unlabelled dihydrotestosterone, testosterone or estradiol. The radioactivity retained by the nuclear envelopes was determined as described in experiment 1. In experiment 3, nuclear envelopes (200 μg protein per assay tube) were incubated with 7.3 nM [^{3}H]dihydrotestosterone in the presence and absence of 100-fold excess unlabelled dihydrotestosterone. The pellets were then extracted with KCl at the specified molarity (0.0, 0.4, 0.6, 1.0 M) for 30 min at 20°C. The radioactivity retained by the nuclear envelopes was determined as described in experiment 1. The values quoted are means ±S.E. of four determinations.

Experimental condition	Specifically bound dihydrotestosterone	
	fmol/mg protein	% decrease
1 Control	4.5±0.2	0
Proteinase	0.0±0.0	100
DNAase	2.8±0.1	38
95°C	0.0±0.0	100
2 Dihydrotestosterone	11.6±0.4	0
Testosterone	2.3±1.1	80
Estradiol	0.0±0.0	100
3 Control	10.3±0.5	0
0.4 M KCl	12.5±0.1	0
0.6 M KCl	6.9±0.3	33
1.0 M KCl	12.0±1.3	0

dase, the plasma membrane marker enzyme, were undetectable in the nuclear envelope fractions of all three cell lines.

Conditions for binding of dihydrotestosterone to nuclear envelopes from the androgen-responsive cell line, TD-2, were determined. Incubation of nuclear envelopes for time intervals from 0 to 18 h at 20°C demonstrated that the specific binding had reached equilibrium by 16–18 h. Specific binding after 2 h was 0.00 fmol DHT/mg protein; at 16 h specific binding was 10.44 fmol DHT/mg protein and at 18 h specific binding was 10.22 fmol DHT/mg protein. Therefore, nuclear envelopes isolated from the TD-2 cell line were incubated with increasing concentrations of dihydrotestosterone for 18 h at 20°C and the specific binding was analyzed according to Scatchard [26]. Fig. 2a shows the total and nonspecific binding of dihydrotestosterone to nuclear envelopes from the TD-2 cell line. Scatchard analysis of the binding of dihydrotestosterone to the nuclear envelopes revealed a class of binding sites with an apparent K_d of 14.2 nM ± 4.2 ($z_{0.025} = 1.96$) and maximum binding capacity of 27.8 fmol/mg protein (Fig. 2b). Nuclear envelopes from the TD-2 cell line displayed saturable, specific binding of androgen to the nuclear envelopes with dihydrotestosterone concentrations of 2.5–45.0 nM. It is evident that there was considerable nonspecific binding of dihydrotestosterone to the membrane preparation. Considerable efforts were made to lower the nonspecific binding but as yet conditions have not been found which succeed in reducing the levels of nonspecific binding below these. Similar levels of nonspecific binding to nuclear envelopes were obtained in the rat liver [15].

The nature of the binding sites for dihydrotestosterone present on the nuclear envelope fractions from the TD-2 cell line was examined. Nuclear envelope fractions previously incubated with radioactive dihydrotestosterone were subjected to proteinase, heat or DNAase treatment for 30 min (Table I). No specific binding was demonstrated following proteinase and heat treatments. This suggests that the nuclear envelope binding sites are protein in nature. DNAase treatment resulted in the loss of 38% of the specifically bound dihydrotestosterone. This loss of binding could not be accounted for by proteolytic activity in the DNAase (legend to Table I).

Steroid specificity of dihydrotestosterone binding to the nuclear envelope fraction was determined by competition with 100-fold excess un-

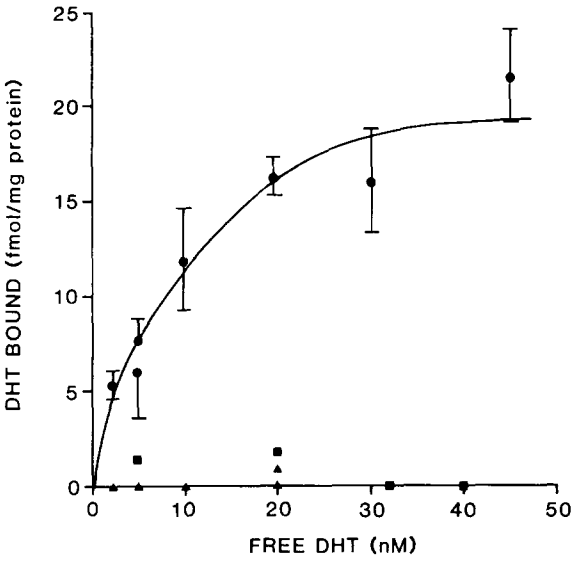

Fig. 3. Saturation analysis of specifically bound dihydrotestosterone (DHT) to nuclear envelopes from the TD-2, S-42 and TAOC-1 cell lines. Nuclear envelopes (200–400 μg protein per assay tube) from the androgen-responsive, TD-2 (●) (mean ± S.E.), and the two autonomous, S-42 (■) and TAOC-1 (▲), cell lines were incubated for 18 h at 20°C with increasing concentrations of [^{3}H]dihydrotestosterone (2.5–45.0 nM) with (to measure nonspecific binding) and without (to measure total binding) 100-fold excess unlabelled dihydrotestosterone. The specific binding was calculated from the difference in binding of ^{3}H-labelled steroid in the presence and absence of unlabelled steroid. Each value is the mean of three to six determinations.

labelled dihydrotestosterone, testosterone or estradiol (Table I). 80% of the specific DHT-binding sites were not competed for by testosterone, whereas estradiol did not displace labelled dihydrotestosterone. Therefore, the binding sites are specific for dihydrotestosterone.

The association of the binding site to the nuclear envelope fraction may be examined by salt-extraction studies. If the binding sites are adsorbed nonspecifically or associated to the membrane fraction with weak electrostatic forces, the binding sites may be removed with salt. Incubation of nuclear envelopes initially labelled with radioactive dihydrotestosterone, with concentrations of KCl up to 1.0 M, did not alter the recovery of specifically bound dihydrotestosterone (Table I).

Having established conditions for the binding of dihydrotestosterone to nuclear envelopes from the TD-2 cell line, the same conditions were used to measure dihydrotestosterone binding to nuclear envelopes from the two autonomous cell lines, S-42 and TAOC-1. Following incubation of nuclear envelopes from the androgen-unresponsive cell lines with concentrations of labelled dihydrotestosterone from 2.5 to 45.0 nM, specific binding could not be detected (Fig. 3). Only the androgen-responsive cell line displayed saturable, high-affinity, low-capacity binding of dihydrotestosterone to the nuclear envelope fractions over the same concentration range.

Discussion

We wish to elucidate the molecular mechanism of steroid translocation across the nuclear envelope and determine whether nuclear envelope components involved in the translocation process play a role in modulating the steroid-responsiveness of tissues. A step toward this is to identify and characterize steroid-binding sites on nuclear envelopes. To investigate whether steroid-unresponsiveness is caused, at least in part, by a lack of or a defect in interaction of the steroid with the nuclear envelope, we are comparing the binding of dihydrotestosterone to nuclear envelopes from an androgen-responsive cell line (TD-2) and two androgen-unresponsive cell lines (S-42 and TAOC-1) from the Shionogi mouse mammary carcinoma. Bruchovsky and Rennie [27] reported that the S-42 and TAOC-1 cell lines have reduced numbers of cytoplasmic receptors and reduced nuclear uptake of androgens, measured in in vivo studies, compared to the androgen-responsive cell line. We have confirmed in in vitro studies that nuclei from the androgen-unresponsive cell lines take up less dihydrotestosterone than nuclei from the androgen-responsive cell line [6]. We have now purified nuclear envelopes from the three tumor cell lines and investigated dihydrotestosterone binding to them.

The preparation of nuclear envelopes from the three variant cell lines of the Shionogi mouse mammary carcinoma by modification of the method of Kay et al. [17] results in a preparation of relatively intact nuclear envelope ghosts as monitored by phase-contrast microscopy. These nuclear envelopes have been characterized chemi-

cally, enzymatically and morphologically to ascertain the purity and integrity of the membrane preparations. The nuclear envelope fractions contain 6–8% DNA. Enzymatic analyses of the nuclear envelopes from the three cell lines revealed no contamination by plasma membranes and mitochondria, whereas low levels of arylesterase and acid phosphatase suggested slight contamination with endoplasmic reticulum and lysosomes. Electron microscopy demonstrated that the nuclear envelope ghosts and membrane sheets retain the inner and outer membranes and that nuclear pore complexes are discernable.

The time-course experiments indicated that the binding of dihydrotestosterone to nuclear envelopes from the TD-2 cell line had reached equilibrium by 16–18 h at 20°C. The steroid-binding sites were therefore measured after incubation of the isolated nuclear envelope fractions with radioactive steroid for 18 h at 20°C. Although these studies did not distinguish between the binding of the labelled steroid to membrane sites previously occupied by endogenous steroid and binding to unoccupied sites, the labelled steroid has probably exchanged with all of the endogenous steroid under these conditions.

Scatchard analysis of the binding of dihydrotestosterone to the nuclear envelope fraction from the TD-2 cell line revealed a class of binding sites with an apparent K_d of 14.2 nM ± 4.2 ($z_{0.025} = 1.96$) and a maximum binding capacity of 28.7 fmol/mg protein. The high level of nonspecific binding, however, makes it impossible to rule out the presence of more than one class of binding sites. It should also be noted that tumor cells do not represent a homogeneous population of cells. In particular, the androgen-responsive cell line probably consists of a mixture of steroid-responsive and steroid-unresponsive cell populations. Therefore, the levels of dihydrotestosterone binding sites that we have demonstrated for the TD-2 cell line do not represent the maximum number of sites but rather exemplify a trend, i.e., steroid-binding sites are detectable on nuclear envelopes from steroid-responsive tissues.

This high-affinity, low-capacity binding site can be compared to nuclear envelope binding sites on nuclear envelope fractions from other tissues. We have reported a saturable high-affinity binding site on purified nuclear envelope fractions from the rat ventral prostate [14] and the rat liver [15]. Under similar binding conditions, the binding of dihydrotestosterone to the inner nuclear membrane of nuclear envelopes from the rat ventral prostate revealed a class of binding sites with a K_d of 8.4 nM and a maximum binding capacity of 3040 fmol/mg protein [14]. Binding affinities of 23.2, 37.7 and 27.2 nM, and binding capacities of 578, 637 and 340 fmol/mg protein were obtained for the binding of dihydrotestosterone to nuclear envelopes from the livers of male rats, castrated male rats and female rats, respectively [15]. These studies show that there are considerably fewer androgen-binding sites on the nuclear envelopes from the TD-2 cell line compared to the nuclear envelopes prepared from the rat ventral prostate and the rat liver, but that the binding affinity is similar.

The androgen-binding sites on the nuclear envelope from the TD-2 cell line also resemble androgen-binding sites present on the nuclear matrix. The nuclear matrix is the residual structure remaining after the removal of most of the chromatin and nuclear envelope phospholipids from purified nuclei [28]. In addition to the ribonucleoprotein network and nucleoli, the matrix consists of remnants from nuclear pore complexes and the lamina, both characteristic of nuclear envelope fractions. Barrack and Coffey [29] have reported the binding of dihydrotestosterone to saturable high-affinity binding sites associated with the nuclear matrix prepared from the rat ventral prostate. Scatchard analysis revealed a class of binding sites with a K_d of 1.6 nM and a binding capacity of 1940 fmol/mg protein. We are now investigating the possiblity that the nuclear envelope and nuclear matrix binding sites are identical in the Shionogi mouse mammary carcinoma.

Proteinase and heat treatment of dihydrotestosterone-labelled nuclear envelopes resulted in complete loss of the dihydrotestosterone binding, whereas DNAase treatment resulted in a loss of 38% of the specifically bound dihydrotestosterone. One explanation for these results is that the protein-binding sites are associated with the residual DNA that is attached to the nuclear envelope fraction. The DNAase treatment would result in

the digestion of some of this residual DNA and the subsequent loss of some protein-binding sites associated with the DNA.

These studies do not distinguish between binding of steroid to a receptor of cytoplasmic or nuclear origin which is in turn bound to a membrane site or to a site which does not involve receptors. The salt-extraction experiments demonstrated that there was no significant loss of binding activity following incubation of the nuclear envelope fraction with concentrations of KCl up to 1.0 M. The binding sites are therefore probably not weakly associated to the membrane and probably do not represent receptor that is nonspecifically adsorbed to the nuclear envelope. At this point, the possibility of artefactual redistribution of steroid-binding sites to the nuclear envelope cannot be discounted. However, as the sites are not weakly associated with the membranes, we believe this to be unlikely. Moreover, in studies of androgen binding to nuclear envelopes prepared from the rat ventral prostate we have compared binding to nuclear envelopes prepared with heparin [14] and DNAase (unpublished data). The affinity and maximum number of binding sites were similar. These studies support the conclusion that steroid-binding sites are not due to redistribution during the preparation. Nuclear receptor is often solubilized by extraction in 0.6 M KCl. The dihydrotestosterone-binding site characterized in the TD-2 cell line nuclear envelope may not be mediated by receptors. This must be investigated further, however, as others have reported that not all bound steroid of androgen-sensitive cells can be extracted with salt [30–32].

We were unable to detect specific binding of dihydrotestosterone to nuclear envelopes from the two autonomous cell lines of the Shionogi mouse mammary carcinoma using the same conditions established for the androgen-dependent cell line nuclear envelopes. It should be borne in mind however that the binding conditions may not be ideal for detection of specific dihydrotestosterone binding to the autonomous cell line nuclear envelopes. Furthermore, the high levels of nonspecific binding may have masked binding sites in the autonomous cell lines. Nevertheless, it can be concluded that the dihydrotestosterone-binding sites on nuclear envelopes from the autonomous cell lines differ from those on the androgen-responsive cell line nuclear envelopes and that in these studies, at least, the binding of androgens to nuclear envelopes of these cells is correlated with the androgen-responsiveness of the cell line.

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References

1 Gorski, J. and Gannon, F. (1976) Annu. Rev. Physiol. 38, 425–450
2 Chan, L. and O'Malley, B.W. (1976) N. Engl. J. Med. 294, 1322–1328
3 Chan, L. and O'Malley, B.W. (1976) N. Engl. J. Med. 294, 1372–1381
4 Jensen, E.V., Polley, T.Z., Smith, S., Block, G.E., Ferguson, D.J. and DeSombre, E.R. (1975) in Estrogen Receptors in Human Breast Cancer (McGuire, W.L., Carbone, P.P. and Vollmer, E.P., eds.), pp. 37–56, Raven Press, New York
5 DeSombre, E.R., Greene, G.L. and Jensen, E.V. (1978) in Hormones, Receptors and Breast Cancer (McGuire, W.L., ed.), Vol. 10, pp. 1–14, Raven Press, New York
6 Lefebvre, Y.A., Caskey, J.J. and Kline, L.D. (1982) J. Steroid Biochem. 17, 609–614
7 King, R.J.B., Gordon, J., Cowan, D.M. and Inman, D.R. (1966) J. Endocrinol. 36, 139–150
8 Maurer, H.R. and Chalkley, G.R. (1967) J. Mol. Biol. 27, 431–441
9 Teng, C.-S. and Hamilton, T.H. (1968) Proc. Natl. Acad. Sci. USA 60, 1410–1417
10 Spelsberg, T.C., Steggles, A.W. and O'Malley, B.W. (1971) J. Biol. Chem. 246, 4186–4197
11 Puca, G.A., Sica, V. and Nola, E. (1974) Proc. Natl. Acad. Sci. USA 71, 979–983
12 Liang, T. and Liao, S. (1974) J. Biol. Chem. 249, 4671–4678
13 Jackson, V. and Chalkley, R. (1974) J. Biol. Chem. 249, 1615–1626
14 Lefebvre, Y.A. and Novosad, Z. (1980) Biochem. J. 186, 641–647
15 Lefebvre, Y.A. and Morante, S.J. (1982) Biochem. J. 202, 225–230
16 Widnell, C.C. and Tata, J.R. (1964) Biochem. J. 92, 313–317
17 Kay, R.R., Fraser, D. and Johnston, I.R. (1972) Eur. J. Biochem. 30, 145–154
18 Parr. E. (1973) Biol. Reprod. 8, 531–544.
19 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275

20 Thomas, P.S. and Farquahar, M.N. (1978) Anal. Biochem. 89, 35–44
21 Goldfine, I.D., Smith, G.J., Wong, K.Y. and Jones, A.L. (1977) Proc. Natl. Acad. Sci. USA 74, 1368–1372
22 Ames, B.N. (1966) Methods Enzymol. 8, 115–118
23 Shephard, E.H. and Hubscher, G. (1969) Biochem. J. 113, 429–440
24 Kun, E. and Abood, L.G. (1949) Science 109, 144–146
25 Kinne, R. and Kinne-Saffran, E. (1969) Eur. J. Physiol. 308, 1–15
26 Scatchard, G. (1949) Ann. N.Y. Acad. Sci. 51, 660–672
27 Bruchovsky, N. and Rennie, P.S. (1978) Cell 13, 273–280
28 Berezney, R. and Coffey, D.S. (1974) Biochem. Biophys. Res. Commun. 60, 1410–1417
29 Barrack, E.R. and Coffey, D.S. (1980) J. Biol. Chem. 255, 7265–7275
30 Van Doorn, E., Craven, S. and Bruchovsky, N. (1976) Biochem. J. 160, 11–21
31 Mainwaring, W.I.P. (1969) J. Endocrinol. 44, 323–333
32 Davies, P., Thomas, P. and Griffiths, K. (1977) J. Endocrinol. 74, 393–404
33 Kunitz, M. (1947) J. Gen. Physiol. 30, 291–310