Extraction of Nuclear Androgen Receptors by Sodium Molybdate from Normal Rat Prostates and Prostatic Tumors

Timothy C. Thompson and Leland W. K. Chung

Pharmacology/School of Pharmacy, University of Colorado, Boulder, Colorado 80309 [T. C. T., L. W. K. C.], and Department of Pharmacology, University of Colorado Health Science Center, Denver, Colorado 80262 [L. W. K. C.]

ABSTRACT

Because sodium molybdate stabilizes steroid receptors, this compound has been included in the homogenizing medium in order to maximize the recovery of measurable steroid receptors in normal and neoplastic tissues. This study demonstrates that sodium molybdate extracts additional androgen receptors from prostatic nuclei in a concentration-dependent manner. Nuclei previously washed with Triton X-100 to remove the outer nuclear membranes released similar numbers of androgen receptors with sodium molybdate as the unwashed nuclei, suggesting that the extracted nuclear androgen receptors are associated with intranuclear matrices. Sucrose density gradient analyses revealed that sodium molybdate-extractable nuclear androgen receptors sedimented similarly to the 0.4 mM KCl extract as 4S receptor complexes under high-salt conditions.

We have compared the amount of nuclear androgen receptors extracted from normal prostates (ventral prostate and dorsolateral prostate) and Noble hooded rat and Dunning prostatic tumors by a sensitive translocation-extraction procedure. This procedure involves the incubation of minced prostatic tissues, isolated from castrated rats, with [3H]R1881 ([3H]methyltrienolone; [6,7-3H]-17β-hydroxy-17α-methylsta-4,9,11-trien-3-one) at 37°C for 2 hr. Crude nuclear pellets were prepared from the minced tissues, and nuclear androgen receptors were extracted with 40 mM Na2MoO4 or 0.4 mM KCl. Results showed that the amount of nuclear androgen receptors present in the prostatic tumor nuclei is lower than that found in the normal. Although the percentage of nuclear androgen receptors extracted by sodium molybdate or KCl is similar between androgen-dependent and androgen-independent prostatic tumors, the absolute amounts of nuclear androgen receptors per mg DNA extracted from the former are 2- to 8-fold higher than those found in the latter.

INTRODUCTION

Since the first demonstration by Nielsen et al. (29), that sodium molybdate stabilizes steroid receptors and prevents the temperature inactivation of cytosolic glucocorticoid receptors obtained from rat liver and thymocyte preparations, this phenomenon has been confirmed in a number of other receptor systems. These receptor systems include rat prostatic (13) and mouse kidney (41) androgen receptors, human prostatic androgen (37) and progesterone receptors (1), estrogen receptors in human breast tumor (23) and bovine uterus (25), avian progesterone receptors (36), rabbit renal aldosterone receptors (21), and rat intestinal vitamin D receptors (22). Because of its generalized stabilizing effect on the cytosolic steroid and vitamin D receptors, sodium molybdate has been widely adopted as an added component in the homogenizing medium to maximize the recovery of measurable steroid receptors in normal (13, 38, 39) and neoplastic (14, 30, 37) tissues.

The purpose of the present study was to examine the interaction between sodium molybdate and isolated prostatic nuclei. Results indicate that sodium molybdate extracts androgen receptors from prostatic nuclei in a concentration-dependent manner. Statistically significant extraction of nuclear androgen receptors was observed with sodium molybdate concentrations as low as 10 mM. The amount of nuclear androgen receptors extracted by sodium molybdate and KCl was compared between normal prostates and Nb3 and Dunning prostatic tumors.

MATERIALS AND METHODS

Animals and Tumors

Nb tumors were obtained from Dr. J. R. Drago (Pennsylvania State University, Hershey, PA) and were maintained in either intact adult male Nb (2PR-129, hormone-dependent Nb tumor) or intact adult female Nb rats (102-PR autonomous Nb tumor) according to published procedures (9). Inbred Nb rats were either obtained from Charles River Laboratories (Wilmington, MA) or bred in the animal facilities of the School of Pharmacy, University of Colorado. Only freshly excised tissues were used in this study in order to avoid variable receptor loss due to tissue storage.

Adult Copenhagen rats bearing Dunning hormone-dependent (R-3327-H) and hormone-independent (R-3327-HI) or anaplastic (R-3327-AT) tumors were obtained from the laboratories of Dr. N. H. Altman (Pan- nicoIou Cancer Research Institute, Miami, FL) and Dr. W. D. W. Heston (Washington University, St. Louis, MO), respectively. Passages were made by s.c. transplantation of small pieces of tumor (1 to 2 cu mm) into adult male or castrated male Copenhagen rats (Charles River Laboratories) according to standard procedures (15, 35).

For comparative purposes, androgen receptor analyses in adult male Nb, Copenhagen, and Wistar rats were also performed.

Androgen Receptor Analysis

Cytosolic Androgen Receptors. All adult male rats were castrated at 24 hr prior to sacrifice. Tumor-bearing Nb female or castrated male Copenhagen rats were used without further surgical treatment. All assays were performed at 0-4°C. Tissues were excised and homogenized in 4 volumes of TEDG buffer [10 mM Tris-HCl, 1.5 mM EDTA, 1.0 mM dithiothreitol, and 10% (v/v) glycerol, pH 7.4] either in the presence or absence of 10 mM Na2MoO4. Cyto-sols were isolated by initial centrifugation at 8000 g for 15 min, followed by recentrifugation of the supernatant fraction at 105,000 g for 1 hr. Aliquots (175 µl) of cytosol were incubated with [3H]R1881 (87 Ci/mmol, 0.03 to 2 nM; New England
Nuclear, Boston, MA) on ice for 20 hr in the presence or absence of radioactive R1881 (1 μM). In some experiments, triamcinolone acetate (1 μM) was added to all samples to mask possible binding of R1881 to progesterone binding sites. The radioactive ligand was purified using Sephadex LH-20 (Pharmacia, Piscataway, NJ) column chromatography (1.1 x 60 cm) eluted with heptane: chloroform (1:1, v/v). The incubation was terminated by the addition of 2 volumes of TEDG buffer containing dextran-coated charcoal (0.25% HCl-washed, activated charcoal (Sigma Chemical Co., St. Louis, MO) and 0.0033% dextran T-70 (Sigma)). Samples were mixed briefly, allowed to stand on ice for 10 min, and then centrifuged at 8000 x g for 10 min. Total radioactivity in aliquots of the supernatant fractions was assessed by a Beckman scintillation counter (LS-3133P) in 5 ml of the scintillation fluid (MaxiFluor; J. T. Baker, Phillipsburg, NJ). Data were analyzed by the method of Scatchard (32), and linear regression lines were calculated and plotted by a Hewlett-Packard Calculator (Model 9821A). Single binding site analysis was assured by the determination of Hill coefficients (24). The counting efficiency for [3H] was determined by internal standard method to be 40%.

Nuclear Androgen Receptor. All operations were performed at 0-4°. Freshly excised Nb tumor tissues (hormone-dependent and autonomous; 0.3 to 0.6 g) were minced with fine surgical scissors and homogenized in 2 volumes of isotonic sucrose buffer (0.32 M sucrose and 1 M MgCl₂, pH was adjusted to 7.4 with K₂HPO₄ solution). The tissue homogenates were centrifuged at 1000 x g for 10 min to obtain the crude nuclear pellets. The nuclear pellets were washed 3 times with the isotonic sucrose buffer (2 ml/wash) and extracted with 2 ml of TEDG buffer containing 10 mM Na₂MoO₄. The crude nuclear pellets were resuspended in this buffer for 15 min with occasional mixing at 5-min intervals. Nuclear suspensions were recentrifuged at 1000 x g for 10 min to yield the supernatant nuclear extracts which were then subjected to androgen receptor exchange assay as described in the methods for cytosolic androgen receptor analysis. Aliquots of nuclear extract containing 0.2 mg of nuclear protein were incubated, and the data were analyzed by the Scatchard method as mentioned previously.

Quantitation of Nuclear Androgen Receptors by an in Vitro Translocation and Extraction Procedure

A procedure described by Traish et al. (38) was used for this study. Samples of freshly excised tissue (0.3 to 0.6 g) were minced with surgical scissors and transferred to small Erlenmeyer flasks (10 ml) containing 2 ml of Dulbecco’s modified Eagle’s medium, pH 7.2, and [3H]R1881 (1 μM). Samples were placed on a gyratory shaker and incubated for 2 hr. Samples were centrifuged at 37°C in an atmosphere of 95% air; 5% CO₂. At the end of incubation, samples were washed twice in ice-chilled Dulbecco’s modified Eagle’s medium and homogenized in 3 volumes of TEDG buffer. All subsequent operations were performed at 0-4°. Crude nuclear pellets were obtained by centrifuging the tissue homogenate at 800 x g for 10 min. The supernatant fractions were centrifuged again at 105,000 x g for 1 hr to obtain the cytosol fraction. The 800 x g supernatant fractions were centrifuged at 1000 x g for 10 min to obtain the crude nuclear pellets. The nuclear pellets were washed 3 times with the isotonic sucrose buffer (2 ml/wash) and extracted with 2 ml of TEDG buffer containing 10 mM Na₂MoO₄. The crude nuclear pellets were resuspended in this buffer for 15 min with occasional mixing at 5-min intervals. Nuclear suspensions were recentrifuged at 1000 x g for 10 min to yield the supernatant nuclear extracts which were then subjected to androgen receptor exchange assay as described in the methods for cytosolic androgen receptor analysis. Aliquots of nuclear extract containing 0.2 mg of nuclear protein were incubated, and the data were analyzed by the Scatchard method as mentioned previously.

Androgen receptor activity in the soluble fraction was analyzed by the HAP adsorption method (40). In brief, 0.5 ml of the HAP slurry (70% v/v; Bio-Rad, Richmond, CA), resuspended in 20 mM Tris-HCl and 1.0 mM K₂HPO₄, pH 7.2, was added to 2 ml of cytosolic or nuclear extract. After 15 min, during which time all samples were resuspended at 5-min intervals, the mixtures were centrifuged at 5000 x g, and the packed HAP pellet was recovered and washed once with 2 ml of TEDG buffer. Each HAP pellet was washed with 2 ml of absolute ethanol. Samples were recentrifuged at 5000 x g, and the supernatant was decanted into scintillation vials and assessed for total radioactivity. Radioactivity associated with the residual nuclear pellet also was determined by solubilization of the nuclear pellet in 2 ml of 1 N NaOH and counting directly in a Beckman scintillation counter as described under cytosolic androgen receptors.

Characterization of Androgen Receptor by Sucrose Density Gradient Centrifugation

To characterize further the androgen receptor extracted from the prostate nuclear pellets with sodium molybdate, ventral prostates isolated from adult male rats that were castrated 24 hr previously were minced and incubated with [3H]R1881 in the presence or absence of the competing radioligand R1881 as described above. The prostatic nuclei were subsequently isolated, and the sedimentation properties of androgen receptors eluted with either sodium molybdate (40 mM) or KCl (0.4 M) were compared by sucrose density gradient centrifugation. A 5-ml linear sucrose gradient, 5 to 20% (w/v), was prepared in TEDG buffer containing both 0.4 M KCl and 40 mM Na₂MoO₄. Samples (0.2 ml) extracted from 0.3- to 0.6-g tissue equivalent of prostatic nuclei were layered on top of sucrose gradients and were centrifuged for 18 hr at 48,000 rpm at 2°C in a SW 50.1 rotor (Beckman). The sedimentation coefficient of androgen receptors was calibrated against 14C-methylated bovine serum albumin (4.6S) and 14C-methylated ovalbumin (3.6S; New England Nuclear, Boston, MA), according to the procedures of Martin and Ames (20).

DNA and Protein Determinations. Total DNA content in tissues was determined by the diphenylamine method using calf thymus DNA as the reference standard (3). Protein concentration was determined by the method of Lowry et al. (19) using bovine serum albumin as the reference standard.

Statistical Methods. Data from experiments designed to test the effects of Na₂MoO₄ on Kₛ and Bₘₜₕ max of R1881 binding to soluble androgen receptors (Table 1) were analyzed by the t test for paired comparisons.

RESULTS

Scatchard Analyses of Cytosolic Androgen Receptors. In an effort to correlate androgen receptor levels and the hormone dependence of the established prostatic tumors, cytosolic androgen receptors were quantified in both the presence and the absence of 10 mM Na₂MoO₄, which were added during tissue homogenization. Table 1 shows that the inclusion of 10 mM sodium molybdate in the homogenizing medium had variable effects on the total number of binding sites (Bₘₜₕₚₚ) and the affinity of soluble androgen receptors in normal prostates and Dunning and Nb prostatic tumors. For example, sodium molybdate increased the total number of cytosolic androgen receptors (Bₘₜₕₚₚ) and enhanced their affinity toward R1881 (decreased Kₛ values) in normal prostates (ventral and dorsolateral prostate) derived from Nb rats, but this same treatment only increased the Bₘₜₕₚₚ max...
Comparison of the Direct Effects of Sodium Molybdate on Cytosolic Androgen Receptors and on the Extraction of Additional Androgen Receptors from Prostatic Nuclei. The observed effects of sodium molybdate on \( B_{\text{max}} \) and \( K_a \) of soluble androgen receptors in normal and neoplastic prostatic tissues (Table 1) can be interpreted as the results of: (a) direct interaction of sodium molybdate with cytosolic androgen receptors; and (b) interaction and/or extraction of nuclear androgen receptors by sodium molybdate; or both. To differentiate these possibilities, 3 experimental approaches were taken: (a) sodium molybdate was added directly to the isolated cytosolic androgen receptor fraction to determine if the molybdate anion may interact directly with cytosolic androgen receptors; (b) tissue-homogenizing buffer containing sodium molybdate was used to isolate cytosolic androgen receptor fraction to determine if molybdate anion may also interact with other androgen-binding subcellular components in the prostatic tissue homogenate; (c) sodium molybdate-containing buffer was added directly to the isolated prostatic nuclear pellet to test if molybdate anion may extract additional androgen receptors from prostatic nuclei.

Results of the first 2 approaches are presented in Chart 2, A and B. In these studies, hormone-dependent (2PM29) and autonomous (102-Pr) Nb tumors were used as the test tissues. Chart 2A showed that direct addition of sodium molybdate to the cytosol of hormone-dependent Nb tumor increased the affinity [Condition A (control), \( K_a = 0.53 \text{ nm} \) versus Condition C (direct addition), \( K_a = 0.28 \text{ nm} \)] but without altering the \( B_{\text{max}} \) [Condition A (control), 5 fmol/mg protein versus Condition C (direct addition) of 5.4 fmol/mg protein] of R1881 binding to cytosolic androgen receptors. However, sodium molybdate, when included in the homogenizing medium prior to cytosol isolation, enhanced both the affinity (decreased \( K_a \)) and capacity (\( B_{\text{max}} \)) of androgen binding [comparison between Condition A (control) and Condition B (addition to the homogenizing medium); also see Table 1].

In the autonomous Nb tumor, direct addition of sodium molyb-
date to the isolated cytosol markedly increased the $B_{\text{max}}$ of androgen binding [Condition A (control), 1.5 fmol/mg protein, was increased to 6.8 fmol/mg protein in Condition C (direct addition)] but with reduced affinity ($K_d$ increased from the control, 0.32 nM, to the direct addition, 0.74 nM; see Chart 2B). Addition of sodium molybdate to the homogenizing medium prior to cytosol isolation increased $B_{\text{max}}$ of R1881 binding but without appreciably altering the $K_d$ (see also Table 1). Because of the marked differences observed in the $K_d$ and $B_{\text{max}}$ of androgen receptor binding between the inclusion of sodium molybdate in the homogenizing medium (condition B) and the direct addition of sodium molybdate to the isolated cytosolic fraction (Condition C), these results suggest that sodium molybdate not only interacts directly with cytosolic androgen receptors but also interacts with particulate fractions of the homogenate such as the prostatic nuclei.

To test the latter possibility, ventral prostates, isolated from adult rats castrated 24 hr previously, were minced and incubated with $[^3H]R1881$ at 37° for 2 hr. Crude nuclear pellets were isolated and were subjected to extraction by homogenizing medium containing 6 concentrations of sodium molybdate ranging from 0 to 40 mM. Results showed that the numbers of nuclear androgen receptors extracted from prostatic nuclei increased with increasing concentrations of sodium molybdate (Chart 3).
Sodium Molybdate and Androgen Receptors

Charts. Concentration-dependent extraction of nuclear androgen receptors from normal prostatic nuclei previously washed or unwashed with a neutral detergent Triton X-100. Prostatic tissues (ventral prostate of Nb rats) were minced and incubated with [3H]R1881 (15 nm) in the presence or absence of radioinert R1881 (1 mM) at 37° (95% air.5% CO2) for 2 hr. Crude nuclear pellets were isolated and washed twice in TEDG buffer with or without 0.2% (w/v) Triton X-100 prior to later receptor extraction with varying concentrations of sodium molybdate (0 to 40 mM). •, Triton X-100-washed nuclei; ◦, buffer-washed nuclei.

Chart 3. Concentration-dependent extraction of nuclear androgen receptors from normal prostatic nuclei previously washed or unwashed with a neutral detergent Triton X-100. Prostatic tissues (ventral prostate of Nb rats) were minced and incubated with [3H]R1881 (15 nm) in the presence or absence of radioinert R1881 (1 mM) at 37° (95% air.5% CO2) for 2 hr. Crude nuclear pellets were isolated and washed twice in TEDG buffer with or without 0.2% (w/v) Triton X-100 prior to later receptor extraction with varying concentrations of sodium molybdate (0 to 40 mM). •, Triton X-100-washed nuclei; ◦, buffer-washed nuclei.

These results taken together suggest that sodium molybdate has dual effects; i.e., direct interaction with cytosolic androgen receptors, and elution of additional high-affinity androgen binding sites from the prostatic nuclei.

Characterization of Salt-extractable Androgen Receptor by Sucrose Density Gradient Centrifugation. Salt-extractable (0.4 M KCI or 40 mM Na2MoO4) nuclear androgen receptors were characterized further by sucrose density gradient centrifugation. Aliquots of these extracts were layered on top of 5 to 20% linear sucrose gradients. Distinct 4S, [3H]R1881-labeled receptor complexes were detected in the 0.4 M KCI extract (Chart 4A). Under the same experimental conditions, 40 mM sodium molybdate extracted labeled receptor complexes with identical sedimentation properties (4S protein) to that of the 0.4 M KCI extracts (Chart 4B).

Comparison of Sodium Molybdate Extraction of Androgen Receptors from Prostatic Nuclei Isolated from Normal Prostates and Prostatic Tumors. Because sodium molybdate is capable of extracting androgen receptors from prostatic nuclei isolated from rat ventral prostate, this finding prompted the comparative studies of sodium molybdate elution of receptors from normal and neoplastic prostatic nuclei. Two types of comparative studies were performed. To determine whether the amount of nuclear androgen receptors associated with hormone-dependent Nb tumors differ from that of the autonomous tumor line, nuclear pellets were isolated from these 2 tumors, extracted with sodium molybdate (10 mM), and analyzed by ligand exchange assay, and data were treated by Scatchard method. Chart 5 shows that significant amounts of androgen receptors (4.3 fmol/mg nuclear protein extracted), kD 0.15 nm, were present in the hormone-dependent, but were absent in the autonomous Nb tumor. To extend these observations, we determined the nuclear androgen receptor content of normal prostates and of

MARCH 1984 1023
Dunning and Nb prostatic tumors by a sensitive whole-tissue assay method which used the procedure of an in vitro translocation of cytosolic androgen receptors to the nuclei and the subsequent extraction of nuclear androgen receptors from isolated prostatic nuclei as described by Traish et al. (38). Table 2 shows that most of the androgen receptor activities are associated with the crude nuclear pellets presumably through cytoplasmic translocation during the in vitro incubation period (31). Similar numbers of androgen receptors can be eluted from the prostatic nuclei by Na₂MoO₄ (40 mM) and KCl (0.4 M) in most of the tissues studied. A substantial amount of androgen receptors (ranging from 23 to 92%), however, was resistant to salt extraction and remained in the residual nuclear fraction. Although the percentage of nuclear androgen receptors extracted did not correlate with the hormone-dependent status of the tumor, the absolute amounts of salt-extractable nuclear androgen receptors were consistently higher (2- to 8-fold) in normal prostates and hormone-dependent prostatic tumors, than the hormone-independent and autonomous tumors. Using this procedure, higher cytosolic androgen receptor levels were detected in Dunning HT than Dunning HIT.

DISCUSSION

Because of the stabilizing effects of sodium molybdate on steroid receptors in general (1, 13, 14, 21, 23, 25, 29, 30, 36–39), many laboratories have adopted a standard procedure which includes sodium molybdate in the homogenizing medium for the isolation of cytoplasmic and nuclear steroid receptors (13, 14, 30, 37–39). The present study explores the possibility that sodium molybdate interacts directly with cytoplasmic androgen receptors and extracts nuclear androgen receptors from both normal prostates and transplantable prostatic tumors. The findings also indicate that molybdate-extracted androgen receptors (number of sites) from prostatic nuclei correlate with the hormone-dependent status of both the Dunning and Nb prostatic tumors.

The following evidence supports the conclusion that sodium molybdate extracts androgen receptors from prostatic nuclei. (a) Sodium molybdate extracts androgen receptors from isolated prostatic nuclei in a concentration-dependent manner. There is no difference in the extraction of nuclear androgen receptors from crude and Triton-washed nuclei, suggesting that the salt-extracted nuclear androgen receptors are associated with intranuclear matrices rather than with the outer nuclear membranes. (b) Sodium molybdate-extracted nuclear androgen receptors banded as a 4S protein under high-salt conditions in a sucrose density gradient as that of the 0.4 M KCl extract. The latter salt concentration is known to extract several types of nuclear steroid receptors from their target tissues (2, 7, 12).

Trachtenberg et al. (37) observed a significant decrease in the amount of salt-extractable nuclear androgen receptors in human prostatic tissues homogenized in buffer containing molybdate, compared to those homogenized in buffer alone. The authors suggested that this reduction in salt-extractable receptors is due to either accelerated destruction of nuclear receptors or a redistribution of the extractable nuclear receptors into either cytosol or the high salt-resistant fraction. Results of the present study in normal and neoplastic rat prostatic tissues favor the latter possibility; i.e., sodium molybdate caused a redistribution of nuclear androgen receptors such that more of these receptors were rendered soluble and appeared in the cytosol. In most of the tissues studied, a fixed percentage (8 to 39%) of nuclear androgen receptors was extracted by 0.4 M KCl and 40 mM sodium molybdate. An exception to this is the Nb ventral prostate, where a greater percentage of nuclear androgen receptors was extracted by 0.4 M KCl (68%) than by 40 mM sodium molybdate (32%). This difference, however, was not observed when normal ventral prostatic nuclei isolated from Wistar rats were used. Because, in most of the studies, the total amount of nuclear androgen receptors recovered in salt-extractable and residual fractions was comparable among TEDG-, 0.4 M KCl-, and 40 mM Na₂MoO₄-treated samples, it is unlikely that sodium molybdate enhanced nuclear androgen receptor destruction.

Effects of sodium molybdate that stabilize against the proteolysis of the untransformed steroid receptors either associated or nonassociated with RNA were determined by Miller et al. (23) and Sherman et al. (33). They demonstrated that the cleavage of tyrosyl- and lysyl-7-amino-4-methylcoumarin derivatives was inhibited by sodium molybdate. The inhibitory actions by 40 mM sodium molybdate were not due to its ionic strength, because .

---

Table 2

<table>
<thead>
<tr>
<th>Source of tissue</th>
<th>Soluble</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nb rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VP Cytosol (3)</td>
<td>28 ± 22</td>
<td></td>
</tr>
<tr>
<td>TEDG extract (3)</td>
<td>3 ± 1</td>
<td>60 ± 21</td>
</tr>
<tr>
<td>0.4 M KCl extract (3)</td>
<td>20 ± 8</td>
<td>42 ± 8</td>
</tr>
<tr>
<td>40 mM Na₂MoO₄ extract (7)</td>
<td>18 ± 4</td>
<td>56 ± 5</td>
</tr>
<tr>
<td>DLP Cytosol (3)</td>
<td>3 ± 1</td>
<td></td>
</tr>
<tr>
<td>TEDG extract (5)</td>
<td>1 ± 0.3</td>
<td>22 ± 14</td>
</tr>
<tr>
<td>0.4 M KCl extract (5)</td>
<td>9 ± 3</td>
<td>14 ± 11</td>
</tr>
<tr>
<td>40 mM Na₂MoO₄ extract (7)</td>
<td>7 ± 1</td>
<td>14 ± 8</td>
</tr>
</tbody>
</table>

* VP, ventral prostate; DLP, dorsolateral prostate; HIT, hormone-dependent Dunning tumor; HDT, hormone-independent Dunning tumor.

* Numbers in parentheses, number of experiments per group.

* Average ± S.E. of 2 to 8 determinations.

---

the control samples treated with 60 mM KCl (same conductivity as 40 mM Na₂MoO₄) failed to suppress the same hydrolytic reactions. A similar conclusion can be drawn relevant to molybdate extraction of nuclear androgen receptors from prostatic nuclei, because it has been shown that 60 mM KCl extracts negligible amounts of steroid receptor complexes (6, 7, 27). The extraction of labeled nuclear androgen receptors from prostatic nuclei has been demonstrated previously by the use of KCl, Cibacron blue, heparin, and pyridoxal phosphate (26). It seems likely that these various reagents interact differently with the chromatin, based on ionic strength considerations.

Recently, Siret and Grant (34) reported that androgen binding sites increased in human hyperplastic prostatic tissue cytosol as molybdate concentration was increased in the tissue-homogenizing buffer. Since molybdate exerted no direct effect (changes of Kᵣ or Bₘₐₓ) on nuclear androgen receptors extracted from human hyperplastic prostate (34), data from their study as well as from the present one are consistent with the proposal that, specifically, molybdate extracts androgen receptor sites from prostatic nuclei. Further substantiation is provided by recent reports of Murakami et al. (27, 28) that sodium molybdate and sodium tungstate extracted glucocorticoid receptors from their bound form in rat liver nuclei or DNA-cellulose matrices.

Comparison of the salt-extractable nuclear androgen receptors in hormone-dependent and independent prostatic tumors showed that androgen-dependent tumors contained more molybdate-extractable receptors than autonomous ones. This reflects the intrinsic differences of the tumors. However, the host hormonal environment may also play a role, because salt-extractable nuclear androgen receptors increased in Nb autonomous tumor when this tumor was allowed to grow in intact male hosts.

Nuclear androgen receptors analyzed by in vitro ligand translocation-extraction method correlated well with the endocrine dependency status of the prostatic tumors. The present results support the current concept that androgen-dependent tumors are more abundant in nuclear androgen receptors than are their androgen-independent counterparts. This concept, however, has been confirmed neither in numerous androgen receptor studies in human prostates (8, 11, 17, 18) nor in the cytosolic androgen receptor assay in the Dunning tumors, as presented herein, using conventional ligand exchange assay method. The consistent difference of cytosolic and nuclear androgen receptors obtained from hormone-dependent and -independent prostatic tumors by the in vitro ligand translocation-extraction assay (see Table 2), therefore, may serve as a basis for future investigation of androgen receptor contents in human prostatic specimens.

It should be stressed that the in vitro ligand translocation followed by salt extraction of nuclear androgen receptors has several advantages over the conventional ligand exchange followed by dextran-coated charcoal treatment method. (a) Nuclear androgen receptor levels in Nb HD tumor analyzed by ligand translocation-extraction assay were 2-fold higher than that analyzed by ligand exchange method; nuclear androgen receptors in Nb HDT were calculated to be 3.19 and 1.65 fmol/50-mg tissue wet weights as analyzed by ligand translocation-extraction and ligand exchange methods, respectively (see Table 2 and Chart 5). This enhanced efficiency of analyzing nuclear androgen receptors by the former method may be due to the more efficient exchange of ligand with occupied receptors at elevated temperatures (37°C for 2 hr versus 0°C for 20 hr). Alternatively, more efficient binding and exchange may occur under conditions of established cellular compartmentalization and less receptor degradation which may result from the presence of steroid ligand which binds stabilized receptors prior to tissue homogenization. This increased efficiency of receptor assay by the use of intact cells (as compared to the use of subcellular fractions) was also observed in glucocorticoid receptor analysis in human leukocytes (16). (b) Most of the salt-extractable [³H]R1881-labeled nuclear androgen receptors (75 to 90%) recovered by ligand translocation-extraction assay were in receptor-bound form (4S) as evidenced by sucrose density gradient analysis. This suggests the presence of lower nonspecific binding and/or less receptor degradation by the use of ligand translocation assay method than by the conventional ligand exchange method. (c) The ligand translocation-extraction assay is rapid (37°C for 2 hr versus 0°C for 20 hr), and the binding takes place under physiological conditions (prostatic tissues incubated under these conditions survived as viable tissue grafts under the kidney capsules (4)). By utilizing the above advantages of the in vitro ligand exchange-salt extraction of nuclear androgen receptors, it is possible to demonstrate that the amount rather than the percentage of nuclear androgen receptors correlates with the hormone dependency status of the Nb and Dunning tumors.

ACKNOWLEDGMENTS

The authors wish to thank Donna Gesumaria and James Matsuura for their expert technical assistance. In addition, helpful discussion with Dr. E. R. Barrack as well as a critical review of the manuscript by Dr. K. B. Horwitz were invaluable.

REFERENCES

13. Gaubert, C. M., Tremblay, R. R., and Dube, J. Y. Effect of sodium molybdate on steroid receptor complexes (6, 7, 27). The present results support the current concept that androgen-dependent tumors are more abundant in nuclear androgen receptors than are their androgen-independent counterparts. This concept, however, has been confirmed neither in numerous androgen receptor studies in human prostates (8, 11, 17, 18) nor in the cytosolic androgen receptor assay in the Dunning tumors, as presented herein, using conventional ligand exchange assay method. The consistent difference of cytosolic and nuclear androgen receptors obtained from hormone-dependent and -independent prostatic tumors by the in vitro ligand translocation-extraction assay (see Table 2), therefore, may serve as a basis for future investigation of androgen receptor contents in human prostatic specimens.

It should be stressed that the in vitro ligand translocation followed by salt extraction of nuclear androgen receptors has several advantages over the conventional ligand exchange followed by dextran-coated charcoal treatment method. (a) Nuclear androgen receptor levels in Nb HD tumor analyzed by ligand translocation-extraction assay were 2-fold higher than that analyzed by ligand exchange method; nuclear androgen receptors in Nb HDT were calculated to be 3.19 and 1.65 fmol/50-mg tissue wet weights as analyzed by ligand translocation-extraction and ligand exchange methods, respectively (see Table 2 and Chart 5). This enhanced efficiency of analyzing nuclear androgen receptors by the former method may be due to the more efficient exchange of ligand with occupied receptors at elevated temperatures (37°C for 2 hr versus 0°C for 20 hr). Alternatively, more efficient binding and exchange may occur under conditions of established cellular compartmentalization and less receptor degradation which may result from the presence of steroid ligand which binds stabilized receptors prior to tissue homogenization. This increased efficiency of receptor assay by the use of intact cells (as compared to the use of subcellular fractions) was also observed in glucocorticoid receptor analysis in human leukocytes (16). (b) Most of the salt-extractable [³H]R1881-labeled nuclear androgen receptors (75 to 90%) recovered by ligand translocation-extraction assay were in receptor-bound form (4S) as evidenced by sucrose density gradient analysis. This suggests the presence of lower nonspecific binding and/or less receptor degradation by the use of ligand translocation assay method than by the conventional ligand exchange method. (c) The ligand translocation-extraction assay is rapid (37°C for 2 hr versus 0°C for 20 hr), and the binding takes place under physiological conditions (prostatic tissues incubated under these conditions survived as viable tissue grafts under the kidney capsules (4)). By utilizing the above advantages of the in vitro ligand exchange-salt extraction of nuclear androgen receptors, it is possible to demonstrate that the amount rather than the percentage of nuclear androgen receptors correlates with the hormone dependency status of the Nb and Dunning tumors.
T. C. Thompson and L. W. K. Chung


Extraction of Nuclear Androgen Receptors by Sodium Molybdate from Normal Rat Prostates and Prostatic Tumors

Timothy C. Thompson and Leland W. K. Chung


Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/44/3/1019

Sign up to receive free email-alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.