Basal and Estrogen-stimulated Hormone Receptor Profiles in Four R3327 Rat Prostatic Carcinoma Sublines in Relation to Histopathology and Androgen Sensitivity

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ABSTRACT

Four sublines (H, HI, G, and AT) of the R3327 (Dunning) rat prostatic carcinoma have different androgen sensitivities and histopathological characteristics. In order to investigate whether these characteristics were associated with differences in the hormone receptor profile and its response to estrogen, we carried out Scatchard analysis on the cytosolic (C) and high-salt nuclear-associated (N) androgen (AR), estrogen (ER), and progesterone (PgR) receptor in each line, carried in control and diethylstilbestrol (DES)-treated animals. In the H line (androgen sensitive, well differentiated) DES treatment resulted in significant increases in total cellular ER and PgR, in redistribution of both receptors between the C and N fractions, and in a marked increased of PgR (>10-fold). The hormone receptor profile and its response to DES was similar in the HI line (androgen insensitive, anaplastic) to that in the H line, but the concentrations were not increased by DES treatment, although redistribution of AR between C and N fractions did occur. Some characteristics of the AR in the AT line (androgen insensitive, anaplastic) contained increased basal concentrations of AR and PgR than in the H line, but the concentrations were not increased by DES treatment, although treatment promoted association of ER with the nuclear fraction. The AT line (androgen insensitive, anaplastic) contained no ER and negligible PgR, but AR was present, although in lower concentrations than in the other lines. Diethylstilbestrol treatment had no effect on the concentration, although redistribution of AR between C and N fractions did occur. Some characteristics of the AR in the AT line differed qualitatively from that in the HI line, but injection of testosterone into castrated animals bearing the AT tumor promoted association of AR with the nuclear fraction, indicating normal activation.

The data suggest that the ability of DES treatment to increase AR and PgR concentrations is associated with differentiation and/or the presence of stroma and that it is unrelated to androgen sensitivity.

INTRODUCTION

The R3327H (Dunning) experimental rat prostatic adenocarcinoma has been widely used as a model for the human disease. It is well differentiated and androgen sensitive, although growth eventually resumes after androgen deprivation due to the presence of autonomous cells within the total cell population (1). In addition to AR, the tumor possesses 5α-reductase and prostate-specific acid phosphatase activity (1). We have demonstrated that this tumor also has some characteristics of an estrogen target organ, in that estrogen treatment results in a marked increase in PgR content, although concentrations of ER are low (2-4). This may indicate a further similarity to the human disease, inasmuch as considerable concentrations of PgR have been demonstrated in human prostatic tissue (5), in spite of the fact that attempts to demonstrate significant quantities of ER have met with limited success (6).

We have now examined the basal and estrogen-treated hor-
presence of AR in the anaplastic AT line tumor. Previous investigators have reported that no AR is detectable in this line (9, 13). We therefore thought it of interest to characterize this AR further. A number of qualitative abnormalities have been noted in the AR of genital skin fibroblasts from some patients with AR-positive androgen insensitivity syndrome, including instability in the presence of sodium molybdate, a faster dissociation of receptor from bound ligand, and a higher affinity for progesterone than the AR from fibroblasts from normal patients (24, 25). We therefore examined these properties of the AR from AT line as compared with the H line. In addition, we gave injections of testosterone or vehicle to castrated animals bearing the AR tumor to investigate the ability of androgen to promote association of the receptor with the high-salt nuclear fraction.

MATERIALS AND METHODS

Animals and Tumors

The H line tumors were derived from a donor Copenhagen × Fischer F1 rat from the Papanicolaou Cancer Research Institute at Miami, Inc. This tumor was passaged through two generations of hybrid rats in our colony before use. The H line was derived from the same parent tumor but had been transplanted into castrated animals from a castrated donor in our colony. The G and AT-2 lines were grown in animals implanted with tumors from donor animals received from the Brady Urological Institute Research Laboratories, Johns Hopkins University, by courtesy of Dr. John Isaacs.

For the hormone receptor profile experiments, animals bearing tumors of approximately 1.5 cm mean diameter were given s.c. injections of either 5 μg DES in 0.125 ml sesame oil/100 g body weight or an equivalent volume of oil for 9 days in 2 consecutive weeks. Each treatment group consisted of 4 or 5 animals. The animals were killed 18–20 h after the final injection, and the tumors were removed. The capsule and any cystic or necrotic portions were discarded, and a representative piece of each tumor was fixed for histological examination. The remainder was rinsed in chilled homogenizing buffer and frozen and stored for not longer than 2 months in liquid nitrogen before assay.

For the experiments on the comparison of AR characteristics in the H and AT lines, tumors were derived from the sources described above, passaged through hybrid animals in our colony for a maximum of 2 tumor generations (H line) and 18 tumor generations (AT-2 line). Animals were castrated via the scrotal route 24 h before use in order to maximize the concentration of cytosolic receptors.

Isotopes and Chemicals

Radioactive isotopes, unlabeled steroids, scintillators, and other chemicals used for ER and PgR assays were obtained and stored as described previously (2). Additional reagents used for the assay and characterization of AR: [1,4]H]estradiol (specific activity, 2–20 Ci/mmol); [3H]estradiol as ligand after adsorption of ER on to hydroxylapatite. Dextran-coated charcoal separation of free and bound steroid was used for the PgR assays, after incubation with [3H]J5020 in the presence of excess cortisol and DHT.

Androgen Receptor Assays. For total cytosol AR, duplicate 200-μl aliquots of stripped cytosol were incubated at 0–4°C for 24 h with 50 μl of [3H]J1881 prepared in buffer B10 to give five final concentrations ranging from 2.5 to 20 nm. Parallel incubations were carried out in the presence of 100-fold radioinert R1881 to correct for low affinity binding. All incubations contained 500-fold TA to prevent binding of [3H]J1881 to PgR. After incubation, DCC suspension (500 μl) was added to each tube, vortexed, and allowed to stand in ice for 15 min. The charcoal was removed by centrifugation twice for 10 min at 2500 × g, and 400 μl of the SN were counted in PCS/toluene for 10 min or to 2% error.

For nuclear AR, 50 μl of stripped nuclear extract were incubated for 16–18 h at 15°C with 125 μl of [3H]J1881 prepared in buffer B10 at five final concentrations ranging from 2.5 to 20 nm. Parallel incubations contained 100-fold radioinert R1881 and all incubations were carried out in the presence of 500-fold TA. Following incubation, duplicate 250-μl aliquots were added to 250 μl of HAP slurry in polystyrene tubes (12 × 75 mm) and incubated on ice for 30 min with vortexing at 5-min intervals. Unbound steroid was removed from the HAP suspension by centrifugation (2500 × g, 3 min) and 4 washes with 1.5 ml of Tris-Tween 80. Bound [3H]J1881 was extracted from the pellet with two ethanol washes at 30°C (total, 2.5 ml; 30 min). Ten ml toluene scintillator were added to the combined ethanol extracts and counted for 10 min or to 2% error.

The data from all receptor assays were analyzed according to the method of Scatchard (27), using the program of Schwarz with a Hewlett-Packard HP-97 calculator (28). Cytosol binding capacity was expressed as fmol/mg of cytosol protein assayed by the method of Lowry et al. (29), using BSA as a standard, and both cytosol and nuclear binding were expressed as fmol/mg DNA in the nuclear pellet. DNA was assayed by the diphenylamine method (30). Previous experiments have established that homogenate and nuclear pellet DNA are virtually equivalent.

Characterization of H Line and AT Line Cytosol AR

H line and AT line tumor cytosols from rats castrated for 24 h were prepared as described in “Materials and Methods,” except that the crude SN was not stripped before adjustment with sodium molybdate. In experiments to determine dissociation and stability and for SDGC, buffer A contained 10% (v/v) glycerol. In the SDGC experiments sodium molybdate concentration varied from 0 to 50 mM as described in the text.

Sucrose Density Gradient Centrifugation in the Presence and Absence of Sodium Molybdate

Linear 5–20% (v/v) sucrose gradients in buffer A plus 10% (v/v) glycerol in the absence or presence of 0.4 mM KC1 and containing sodium
molybdate (0–50 mM) were prepared in 5-mI polyallomer tubes and allowed to stand at 0–4°C overnight. The pH of the buffer containing 50 mM molybdate was readjusted to pH 7.4 before formation of the gradients. Cytosol was incubated at 0–4°C for 16 h with [3H]R1881 plus 500-fold TA in the presence or absence of 100-fold radioinert R1881 to give a final concentration of 5 nM [3H]R1881. Unbound steroid was removed with DCC pellets followed by centrifugation to remove DCC. Two hundred µl of incubated cytosol, equivalent to 0.9 mg protein and containing [14C]BSA (4.65) and/or [14C]globulin (7S) to act as internal sedimentation markers, were layered onto each gradient. The gradients were centrifuged in a Beckman SW 55 Ti rotor at 49,000 rpm for 20 h. Fifteen-drop fractions (0.25 ml) were collected in scintillation vials; 0.25 ml of distilled water and 10 ml of PCS were added to each vial and radioactivity was counted by using a dual label program. In one experiment 10 mM sodium molybdate was added to the homogenizing buffer instead of the crude SN.

Steroid Specificity

For this experiment [3H]DHT was used as ligand to avoid the necessity for using TA and to allow progesterone and cortisol to be investigated as competitors for binding to AR. Two hundred µl aliquots of cytosol were incubated at 0–4°C for 16 h with [3H]DHT in 50 µl of buffer B50 to give a final concentration of 5 nM in the absence or presence of a number of steroids at six concentrations ranging from 5 nM to 0.5 µM. The competitors used were DHT, testosterone, estradiol, R5020, progesterone, and cortisol. The percentage reduction in high affinity binding at each concentration of each competitor was calculated. Relative binding affinity was defined as the ratio of the concentration of DHT to competitor required to reduce high-affinity binding of [3H]DHT by 50%.

In addition, Scatchard analysis of cytosol and nuclear AR in AT line tumor extracts were carried out by using [3H]DHT and [3H]R1881 in the presence of excess TA as ligands in order to compare the binding characteristics of the two ligands. Ligand concentrations ranged from 2.5 to 25 nM with 100-fold radioinert DHT or R1881 to correct for low-affinity binding.

Stability and Dissociation of [3H]R1881 and [3H]DHT Binding to Cytosolic AR

Aliquots of H and AT tumor cytosols (200 µl) were incubated at 0–4°C for 16 h with 50 µl of [3H]R1881 or [3H]DHT contained in buffer B50 to give a final concentration of 5 nM. Parallel incubations for both 3H-ligands were performed with 100-fold excess of the appropriate radioinert steroid. In addition, all incubations with [3H]R1881 contained 500-fold TA. Following incubation, excess radioinert R1881 (5 µmol in 50 µl of buffer) was added to one half of the [3H]R1881 ± 100-fold R1881 incubations to measure dissociation and 50 µl of buffer only was added to the other half to determine stability.

Similarly, radioinert DHT or buffer only was added to the [3H]DHT ± 100-fold DHT incubations. All tubes were vortexed and kept at 15°C. At intervals over a 24-h period, 500 µl of DCC suspension were added to duplicate tubes from each set of incubations, vortexed, and allowed to stand in ice for 15 min. Charcoal was removed and the SN was counted in a Beckman spectrum. The percentage of specifically bound [3H]R1881 or [3H]DHT was plotted on a log scale against time with 100% representing the amount of specifically bound radioactive ligand at zero time.

Promotion of Association of AR with High-Salt Nuclear Fraction in Vivo

To determine whether AT line tumors were able to promote association of AR to the high-salt nuclear fraction, rats bearing AT line tumors were castrated 24 h before injection of either 10 µg of testosterone one in 0.5 ml of 10% ethanol in saline or vehicle only. One h after injection blood was taken from the aorta for assay of serum testosterone, tumors were removed, and total cytosol and nuclear AR were determined by using single saturating dose assays, using a concentration of 20 nM [3H]R1881 plus 10 µM TA. Results were expressed in terms of nuclear pellet DNA and as a percentage of total cell binding.

Statistical Techniques. Comparisons between group means were carried out by using the Mann-Whitney nonparametric test.

Testosterone Assay. Radioimmunoassay of serum testosterone was carried out in the Department of Clinical Biochemistry, Sunnybrook Medical Centre, by courtesy of Dr. A. Malkin. The lower limit of sensitivity of the assay as carried out for these animals was 1 ng/dl.

RESULTS

Protein and DNA Yields. Mean cytosol protein yields from control H, HI, G, and AT line tumors were 29.0 ± 5.4 (SD), 29.0 ± 3.8, 32.7 ± 3.3, and 30.1 ± 2.2 mg/g tumor, respectively. None of these differences was statistically significant. After DES treatment, there was a significant increase in mean cytosol protein yield in the H line to 38.3 ± 3.7 mg/g (P < 0.001 compared with the control value), while in the G line, there was a marginal decrease to 26.9 ± 4.5 mg/g (P < 0.05 versus control). There was no significant change in the HI and AT lines. DNA yields in control tumors were slightly lower in the HI than in the H line (10.8 ± 1.2 versus 12.0 ± 0.3 mg/g; P < 0.05) and considerably lower in the G and AT lines (6.6 ± 0.5 and 7.8 ± 0.5 mg/g, respectively) in spite of the reported hyperdiploidy, suggesting that these lines have a higher proportion of tissue constituents other than DNA than the H and HI lines. DES treatment decreased mean DNA yield significantly in the H, HI, and G lines to 10.9 ± 0.4, 8.4 ± 0.3, and 5.3 ± 0.5 mg/g, respectively (P < 0.005 versus control values). In the AT line there was a slight increase to 8.6 ± 0.3 mg/g (P < 0.025 versus control value). The net consequence of these changes after DES treatment was a significant increase in the mean cytosol protein:DNA ratios in the H and HI lines from 2.6 ± 0.2 and 2.7 ± 0.3 mg/g in control tumors to 3.5 ± 0.5 and 3.2 ± 0.2 mg/g in treated tumors (P < 0.01 versus control values). There were no significant differences in the cytosol protein:DNA ratios between control and treated G and AT line tumors. These ratios were, however, significantly higher than in the H and HI lines in control and treated G line tumors (5.0 ± 0.9 and 5.1 ± 0.7) and in control AT line tumors (3.9 ± 0.4) (P < 0.001).

Hormone Receptor Profiles in Control and DES-treated Tumors

Table 1 summarizes the data on the concentration and distribution between cytosolic and nuclear fractions for all three receptors. These are expressed in terms of nuclear pellet DNA in order to demonstrate changes in receptor distribution with treatment. Further details, including expression of cytosolic receptor in terms of cytosol protein and information regarding binding affinity, are as follows.

Estrogen Receptor

No ER was detected in the AT tumors, and in the control H and G line tumors concentrations of ER were low, cytosolic values ranging from 4 to 21 fmol/mg protein. In the control HI tumors, cytosolic values were significantly higher (17–51 fmol/mg protein); this was associated with a significantly higher mean cytosol protein:DNA ratios between control and treated G and AT line tumors. These ratios were, however, significantly higher than in the H and HI lines in control and treated G line tumors (5.0 ± 0.9 and 5.1 ± 0.7) and in control AT line tumors (3.9 ± 0.4) (P < 0.001).
in the estrogen-treated H line tumors over the untreated tumors. An increase of mean total ER was also observed in the G line tumors, but this was not statistically significant owing to the high variability of the values observed. No increase of total ER was found in the estrogen-treated HI tumors.

A marginally higher $K_d$ was observed for cytosolic ER in treated as compared with control H line tumors (1.0 ± 0.5 versus 0.5 ± 0.1 nM, $P = 0.05$). However, DES treatment did not affect binding affinity for estradiol in either the cytosol of the other lines or in the nuclear fraction of any line. No significant differences in nuclear $K_d$ values were observed between tumor lines, except that the nuclear binding in the G line showed consistently higher $K_d$s after DES treatment than in the H and HI lines (2.1 ± 0.3 nM versus 0.5 ± 0.2 nM and 0.9 ± 0.5 nM; $P < 0.001$, $<0.005$, respectively).

**Progesterone Receptor**

Progesterone receptor was detected in the cytosolic fraction of only one control and one DES-treated AT line at concentrations of 30 and 29 fmol/mg protein, respectively, and in the high-salt nuclear fraction of 3 control tumors at concentrations of 21–26 fmol/mg DNA. Thus the PgR content of AT line tumors is negligible. High concentrations of cytosolic PgR were observed in all tumors of the other 3 lines. The mean concentration in control H line tumors was significantly higher than in HI tumors (193 ± 33 versus 107 ± 29 fmol/mg protein, respectively, $P < 0.01$), and that in G line tumors was significantly higher than in any of the other lines (251 ± 46 fmol/mg protein, $P < 0.025$). The $K_d$ for cytosolic binding in the control H line tumors was somewhat higher than that in the HI and G lines (1.8 ± 0.9 versus 0.9 ± 0.3 and 0.9 ± 0.2, respectively; $P < 0.05$). Progestosterone receptor was absent from the nuclear fraction of control G line tumors, and nuclear PgR was observed in only three H line tumors and one HI line tumor, all at concentrations of <40 fmol/mg DNA. Treatment with DES resulted in increases in mean cytosolic PgR concentrations in the H and HI lines to 9- and 11-fold the control values, respectively. No significant change was observed in the G and AT lines. No consistent changes in cytosolic $K_d$ values were observed after DES treatment; in the H line, the mean $K_d$ was slightly higher after treatment (2.5 ± 1.4 versus 1.8 ± 0.9 nM in control tumors, $P < 0.05$), while in the G line it was slightly lower (0.6 ± 0.2 versus 0.9 ± 0.2 nM in control tumors, $P < 0.025$), and in the HI line no significant difference was observed (1.2 ± 0.5 versus 0.9 ± 0.3 nM in control tumors).

Nuclear PgR was detected in only one treated G line tumor (117 fmol/mg DNA) but was observed in 3 of 4 treated HI line tumors and in all 5 treated H line tumors at concentrations >300 fmol/mg DNA. However, the mean nuclear concentrations were <10% of the total tissue PgR in all groups.

**Androgen Receptor**

In the control H line tumors, the mean cytosolic AR was 105 ± 32 fmol/mg protein, and the mean total AR was 337 ± 86 fmol/mg DNA, not significantly different from the corresponding HI line values (136 ± 24 fmol/mg protein and 364 ± 51 fmol/mg DNA, respectively). Mean cytosolic AR in the G line, when expressed in terms of protein (111 ± 20 fmol/mg), was not significantly different from that in the H line but, when expressed in terms of DNA, both mean total and mean cytosolic AR were significantly higher than in the other lines (total AR, 609 ± 118 fmol/mg DNA, $P < 0.005$ compared with the H line). The control AT line tumors, on the other hand, had significantly lower mean cytosolic and total AR concentrations than the other lines however these were expressed (cytosolic, 42 ± 10 fmol/mg protein, $P < 0.001$; total, 220 ± 26 fmol/mg DNA, $P < 0.025$ compared with the H line). In the control H and AT lines approximately 25% of the total AR was detected in the high-salt nuclear fraction, while in the G line only 0–15% was nuclear; however, in terms of absolute concentration, there were no significant differences in mean nuclear AR concentration among the H, G, and AT lines. No nuclear AR was detected in the HI line, which was carried in castrated animals.

In the other 3 lines, DES treatment reduced the nuclear AR concentrations to <25 fmol/mg DNA. In the H and HI lines, significant increases in mean total AR after DES treatment were observed ($P < 0.001$ and 0.05, respectively), while no significant changes in total AR were detected in the G and AT lines. No significant differences in mean cytosolic or nuclear $K_d$ values between control and treated groups were observed in any of the tumor lines, and there were no significant differences in mean $K_d$ values between the H line and any of the other lines, apart from a marginally lower mean cytosolic $K_d$ in the treated HI tumors ($K_d$ 0.4 ± 0.3 versus 0.8 ± 0.5 nM in the H line, $P < 0.05$).

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**Table 1** Concentration of cytosolic and nuclear receptors in four R3327 sublines from vehicle- and DES-treated animals

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Characterization of AR from the AT-2 Tumor

Sedimentation Analysis in the Presence and Absence of Sodium Molybdate. The results of this analysis are presented in Fig. 1. Cytosol prepared and centrifuged in the absence of molybdate showed a peak of high-affinity binding for [3H]R1881 in the presence of TA at 7S when KCl was omitted from the gradient, and at 4.6S when 0.4 M KCl was included (Fig. 1, A and B). When 10 mM sodium molybdate was included in the homogenization buffer or added to the crude SN, the high affinity binding peak was shifted to a sedimentation rate slightly greater than 7S, and binding activity was somewhat higher than in the absence of molybdate (Fig. 1, A and C). These two gradients were derived from cytosols from the same crude SN, and 10 mM molybdate increased the high-affinity binding detected from 23 to 30 fmol/mg protein. When cytosol was prepared in the presence of 10 mM molybdate and centrifuged in the presence of both molybdate and KCl, a high-affinity binding peak at 4.6S was observed identical to that found in the absence of molybdate. When the concentration of molybdate in the cytosol preparation and in the gradient was progressively increased, a progressive shift of the high-affinity binding peak toward 7S was observed in the presence of KCl (Fig. 1D). At 50 mM molybdate, the peak was virtually identical to that observed in the absence of KCl. Parallel experiments using cytosol from the H line tumor gave identical results, except that the binding activity was approximately 3-fold higher (data not shown). Thus the cytosol from both lines was stabilized to a minor extent by 10 mM molybdate at 0°C, KCl produced the expected shift in sedimentation constant, and molybdate at concentrations above 20 mM prevented this shift.

Steroid Specificity. The comparison of [3H]DHT and [3H]-R1881 as ligands for AR in the AT line showed that both the $B_{max}$ and $K_d$ were similar for both ligands. For cytosol AR, $B_{max}$ and $K_d$ for [3H]DHT were 47 fmol/mg protein, 3.4 nM, and for [3H]R1881, 43 fmol/mg protein, 1.5 nM. For nuclear AR, values were 113 fmol/mg DNA, 2.0 nM, and 82 fmol/mg DNA, 1.1 nM for each ligand, respectively.

The relative binding affinities of steroids used as competitors for [3H]DHT binding had the same ranking in cytosols from both the H and AT lines (Table 2). However, the progestins used appeared to have considerably greater competitive activity in the AT than in the H line cytosol. When progesterone and R5020 were used at 100-fold the concentration of [3H]DHT, both reduced [3H]DHT high-affinity binding in the H line cytosol by 39% and in the AT line cytosol by 53 and 62%, respectively.

Stability and Dissociation of AR. Under the conditions used, cytosolic AR from both the H and AT lines showed high stability after incubation with either [3H]DHT or [3H]R1881. After a postincubation time of 24 h at 15°C, 80–90% of the original radioactivity remained bound to receptor. In the presence of excess radioinert androgen, dissociation of the AR complex in the AT line cytosol occurred more rapidly than in the H line cytosol, when AR was bound to [3H]R1881. Dissociation was biphasic, consisting of fast and slow components (Fig. 2). With R1881, dissociation of both components was more rapid in the AT than in the H line.

Promotion of Association of AR with the High-Salt Nuclear Fraction in Vivo by Testosterone Stimulation. The results presented in Table 3 show clearly that testosterone stimulation of castrated rats bearing AT line tumors was able to promote association of cytosolic AR with nuclear components. In the unstimulated animals a mean of only 4% of the total tissue AR was found in the high-salt nuclear fraction, while stimulation with 10 µg testosterone increased this proportion to 30%, a fraction close to that we observed in the intact AT and H line tumors in Fig. 1.

DISCUSSION

Interpretation of the results concerning the hormone receptor profiles in control and DES-treated animals is complicated...
somewhat by the fact that a significant degree of aneuploidy at 15°C was calculated. H line cytosol; O, AT line cytosol. Due to the hyperploidy of these two lines, individual cells of the G line have been reported in the G and AT line tumors (13, 15). Due to the hyperploidy of these two lines, individual cells of the G and AT lines contain more DNA than those of the H and HI lines, and each cell in the former lines must contain more receptor sites than H and HI line cells for the same concentration of bound activity expressed in terms of DNA. A further complication is the difference in protein and/or DNA yields between control and treated tumors of all four lines. The increase in cytosol protein:DNA ratio in the H and HI lines after DES treatment suggests that treatment may have stimulated synthesis of soluble protein and/or depressed DNA synthesis. The latter would be expected only in androgen-sensitive tumors. In the G and AT lines, cytosol protein:DNA ratios were higher than in the control or treated H and HI lines and were not changed by DES treatment. Because of this, cytosol receptor concentrations expressed in terms of DNA appear higher in the G and AT lines relative to that in the well-differentiated lines than if they were expressed in terms of cytosol protein. Both cytosol and nuclear receptor data have been expressed in terms of DNA in Table 1 to permit examination of the cellular distribution of receptor. The conclusions drawn from these data do not change when they are examined in the light of the caveats expressed above.

The most striking effect of DES treatment was the marked increase of PgR concentration in the two well-differentiated lines. This confirms our previous findings for the H line (2, 3). In the poorly-differentiated/anaplastic lines, PgR concentration was negligible (AT) or was initially high and not increased by treatment (G line). Concomitant with the increase in PgR, although not as marked, was an increase in total AR in the H and HI lines. Increases in the AR content after estrogen treatment have also been reported in the rat and dog prostates and in the chick oviduct (31–34). DES treatment also resulted in significant increase of total ER in the H line. Thus in these tumors, the ability of DES to induce PgR and AR synthesis was associated with the presence of stroma and/or good differentiation, irrespective of androgen sensitivity, while increase of ER was found only in the H line, which is well differentiated and androgen sensitive. In the G line, PgR concentrations appear to be constitutively high. It appears then that the use of a cytotoxic agent designed to use PgR (possibly induced by estrogen treatment) as a target may be a treatment modality worthy of consideration for prostatic carcinomas, whether or not they are androgen sensitive. Such an agent might be particularly valuable for tumors which have relapsed from other hormonal therapies. At present there is a serious lack of effective therapy for androgen-insensitive prostatic carcinoma.

The lack of correlation between total AR content and androgen sensitivity in these 4 tumor lines is consistent with the results of Diamond and Barrack (9), although these workers did find an association between androgen sensitivity and salt-resistant nuclear AR content. Due to the inclusion of DTT in our homogenization buffer, it is likely that the majority of the nuclear AR in the tumors used here would have been extracted with the 0.4 M KCl buffer (35).

The presence of AR, PgR, and ER receptor in the G line tumors, which lack significant stroma, indicates that prostatic lines have a relatively high concentration of these receptors. This suggests that the tumors may respond to estrogen treatment, which could be effective for tumors with high AR content. However, the lack of correlation between total AR content and androgen sensitivity in these 4 tumor lines highlights the complexity of androgen signaling in prostatic carcinomas. Further studies are needed to better understand the mechanisms underlying these findings and to develop effective therapeutic strategies for androgen-insensitive prostatic carcinoma.
epithelial cells may contain all 3 receptors, and the lack of response to DES treatment suggests that a controlling mechanism, possibly mediated by stroma, was absent.

The differences in mean $K_a$ values observed between tumor lines, or between control and treated tumors, were generally minor, and any biological significance is doubtful.

Our findings with regard to the presence of $PgR$ in untreated H line tumors, and of AR in the AT-2 line do not agree with all previous reports on this topic (see “Introduction”). Receptor assay methodology has improved in recent years, resulting in greater conservation of receptor, and differences in methodology may account for discrepancies with some of the earlier reports. However, we cannot account for the difference in results regarding AR in the AT-2 line from those of Diamond and Barrack (9). We found AR concentrations in this line to be significantly lower than in the others, but it was detected in all nine tumors examined. Qualitatively, the biphasic dissociation curves observed using both $[H]DHT$ and $[H]R1881$ as ligands, and the effects of molybdate and KC1 on the SDG profile were similar to those described by Traish et al. (36) for AR from normal rat ventral prostate. However, the AT-2 AR did appear to be different from H line AR in that it had a higher affinity for progesterins and a faster dissociation rate at $15^\circ$C from $[H]R1881$ than H line AR. The difference in dissociation rates for $[H]R1881$ and $[H]DHT$ at $15^\circ$C was also considerably greater in the AT line than in the H line, although Scatchard analysis at $0^\circ$C did not reveal this. The biological significance of these observations is uncertain, inasmuch as exogenous testosterone administered to castrated animals bearing AT line tumors was able to promote association of the receptor with the nuclear fraction to an extent similar to that observed in intact animals bearing the H line tumor, in spite of the low $5\alpha$-reductase activity reported in the AT line (7). Possibly testosterone itself is effective in binding AR and promoting its association with the high-salt nuclear fraction, as has been suggested for the G line, which also has low $5\alpha$-reductase activity (8). Thus it seems likely that the main causes of androgen insensitivity in the AT tumors are factors other than deficiencies in $5\alpha$-reductase activity or in receptor content or nuclear binding capacity.

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REFERENCES

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