Regulation of Estrogen and Progestin Receptor Concentrations in an Experimental Rat Prostatic Carcinoma by Estrogen, Antiestrogen, and Progesterone

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ABSTRACT

In order to assess prostatic tissue as a target for receptor-mediated estrogen action, we have examined the regulation of estrogen (ER) and progestin receptors (PgR) by estrogen, antiestrogen, and progesterone in prostatic adenocarcinoma of the rat. Twenty μg diethylstilbestrol (DES) with or without 800 μg tamoxifen (Tam) were injected s.c. in oil 5 times weekly for 2 weeks. Controls were given oil only. Estrogen receptor assays were carried out using [3H]estradiol and a hydroxylapatite exchange method. Progestin receptors were assayed using [3H]R5020 and dextran-coated charcoal to separate free and bound steroid. All binding data were evaluated by using Scatchard analysis.

Treatment with DES depleted cytosolic ER, promoted association of ER with the nuclear fraction, and concomitantly increased PgR concentrations in amounts proportional to nuclear ER. Treatment with Tam alone resulted in higher nuclear ER concentrations than treatment with DES, but induced only one-fifth the amount of PgR. Treatment with DES plus Tam resulted in similar nuclear ER concentrations as with Tam alone, but PgR concentrations were intermediate between those observed with DES alone and Tam alone. Thus Tam exhibited both estrogenic and antiestrogenic properties.

In a separate experiment, administration of progesterone with DES decreased the concentration of nuclear ER to less than one-half that observed after administration of DES alone, with proportional decreases in both cytosolic and nuclear PgR. All these observations indicate that the control of ER and PgR concentrations in this prostatic tumor is identical to that observed in female rat target organs, that monoclonal antibody to ER reacts differently with the TAM-bound ER complex than with the estradiol-bound ER complex.

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INTRODUCTION

Estrogens have been observed to have multiple effects on the prostate gland, especially when administered in pharmacological doses, as for the treatment of advanced prostatic carcinoma. Both direct effects, such as the inhibition of 5α-reductase activity (1), and indirect effects due to the suppression of testicular androgen synthesis secondary to the feedback inhibition of gonadotropin release, have been demonstrated. However, even though the prostate gland in several species contains ER (2–6), none of these effects is thought to be mediated by prostastic ER and no physiological role for this receptor has been determined. In analogy with female target organs for estrogen, the presence of functional ER should be indicated by the induction of PgR synthesis under estrogen stimulation.

In an investigation of the effects of estrogen treatment on the R3327H experimental carcinoma, we have recently been able to show that injection of diethylstilbestrol in amounts equivalent to a pharmacological dose in men resulted in an increase in nucleus-associated ER, with a concomitant proportional increase in cellular PgR content (7). We have continued to investigate the control of both ER and PgR in this tumor by examining the effects of treatment with the antiestrogen Tam, alone and in combination with DES, and of treatment with progesterone alone and with DES. The results demonstrate conclusively that this prostate tumor has the characteristics of an estrogen target organ with regard to the control of PgR content. In addition, we have compared the results of a radioligand assay for ER in cytosolic and nuclear fractions of the tumor with those of an enzyme immunoassay using monoclonal antibodies to ER, and have also used an immunohistochemical method for the localization of ER in sections of control and DES-treated R3327H tumors.

MATERIALS AND METHODS

Animals and Treatment. Male Copenhagen × Fischer F1 rats bearing bilateral s.c. implants of the R3327H tumor were obtained from the Panacoloucarc Cancer Research Institute at Miami, Inc. When the tumors reached a mean diameter of approximately 2.0 cm, animals were assigned in turn to one of 3 treatment groups or a control group.

At the initiation of the treatment, the mean body weight of all animals was 368 ± 35 (SD) g. The treated groups were given injections of 20 μg DES alone, 800 μg Tam alone, or both DES and Tam at separate sites. Injections were given s.c. in 0.5 ml sesame oil, 5 times weekly for 2 weeks. The control group was given injections of oil only. The animals were killed 4–6 h after the last injection by drawing blood from the aorta under anesthetic. Serum was separated and frozen for testosterone assay. The tumors were dissected out and rinsed in ice-cold homogenization buffer. Subsequent handling of the tumors was carried out on ice. The capsule was removed and any necrotic portions were discarded. The remaining tumor tissue from each rat was pooled. Representative slices were fixed for histological examination, or frozen at −70°C for ER enzyme immunoassays and ER immunohistochemical localization. The remaining tissue was snap-frozen and stored in liquid nitrogen for not more than 3 months before assay.

A small batch of tumors from a different transplant generation was used to investigate the effects of progesterone treatment on the ER and PgR content and distribution in DES-treated tumors. Animals with tumors approximately 2 cm in diameter were given injections s.c. of 20 μg DES with or without a separate injection of 4 mg progesterone, both in 0.5 ml oil, 5 times weekly for 2 weeks. Tumors were harvested 4–6 h after the last injection and frozen for ER and PgR radioligand assays

1Supported by grants from the National Cancer Institute of Canada (B. G. M., I. E. J.), and from the NIH, Grants CA 14599 and HD 15513 (E. R. D.).

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3The abbreviations used are: ER, estrogen receptor; PgR, progesterone receptor; Tam, tamoxifen; DES, diethylstilbestrol; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; DCC, dextran-coated charcoal; HAP, hydroxylapatite; SN, supernatant; EIA, enzyme immunoassay; PBS, phosphate-buffered saline.
as described above. Serum testosterone assays were not carried out for these animals.

Isotopes and Chemicals. [3H]Promegestone (RS020; specific activity, 70–87 Ci/mmol; [2,4,6,7-3H]estradiol (specific activity, 90–115 Ci/mmol), radioiodinert RS020, PPO, and POPOP were obtained from New England Nuclear. On arrival, the labeled steroids were diluted to 50 μCi/ml in redistilled benzene:ethanol (9:1, v/v) and stored at −4°C for not more than 3 months. Other unlabeled steroids, DES, DTT, PMSF, protein bovine serum albumin, and DNA (Type III, from salmon testes), for use as standards, and Tween 80 were obtained from Sigma Chemical Co. (St. Louis, MO); dextran T 70 was from Pharmacia (Montreal); charcoal (Norit-A) was from BDH, Cincinnati, OH; DNA-grade HAP was from Bio-Rad laboratories (Richmond, CA). The scintillators used were PCS (Amersham), or PPO (5 g) and POPP (100 mg) per liter of toluene. All other chemicals were of the highest purity available. Enzyme immunoassay kits were obtained from Abbott Laboratories, Chicago, IL.

Buffers and Solutions. Buffer A: 5 mM sodium phosphate buffer, pH 7.4, containing 1 mM DTT, 0.1 mM PMSF; Buffer B: Buffer A containing 40% glycerol (v/v), pH 7.4; Buffer C: Buffer A containing 10% glycerol (v/v), pH 7.4; Buffer D: 10 mM Tris, 0.6 mM KCl, 1 mM DTT, 0.1 mM PMSF containing 10% glycerol, pH 8.5. DCC pellet: 1% charcoal, 0.05% dextran T 70 for 10 min at 3000 × g for 10 min; DCC 20G: 0.33% charcoal, 0.03% dextran T 70, in Buffer A containing 20% glycerol (v/v); HAP slurry prepared in 5 mM sodium phosphate (pH 7.4), ratio HAP: buffer, 0.7. Tris-Tween: 10 mM Tris, 1% Tween 80, pH 7.4.

Homogenization and Preparation of Cytosol and Nuclear Extracts. All tissue handling and assay procedures were carried out at 0–4°C with precooled equipment, glassware, and buffer solutions. Frozen tissue was pulverized in an Auto-pulverizer (Redi Industries Corp., Hempstead, NY), cooled with liquid nitrogen, and then homogenized in 10 volumes of Buffer A using a Polytron P-10 homogenizer (Brinkman Instruments, Inc.) for 2 bursts of 10 s (setting 3.5) with a 30-s cooling interval. The homogenate was centrifuged at 3500 × g for 10 min to yield a crude nuclear pellet and a crude SN. The nuclear pellet was washed three times with Buffer C, and then extracted for 1 h with 5 volumes of Buffer D, vortexing every 10 min. Since DTT was used in the homogenization and extraction buffers, most of the otherwise "salt-resistant" nuclear receptor is likely to have been extracted by this procedure (8). The pellet was precipitated by centrifugation at 12,000 × g for 10 min, and the SN from this spin was combined with that from a further washing for 10 min with 5 volumes of Buffer A. The pellet was frozen for DNA assay by the diphenylamine method (9). To eliminate possible interference by DES and/or Tam in the receptor assays, both the nuclear extract and the crude SN from the homogenate of each tumor were stripped by vortexing with DCC pellets and standing for 10 min. The tubes were centrifuged for 2 min at 2300 rpm and the SNs were removed by aspiration. The HAP pellets were incubated after vortexing with 200 μl Buffer A containing [3H]estradiol at five concentrations in the range of 1–10 nM overnight at 0–4°C, followed by 3 h at 30°C (cytosol) or 5 h at 30°C (nuclear extract). Parallel incubations were carried out in the presence of 500-fold DES, and all aliquots contained radioinert dihydrotestosterone at concentrations 10-fold that of [3H]estradiol. After incubation, unbound steroid was removed from the HAP suspension by centrifugation and 4 washes with 1.5 ml Tris-Tween 80. Bound [3H]estradiol was extracted from the pellets with two ethanol washes at 30°C (total, 2.5 ml, 30 min). Ten-ml toluene scintillator was added to the combined ethanols extracts and counted for 10 min or to 2% error.

Progestosterone Receptor Assays. Duplicate 200-μl aliquots of stripped cytosol or nuclear extract were incubated overnight with 50 μl [3H]-RS020 prepared in Buffer B, at five final concentrations in the range of 1–10 nM. Parallel incubations were carried out in the presence of 100-fold radioinert RS020 to allow correction for low affinity binding, and all aliquots contained cortisol and dihydrotestosterone at concentrations 100- and 10-fold that of [3H]RS020, respectively. After incubation, DCC 20G (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 aliquots of the SN were counted in PCS for 10 min or to 2% error.

The data from all receptor assays were analyzed according to Scatchard (12), using the program of Schwarz with a Hewlett-Packard HP-97 calculator (13). Cytosol-binding capacity was expressed as fmol/mg cytosol protein, and both cytosol and nuclear binding were expressed as fmol/mg DNA in the nuclear pellet. Preliminary experiments established that no significant loss of DNA occurred during pellet preparation from the homogenate, so that homogenate DNA and pellet DNA were virtually equivalent. ER Enzyme Immunoassays. Cytosolic and nuclear ER-EIA were carried out on representative portions of the same tumors used in the investigation of the effects of DES and Tam treatments alone and in combination with H2O2. Cytosol and nuclear ER were measured by the techniques as described for the ER radioligand assays and used for ER-EIA as described in the instructions included in the Abbott ER-EIA monoclonal kit. Duplicate 100-μl aliquots of cytosol or nuclear extract were mixed with 100 μl of diluent and incubated with polystyrene beads coated with anti-ER antibody (rat monoclonal, D547) at 4°C for 18 h to bind ER to the solid phase. The beads were then washed with distilled H2O to remove unbound materials, then incubated at 37°C for 1 h with 200 μl of a second ER antibody preparation (rat monoclonal, H222) conjugated with horseradish peroxidase which binds to ER on the beads. The beads were washed with distilled H2O to remove unbound conjugate, then incubated for 30 min at room temperature with 300 μl of enzyme substrate solution (H2O2 and o-phenylenediamine•2HCl) to develop a color proportional to the amount of ER conjugate present. The reaction was stopped by the addition of 1 ml of 1 N H2SO4 and the absorbance at 492 nm was read on a Quantum II spectrophotometer supplied by Abbott.

As the ER-EIA was developed for use with cytosols, the conditions for the nuclear ER-EIA were established in this laboratory. It was found that use of the buffers used for the radioligand assay instead of the buffer supplied with the kit for reconstitution of the standard lyophilized cytosol did not affect the ER values obtained. Using nuclear pellets derived from rat uterus and extracted with Buffer D as described above, a linear correlation was established between the ER values and nuclear protein concentrations ranging from 0.15-3.0 mg protein/ml nuclear extract. Nuclear ER-EIA on the tumors were therefore carried out on nuclear pellets from approximately 0.5 g tumor, extracted with a total of 5 ml Buffer D, typically yielding approximately 0.9 mg protein/ml extract, and approximately 11 mg DNA/g tumor. The results from both cytosol and nuclear assays were expressed in terms of protein in the extracts and in terms of DNA in the nuclear pellets.

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

Statistical Methods. Since the number of tumors in each experimental group was small, between-group comparisons were made using the Mann-Whitney nonparametric test.

ER Immunocytochemical Localization. This was carried out on control and DES-treated tumors, by a technique in which tissue sections are incubated with a biotinylated monoclonal antibody (H222) to ER, followed by incubation with an avidin-biotin-peroxidase complex (14). This avoids use of a bridging antibody directed against rat immunoglobulins, which would give rise to nonspecific staining in rat tissue. Representative portions of the tumors were shipped from Toronto to the Ben May Laboratory on dry ice. Still in the frozen state, the specimens were attached to cryostat chucks with OCT compound. Sections 8-μm thick were cut at −20°C, thaw mounted on poly-L-lysine-
coated microscope slides and immediately fixed for approximately 2 min in Zamboni's fixative (picric acid-parafomaldehyde) (15). The sections were washed for 20 min with PBS, incubated with hydrogen peroxide in PBS to quench any endogenous peroxidase activity and, following further PBS washes, were blocked by two 30-min incubations with 10% goat serum in PBS with intermittent and subsequent PBS washes. Biotinylated H222 (prepared by reaction of H222 with biotin succinimide) was added at 3 or 10 μg/ml PBS and incubated with the sections overnight, washed three times for 5 min with PBS and stained for 2 h with Vectastain (avidin plus biotinylated peroxidase complex). As a negative control some sections were incubated with 10 μg rat biotinylated IgG/ml instead of biotinylated antibody.

After three more 5-min PBS washes the sections were incubated with diaminobenzidine and hydrogen peroxide for 20 min to develop the peroxidase color, washed with PBS, lightly counterstained with hematoxylin, washed in water, dehydrated, and mounted.

RESULTS

Serum Testosterone Levels. As we have previously observed in these F3 hybrids, serum testosterone levels in the control animals were very variable [mean, 215 ± 110 (SD) ng/dl]. A similar range of values has been observed by Grossman et al. (16) in pure-bred Copenhagen rats. Testosterone levels were drastically reduced by DES treatment alone or in combination with Tam (7 ± 1 and 7 ± 2 ng/dl, respectively). Tam administered alone also depressed the levels very significantly (49 ± 13 ng/dl).

Effects of DES and Tamoxifen Treatment on ER and PgR Content and Distribution. The effects of treatment on receptor content and distribution between cytosolic and nuclear fractions are summarized in Table 1. With regard to ER, linear Scatchard plots were obtained from cytosols of all control tumors except one, which yielded a curved plot, resolvable into high and low affinity components by the method of Rosenthal (17). High affinity binding in the nuclear fraction was detected at very low concentrations in only 3 of the 6 control tumors. Cytosolic ER was totally depleted in all the treated tumors, but linear Scatchard plots were obtained from all nuclear fractions. In the DES-treated group, the nuclear (total) concentration of ER was not significantly different from the total (cytosol plus nuclear) in the control group. However, in both Tam- and DES- plus Tam-treated tumors, both the ER content and the Ka's were significantly higher than in the control and DES-treated tumors. Thus Tam, whether administered alone or with DES, appeared to result in an accumulation of nuclear-associated ER, although the tightness of this association was somewhat less than that in tumors treated with DES alone.

Curved Scatchard plots were derived from all PgR data from the control tumor cytosols. These could be resolved into high and lower affinity components. The lower affinity component was present at approximately 3.5 times that of the high affinity one, with a Ka of approximately 7 nM. Its significance is not clear. Data from all the treated groups yielded linear Scatchard plots with markedly increased Bmax values compared with the control tumors. Tam treatment alone resulted in a smaller increase than DES alone, while the combined treatment resulted in an intermediate increase. Thus the increase in nuclear ER observed after Tam treatment did not result in an increase in PgR proportionate to that resulting from DES treatment. Nucleus-associated PgR was detectable at very low concentrations by Scatchard analysis in only 3 of the 6 control tumors, but in all the treated tumors linear Scatchard plots were obtained for nuclear PgR at concentrations 4-13% of that in the corresponding cytosols when expressed in terms of DNA equivalents. The differences between Bmax values for both cytosol and nuclear PgR in the different treatment groups were all highly significant.

It was observed that the Ka's for cytosolic PgR in the DES-treated group were higher than for the other groups; the significance of this is not clear. The Ka's for nuclear PgR in the vehicle-treated group were higher than in the experimental groups; this was probably due to the extremely low concentrations resulting in bound:free ratios of <0.01. Since nuclear ER and PgR concentrations were undetectable in three of the vehicle-treated tumors and negligible in the other three, these Ka's are not felt to be very meaningful.

Effect of Progesterone on ER and PgR Content in DES-treated Tumors. The results of this experiment are presented in Table 2. As already shown, cytosolic ER was totally depleted by DES treatment, and this was not changed when progesterone was administered with DES. However, the mean nuclear ER concentration was reduced by more than 50% by the addition of progesterone to the DES treatment, and this was accompanied by proportional decreases in both cytosol and nuclear PgR. The binding affinity of ER and PgR for their respective ligand was not altered by the addition of progesterone to the DES treatment.

Comparison of ER Values Obtained by Radioligand and Enzyme Immunoassay in DES- and Tam-treated Tumors. The results of this experiment are shown in Table 3. No significant differences were observed between cytosol or nuclear ER concentrations obtained by the HAP and EIA methods in the control or DES-treated tumors. However, in both Tam-treated groups, in which cytosolic ER was undetectable by the HAP method, immunoreactive ER was detected in the cytosol. The concentration in the Tam-treated group was significantly higher.
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Table 2 Down regulation of ER and PgR in R3327H tumors after DES treatment with progesterone

<table>
<thead>
<tr>
<th>Treatment (no. of animals)</th>
<th>ER</th>
<th>PgR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cytosol (fmol/mg DNA equivalent)</td>
<td>Nuclear (fmol/mg DNA)</td>
</tr>
<tr>
<td>DES (3)</td>
<td>0</td>
<td>47*</td>
</tr>
<tr>
<td>DES + progesterone (3)</td>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>

* Data are presented as means with ranges in parentheses.

Table 3 Concentration of ER assayed by HAP exchange and enzyme immunoassay methods after treatment with DES and tamoxifen alone and combined

<table>
<thead>
<tr>
<th>Treatment (no. of animals)</th>
<th>Cytosolic ER (fmol/mg cytosol protein)</th>
<th>Nuclear ER (fmol/mg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HAP exchange</td>
<td>EIA</td>
</tr>
<tr>
<td>Vehicle (6)</td>
<td>20 ± 8*</td>
<td>29 ± 19</td>
</tr>
<tr>
<td>DES (5)</td>
<td>&lt;2 (0)</td>
<td>65 ± 8</td>
</tr>
<tr>
<td>Tam (5)</td>
<td>23 ± 9*</td>
<td>109 ± 26</td>
</tr>
<tr>
<td>DES + Tam (6)</td>
<td>11 ± 5*</td>
<td>109 ± 22</td>
</tr>
</tbody>
</table>

* Data are presented as mean ± SD.
* Means value from 3 tumors only. No high affinity binding was detected in the other vehicle-treated tumors.
* Means for EIA assay significantly higher than that for HAP assays, P < 0.001.
* Means for EIA assay significantly higher than that for HAP assays, P < 0.005.

DISCUSSION

These results demonstrate conclusively that estrogen is able to bring about effects characteristic of estrogen target tissues in a prostatic tumor. The control of ER and PgR concentration and cellular distribution in the R3327H tumor is similar to that in female rat target organs such as the uterus and dimethylbenz(a)anthracene-induced tumor. As we have previously observed, DES treatment of the R3327H tumor promotes depletion of cytosolic ER, with a concomitant increase of nuclear-associated ER, and an increase in PgR concentration (7). In the present group of tumors, the increases in PgR concentration were even more marked than in the batch of tumors used in the previous study.

In the female, the effects of Tam are complex and depend on the species and tissue under investigation (18). In the uterus of the immature or ovariectomized rat (i.e., in the absence of estrogen) Tam has been found to be a partial estrogen agonist, increasing PgR concentration (19). If administered with estrogen, on the other hand, Tam antagonizes the effects of the latter (18). Tam has been observed to deplete cytosolic ER and concomitantly increase nuclear-associated ER in the dimethylbenz(a)anthracene-induced tumor as we have observed in the R3327H tumor (20). The significantly higher concentration of ER as measured by the HAP assay in the Tam-treated groups as compared with the DES only-treated group may be due to prolonged retention of ER in the nuclear fraction, as has been observed in female target organs (18). It has also been observed that Tam-occupied ER is completely extractable by salt (i.e., there is no salt-resistant form), whereas estradiol-occupied ER is partially salt resistant (18). Under certain conditions therefore, the nuclear extraction buffer may extract Tam-occupied ER more efficiently than estradiol-occupied ER. Although tissue homogenization in our experiments was carried out in buffer containing DTT, which renders the majority of the salt-resistant ER extractable (8), it is possible that the "excess" of Tam-induced over DES-induced nucleus-associated ER is partly due to differential extractability after the two treatments.

An important difference between this tumor model and female target organs is that estrogen has not been observed to stimulate growth of the R3327H tumor in spite of the increase in nuclear ER concentration; this is consistent with observations which suggest that the growth-stimulating effect and the PgR induction effect of estrogen can be expressed independently (21–24). Long-term treatment with Tam has been shown to reduce the growth rate of the R3327H tumor (25); this is presumably due to the reduction in serum testosterone levels, as observed by Bartke et al. (26) and confirmed in this paper. In men, Tam treatment tends to increase circulating luteinizing hormone and testosterone levels, presumably by antagonizing feed-back inhibition by estrogen at the hypothalamic-pituitary axis (27, 28). In human prostatic tissue also, Tam appears to act as an antiestrogen, since PgR levels have been demonstrated to be significantly lower in benign hypertrophic tissue from patients treated with Tam than in untreated patients (29).

The effect of progesterone on the R3327H tumor when administered in the presence of estrogen is also consistent with that in female target organs; in both, high serum progesterone levels result in reduced levels of nuclear ER (30). At the same time, both cytosol and nuclear PgR levels in the tumors were reduced in comparison with tumors treated with DES alone. This effect may be secondary to the reduction in nuclear ER. The distribution of PgR between cytosolic and nuclear fractions

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was not substantially altered by the addition of progesterone to
the DES treatment.
It has been shown previously that the monoclonal antibodies
commercially available for enzyme immunoassay of ER in
human tissue cytosols also react with rat ER (31, 32). The
comparison of cytosol and nuclear ER values after DES and/
or Tam treatment obtained by the HAP and enzyme immunoassay is of particular interest, since it may have implications
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beyond the particular experimental tumor we have examined. In the control and DES-treated tumors, mean ER values obtained by EIA were slightly, but not significantly, higher than those obtained by the HAP assay. This indicates that both methods were measuring the same protein, and that the HAP assay did not seriously underestimate the ER concentration. However, in both Tam-treated groups, very significant differences were observed between the HAP and EIA data. The EIA detected ER in the cytosol, whereas no high affinity binding of \(^{1}H\)estradiol was observed with the HAP assay. Nuclear-associated concentrations of immunoreactive ER were significantly higher than high affinity \(^{1}H\)estradiol-binding concentrations, and the difference was more marked in tumors which had been treated with Tam alone than with Tam plus DES. Induction by Tam of an immunoreactive, but non-estradiol-binding ER-like protein has also been observed in rat uterine extracts (32). In addition, Eckert and Katzenellenbogen (33) have examined the properties of nuclear antiestrogen-receptor complex in the MCF-7 human breast cancer cell line, and have found that they are consistent with the association of the complex with an additional protein not associated with the ER complex. It is tempting to speculate that such a protein may be associated (a) with the appearance of immunoreactive (but non-hormone binding) ER-like antigen in the nucleus after Tam treatment, and (b) with a reduced capacity for PgR induction during Tam treatment as compared with estrogen treatment. In these R3327 tumors, the highest concentration of immunoreactive, but non-hormone binding ER was associated with the lowest concentration of PgR observed after any of the three treatments investigated.

The results of the localization of ER with the H222 antibody were of interest in view of the controversy concerning the putative site of estrogen action in the prostate. Investigation of the localization of estradiol and estradiol binding in fractionated accessory sex glands of experimental animals and in human benign hypertrophic prostatic tissue using biochemical methods (33–38), has indicated that the main site of estrogen action is probably the stroma. However, Eaton et al. (39) demonstrated that specific staining with the immunohistochemical method was virtually all nuclear, although slight cytoplasmic coloration was observed. This was also detectable in the control slides. Somewhat more cytoplasmic coloration is generally observed with the directly biotinylated antibody than with the conventional (Sternberger) sandwich technique.

The apparent reduction in ER staining in epithelial nuclei after DES treatment would at first analysis appear to be inconsistent with the apparent increase in nuclear ER found by both steroid binding and EIA (Table 3). However, a similar decrease in the intensity of nuclear staining was seen on treatment of MCF-7 cells with estradiol in vitro. It is possible that in the cell, the physical location of the “nuclear ER,” that is the ER complex tightly bound in the nucleus, makes it inaccessible to the antibody. It must be kept in mind that previous studies of breast cancer tissue (43) showed good correlation between the amount of “cytosolic” ER found by steroid-binding assay with the nuclear staining by ERICA. While it is known that antibody H222 has high affinity for the salt extractable nuclear receptor as well as for the low salt extractable cytosolic ER, the nature of the subcellular localization of the nuclear ER or its accessibility in the nucleus is unknown.

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