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Specific Binding of Estradiol and Dihydrotestosterone in Human Mammary Cancers[1]

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SUMMARY

Estrogen and dihydrotestosterone receptors were detected by agar gel electrophoresis in 224 human mammary tumor biopsies. Approximately half of the tumor specimens revealed measurable amounts of estradiol receptors, whereas only 20% contained dihydrotestosterone receptors. The average concentration of spare estrogen receptors in primary tumors was markedly higher in the postmenopausal than in the premenopausal group, but this was not found to be the case for androgen receptors. The findings presented are discussed with regard to the clinical relevance of multiple assays for various types of receptors in a single tumor.

INTRODUCTION

Recently, Jungblut *et al.* (5), Giannopoulos (3), and Heyns (4) described the simultaneous occurrence of E-R[2] and androgen receptors in uterine tissue of various animal species. Subsequently, the existence of binding proteins for these 2 steroids was reported for human mammary cancer in a preliminary study by Wagner *et al.* (11). At present, there is much evidence that E-R are involved in the hormonal regulation of growth of human breast cancer and that receptor content of tumor biopsies can be useful in predicting the hormone dependence of the tumor. In a recent evaluation of the current status of this field, McGuire *et al.* (9) showed a receptor-positive tumor response to endocrine therapy in approximately 55% of cases tested and receptor-negative carcinomas in about 7% of the cases.

This paper details our experience in measuring androgen receptors in human breast cancers. In addition, the question whether the additional determination of this parameter could help to increase the rate of correlation between receptor assay and clinical response of the tumor will be discussed.

MATERIALS AND METHODS

17β[6,7-^{3}H]-Estradiol (specific activity, 48 Ci/mmole) and 5α-[1,2-^{3}H]DHT (specific activity 49 Ci/mmole) were purchased from New England Nuclear, Boston, Mass. The purity (>94.0%) was checked by thin-layer chromatography. The antiestrogenic compound U. 11,100 (Nafoxidine) was a gift from the Upjohn Company, Kalamazoo, Mich. Cyproterone acetate was provided by Schering AG, Berlin, Germany. Agar purum, human serum albumin, and rabbit antiserum against human serum albumin were obtained from Behringwerke, Marburg/Lahn, Germany. All other chemicals were products of Merck, Darmstadt, Germany, or Boehringer Mannheim, Mannheim, Germany, and were of analytical grade.

Preparation of Extracts. Tumor tissue was dissected free of fat and normal tissue after excision. The tissue sample was then immersed in liquid nitrogen and either processed immediately or stored at −20° until assayed, but for no longer than 2 to 3 days. Frozen tissues were pulverized in a Micro-Dismembrator (B. Braun Apparatebau, Melsungen, West Germany), and the powder was extracted with Tris-HCl buffer (0.01 M Tris, pH 7.5; 1 mM NaN_3), 4 v/w. After being thawed, the sample was centrifuged at 2° and 40,000 rpm (157,000 × $g_{average}$) in a Beckman SW 56 rotor (Model L2-65 B; Beckman Instruments, Inc., Fullerton, Calif.) for 90 min. The supernatant was removed by pipetting and was used immediately. Aliquots of the extract were incubated overnight in glass tubes with 5α-[1,2-^{3}H]DHT or 17β-[6,7-^{3}H]estradiol. To exclude nonspecific binding, 1 part of the extract was heated at 48° for 60 min or incubated with a mixture of the labeled steroid and a 10^3-fold excess of the suitable competitor (Nafoxidine or cyproterone acetate, respectively). At the end of the incubation period, aliquots of each sample were subjected to agar gel electrophoresis. This was performed according to the method of Wagner and is described in detail elsewhere (10).

Gel layers were prepared with a 1% agar solution (0.05 M Michaelis buffer, pH 8.2). In the center line of the gel plate, wells were punched out, and 50-μl aliquots of the labeled tissue extracts were applied. The prepared gel plates were then placed on a Teflon-coated, cooled (1°) brass plate within an airtight electrophoresis chamber. Electrophoresis was carried out for 90 min at 110 ma/300 V. The gel was then divided lengthwise and cut into sections 3-mm wide. Radioactivity was eluted from the strips with scintillation fluid according to the method of Hayes (7.0 g PPO, 0.3 g dimethyl-POPOP, and 100 g naphthalene in 1000 ml dioxane) for at least 4 hr before being counted in a Tri-Carb liquid scintillation spectrometer.

Total protein content of the cytosol was determined by the method of Lowry *et al.* (6). The amount of serum contamination was estimated by measuring the albumin con-

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[2] The abbreviations used are: E-R, estrogen receptors; 5α-[1,2-^{3}H]DHT, 5α-dihydrotestosterone; DHT-R, dihydrotestosterone receptors; SHBG, sex hormone-binding globulin.

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centration by immunodiffusion, according to the method of Augustin and Hayward (1).

Evaluation. The difference between the 2 anodal peaks (incubation with and without Nafoxidine or cyproterone acetate, respectively) was used as a measure of the steroid-binding capacity. The tumor specimen was classified as receptor positive when this value amounted to 100 cpm or more. One-hundred cpm are equivalent to about 2 fmoles of bound steroid, which corresponds to the double value of the detection limit of the method used. This was set as a borderline value for the classification of tumor tissue with respect to the presence or absence of receptors. The coefficient of variation in repeat estimates was 12% (n = 6). Spare receptor (receptor sites that are not saturated by endogenous hormone) concentration was expressed as fmoles/mg soluble tissue protein.

RESULTS

A total of 224 specimens of breast cancer tissue were assayed both for E-R and for DHT-R. Agar gel electrophoresis was used to characterize the steroid-receptor complexes, since this is the only procedure currently available for discriminating between specific receptor proteins and the SHBG of the serum (13). This is of some importance, since SHBG has a high affinity for androgens, and contamination of cytosols by serum proteins is occasionally very high (7, 13). This phenomenon became apparent in the electrophoretic analysis of a human breast cancer extract, shown in Chart 1*B*. The SHBG peak was higher than the anodal peak, which corresponds to DHT-R. The specificity of receptor was demonstrated by competition with cyproterone acetate and heat inactivation, respectively. (SHBG is usually heat stable under the experimental conditions used here.) The free labeled steroid was shifted toward the cathode. As seen in Chart 1*A*, the cancer of this patient did not contain any estradiol-binding capacity.

Chart 2 shows the electrophoretic pattern of an extract derived from an axillary neoplastic lymph node. In contrast to the specimen analyzed in Chart 1, this tumor tissue contained estrogen (66.0 fmoles/mg tissue protein) as well as DHT-R (40.0 fmoles/mg tissue protein). Also of note are the different levels of binding of 5α-DHT to SHBG, apparent in Charts 1 and 2. This phenomenon was due to the difference in ages of the patients whose tumors were examined (42 and 83 years), since the concentration of SHBG in serum seems to decrease with age (13).

The E-R and DHT-R values from the 224 tumor specimens are summarized in Table 1. In 15.2%, both E-R and DHT-R were observed in the same tumor. DHT-R alone was present in only 4%, whereas 34.4% of all tumors examined contained E-R alone. The proportion of receptor-positive cases showed no significant difference between primary tumors and metastases.

In Table 2 the same material was analyzed exclusively with respect to the presence of DHT-R. Of a total of 224 tumors, 43 biopsies (approximately 20%) were considered to have positive DHT-R levels. In contrast to this value, the simultaneous determination of E-R in the same group yielded a positive result in nearly 50% of the cases. When the rates of DHT-R-positive cases were compared to the hormonal status of the patient, similar data were obtained, with the exception of the premenopausal patients with metastatic desease (50% DHT-R positive). The number of specimens in this group was rather small; therefore the rate might not be representative.

Table 3 lists the quantitative levels of E-R and DHT-R for

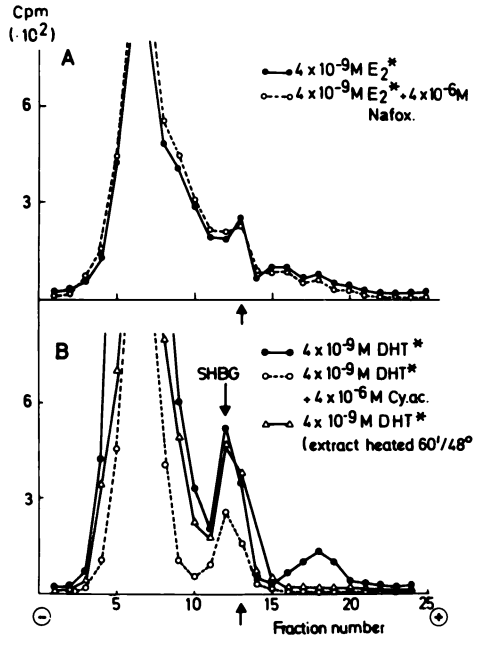

Chart 1. Determination of estradiol and 5α-DHT binding in human breast cancer tissue by agar-gel electrophoresis. Identical samples of the cytosol were incubated overnight at 4° either with 4 × 10^{-9} M [^{3}H]estradiol (*A*) or with 4 × 10^{-9} M 5α-[1,2-^{3}H]DHT (*B*) in the absence or presence of 4 × 10^{-6} M Nafoxidine (*Nafox.*) or 4 × 10^{-6} M cyproterone acetate (*Cy.ac.*), respectively. Another aliquot of the extract was inactivated by heat (48°/60 min).

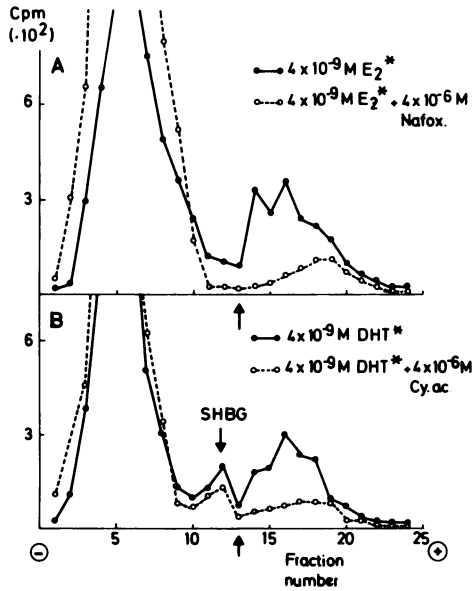

Chart 2. Determination of estradiol and 5α-DHT binding in human breast cancer tissue (axillary lymph node) by agar-gel electrophoresis. Identical samples of the cytosol were incubated overnight at 4° either with 4 × 10^{-9} M [^{3}H]estradiol (*A*) or with 4 × 10^{-9} M 5α-[1,2-^{3}H]DHT (*B*) in the absence or presence of 4 × 10^{-6} M Nafoxidine (*Nafox.*) or 4 × 10^{-6} M cyproterone acetate (*Cy.ac.*), respectively.

Table 1
E-R and DHT-R in human breast cancer tissue

Tissue for assay	n	E-R positive	DHT-R positive	E-R positive/DHT-R positive
Primary tumors				
Premenopause	35	13	2	4
Postmenopause	81	34	1	15
	116	47	3	19
Metastases				
Premenopause	14	0	3	4
Postmenopause	94	30	3	11
	108	30	6	15
Total	224	77 (34.4)[a]	9 (4.0)	34 (15.2)

[a] Numbers in parentheses, total measured as percentage.

Table 2
DHT-R in human breast cancer tissue

Tissue for assay	DHT-R positive	
	Rate	%
Primary tumors		
Premenopause	6/35	17.2
Postmenopause	16/81	19.8
	22/116	19.0
Metastases		
Premenopause	7/14	50
Postmenopause	14/94	14.9
	21/108	19.5
Total	43/224	19.2
E-R positive	111/224	49.6

the various groups of patients. There was no relationship between the average concentration of DHT-R protein and ovarian function, but this was not the case for E-R. In primary tumors, the concentration of E-R was significantly higher in the post- than in the premenopausal group.

To date, 29 trials of endocrine treatment have been evaluated. The majority of our patients received an endocrine-additive therapy (most were given a course of ethinyl estradiol, 3 mg/day). The other patients were treated with antiestrogens or androgens. Only a minority of patients were subjected to ovariectomy or hypophysectomy. The correlations between the therapeutic response and the result of receptor assays are summarized in Table 4. For the purpose of comparison, this table also contains the analogous data of Engelsman (2).

DISCUSSION

Simultaneous determinations of E-R and DHT-R were carried out in 224 tumor specimens and confirm the existence of DHT-R in human breast cancer. In comparison to E-R (50%), the rate of DHT-R-positive cases within the same group was significantly lower (approximately 20%). Wirtz *et al.* (14) likewise found DHT-R in about 22% of mammary cancers, while the rates of DHT-R-positive cases obtained by Wagner *et al.* (11, 12) and by Engelsman (2) were considerably higher (60 to 70% and 40 to 50%, respectively). These differences are difficult to explain. A misinterpretation of DHT-R-"positive" samples caused by contamination with SHBG can be excluded since all these researchers used agar gel electrophoresis, which clearly differentiates between these 2 binding proteins. The increase of the final 5α-[1,2-^{3}H]DHT concentration in the incubation medium (from 5×10^{-9} to 10^{-8} M) also did not alter the portion of DHT-R-positive cases (not illustrated here).

Our data gave no indication that the presence of DHT-R depended on the site of tumor lesion, menopausal status (Table 2), or age of patient (not illustrated here). Therefore it is unlikely that these factors were responsible for the different DHT-R rates reported by the various study groups, especially since the characteristics of patients selected for this study seem similar.

Of the patients whose tumors were assayed for E-R and DHT-R, a limited number received some type of endocrine therapy. Data correlating the receptor test with the clinical response (Table 4) indicated an excellent prediction in receptor-negative cases. In patients with E-R- and DHT-R-positive tumors, we observed an objective response rate of 66.6 and 58.3%, respectively. The number of patients in

Table 3
E-R and DHT-R concentrations in cytosols of primary and metastatic breast cancer tissue

Tumor	n	E-R[a]	n	DHT-R[a]
Primary				
Premenopause	17	48.7[b] (12–170)[d]	6	29.3[c] (5–59)
Postmenopause	48	90.3 (10–450)	15	31.1 (3.3–100)
Metastases				
Premenopause	6	99.3[c] (13–430)	5	28.8[c] (15–40)
Postmenopause	40	63.7 (3,1–640)	14	26.5 (7–105)

[a] Each value indicates the mean receptor content (fmoles/mg of soluble tissue protein).
[b] Significant difference ($p < 0.05$) from the analogous value of postmenopause.
[c] No significant difference from the analogous value of postmenopause.
[d] Numbers in parentheses, range.

Table 4
Tumor regression to endocrine treatment according to E-R and DHT-R assay

Figures [our data and those of Engelsman (2)] represent the number of objective remissions/number of trials of endocrine treatment.

Receptor assay	E-R positive/DHT-R positive	E-R positive/DHT-R negative	E-R negative/DHT-R positive	E-R negative/DHT-R negative
Remissions	4/6 (66.6)[a]	7/12 (58.3)	0	0/11 (0)
Engelsman (2)	9/12 (75)	9/16 (56)	7/16 (44)	2/24 (8)

[a] Numbers in parentheses, response rates (percentage).

each receptor-positive category was too small for statistical evaluation. A similar trend was observed by Engelsman (2), who suggested that patients with tumors containing both E-R and DHT-R have a better chance for clinical response than do those with tumors containing only E-R.

At present, we have no patients in whom we can correlate the existence of DHT-R alone to clinical response. More patients are necessary to obtain a definitive statement about the relevance of multiple assays for various types of receptors in a single tumor. The preliminary data reported here for DHT-R and those presented by McGuire (8) for progesterone receptors would seem to justify further investigations in this direction.

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