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Effect of Drostanolone Propionate on the Binding of Oestradiol and Dihydrotestosterone by Normal and Malignant Target Tissues*

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Abstract—*The influence of drostanolone propionate, an anticancer agent, was tested on the binding of 17β-oestradiol and dihydrotestosterone to specific receptor proteins in tissue of normal and neoplastic target organs. Steroid binding capacity was measured by agar gel electrophoresis of tissue extracts.*

Drostanolone was found to compete with androgen binding sites but not with oestrogen receptors. Therefore it is unlikely that the growth inhibitory effect of drostanolone propionate in human breast cancer is mediated through interaction with oestradiol binding proteins as suggested earlier by other authors.

INTRODUCTION

DROSTANOLONE propionate (2α-methyl-dihydrotestosterone propionate, Masterid,® Fig. 1) has been reported to be an anti-cancer agent for advanced human breast cancers [1–4] and also for 7,12-dimethylbenzanthracene(DMBA)-induced mammary tumours of the rat [5].

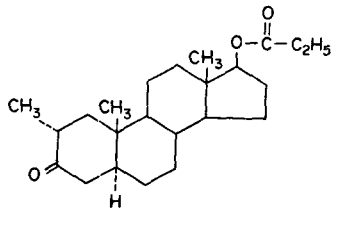

Fig. 1. Structure of 2α-methyl dihydrotestosterone propionate.

Deshpande *et al.* [6] had shown that pretreatment with this compound decreased the uptake of injected ^{3}H-oestradiol in human mammary tumors compared with normal breast or other tissues. Therefore they draw the conclusion that the growth inhibitory effect of drostanolone propionate is mediated through a reduced uptake of oestradiol by the tumor. Under this aspect it was of interest to study the influence of drostanolone propionate on the binding of 17β-oestradiol and 5α-dihydrotestosterone (5α-DHT) to specific cytoplasmic receptor proteins and to the sex hormone binding globulin (SHBG) of the plasma.

MATERIAL AND METHODS

Chemicals

(6,7-^{3}H) 17β-oestradiol (spec.act. 48 Ci/mM) and (1,2-^{3}H)-5α-dihydrotestosterone (spec.act. 49 Ci/mM) were purchased from New England Nuclear Corp., Boston, Mass. The purity (> 94·0%) was checked by thin layer chromatography. The anti-oestrogenic compound U. 11·100 (nafoxidine) was a gift from the Upjohn Company, Kalamazoo, Mich., drostanolone propionate was provided by the Chemie Grünenthal, Stolberg, and cyproterone acetate by the Schering AG, Berlin. All other chemicals were purchased from E. Merck, Darmstadt or Boehringer, Mannheim and were of analytical grade.

Tumour induction

Mammary carcinomas were induced in female Sprague–Dawley rats by a single feeding of 50 mg DMBA at day 50. DMBA was

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dissolved in sesame oil (50 mg/ml). Starting 4 weeks after the carcinogen appearance of tumors was assessed by palpation. Tumor sizes were measured weekly by calipers in the two greatest dimensions (D and d). Tumor mass was calculated by the formula $V = D \times (d)^2/2$ [7]. Ovariectomy in rats was performed at the time when tumors had reached a diameter of at least 2 cm. Those tumors which regressed after removal of the ovaries were called "hormone dependent".

Receptor assay by agar gel electrophoresis

Tissue was frozen in liquid nitrogen immediately after removal and pulverized with the Mikro-Dismembrator (Braun, Melsungen). The fine powder was transferred to a centrifuge tube and four volumes (vol/weight) Tris–HCl buffer (0·01 M, pH 7·5, 1 mM NaN_3) were added. After thawing the sample was centrifuged for 90 min at 40,000 rev/min. (157,000 g_{av}) and 2°C (L2–65B, Beckman Instr.). The supernatant was removed by pipetting and used immediately. Aliquots of the extract were incubated at 4°C overnight with (6·7-^{3}H)-oestradiol or ^{3}H-5α-DHT in presence or absence of radioinert compounds without shaking. The concentrations used are defined in the legends of the figures. At the end of the incubation period, aliquots (50 μl per well) of each sample were subjected to gel electrophoresis, which was performed according to Wagner [8]. Gel layers were prepared with a 1% agar solution (0·05 M Michaelis buffer, pH 8·2). In the centre line of the gel plate, wells were punched out and 50-μl aliquots of the labelled extracts were applied. Two wells were charged with material from one sample. The prepared gel plates were then placed on a teflon coated brass plate within an airtight electrophoresis chamber. The plate was cooled to 2°C. Electrophoresis was carried out for 90 min at 110 mA/300 V. After the run, the gel was divided lengthwise and then cut into 3 mm wide sections. Radioactivity was eluted from the strips with scintillation fluid (7·0 g PPO, 0·3 g dimethyl-POPOP, 100 g naphthalene in 1000 ml dioxane) for at least 4 hr before counting.

Total protein content of tissue extracts was determined according to Lowry *et al.* [9].

RESULTS

In a first series of experiments we tested the effect of drostanolone on the oestrogen binding capacity of normal and neoplastic target organs. Aliquots of cytosols prepared from calf uterus or human breast cancer tissue were incubated with ^{3}H-oestradiol in presence or absence of Nafoxidine or drostanolone propionate. The electrophoretic analyses of the labelled extracts are demonstrated in Figs. 2 and 3. The anodical peak, stretching from the starting line (indicated by the arrow) to fraction 20, represents the receptor bound steroid, while the free hormone is shifted towards the cathode (left side in the figures).

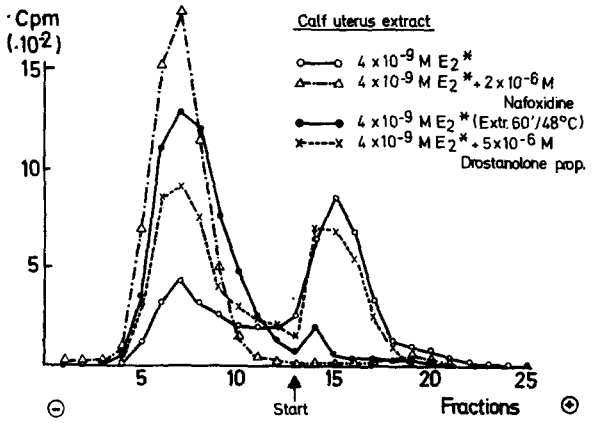

Fig. 2. Determination of oestradiol binding in calf uterus cytosol by agar gel electrophoresis in absence and presence of Nafoxidine and drostanolone propionate, respectively. Protein content of the cytosol was 8·2 mg/ml.

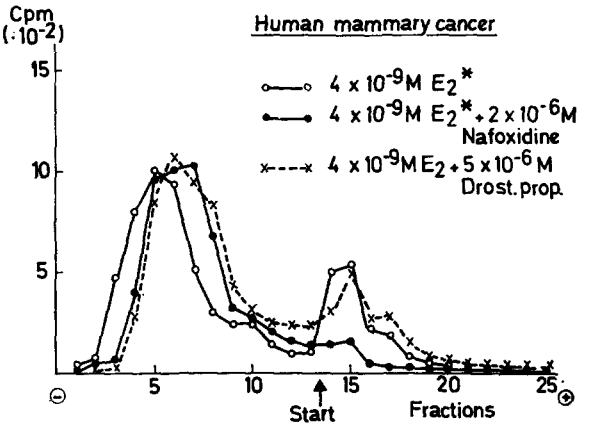

Fig. 3. Determination of oestradiol binding in human mammary cancer cytosol by agar gel electrophoresis in absence and presence of Nafoxidine and drostanolone propionate, respectively. Protein content of the cytosol was 12·4 mg/ml.

Inactivation of the binding protein by heat (60 min at 48°C) or displacement of the labelled oestradiol by the anti-oestrogenic compound Nafoxidine results in a disappearance of the receptor bound labelled hormone and in an increase of the cathodical peak by the liberated steroid. In contrast to this finding drostanolone propionate added in a 1000-fold excess does not compete at the oestrogen-specific binding sites. This holds true both for

calf uterus (Fig. 2) and for human breast cancer tissue (Fig. 3) demonstrated by the persistence of the receptor peak (broken line).

With respect to the fact that drostanolone propionate is a testosterone derivative it was obvious to test this compound on its androgen binding characteristics. As the simultaneous occurrence of oestrogen and androgen receptors was described as well for calf uterus [10] as for human mammary cancer [11–14] the cytosols of these tissues were likewise assayed for their androgen binding capacity. Extracts were incubated with ^{3}H-5α-DHT and analysed electrophoretically in the same manner as described above (Figs. 4 and 5). The 5α-DHT-receptor-complex is characterized by the same mobility as the oestrogen binding protein. The analysis of the tumour extract (Fig. 5) moreover illustrates the clear discrimination between receptor protein and the sex hormone binding globulin (SHBG) from the serum. The last one binds favouredly 5α-DHT and migrates towards the cathode. Addition of a

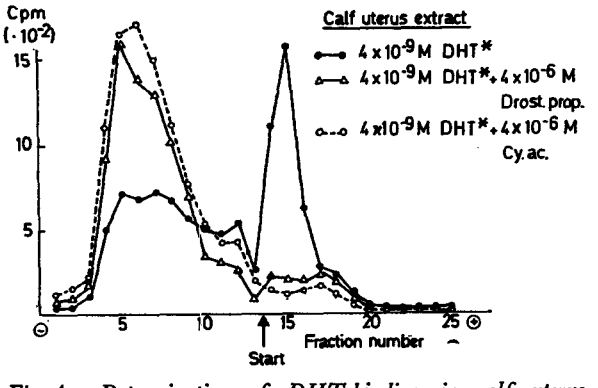

Fig. 4. Determination of DHT-binding in calf uterus cytosol by agar gel electrophoresis in absence and presence of drostanolone propionate and cyproterone acetate, respectively. Protein content of the cytosol was 7·2 mg/ml.

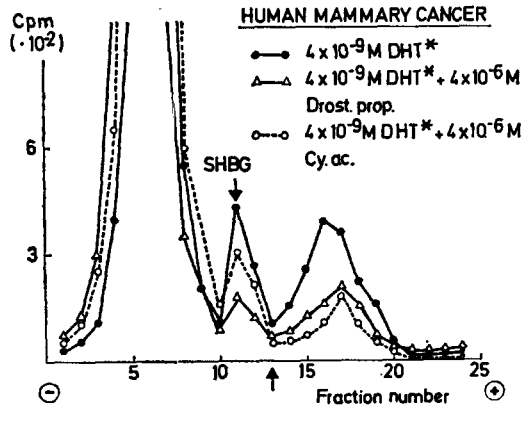

Fig. 5. Determination of DHT-binding in human mammary cancer cytosol by agar gel electrophoresis in absence and presence of drostanolone propionate and cyproterone acetate, respectively. Protein content of the cytosol was 8·0 mg/ml.

1000-fold excess of the anti-androgen cyproterone acetate or of drostanolone propionate results in a decrease of radioactivity at the anodical peak, which is due to competition at specific receptors sites. This competition is less pronounced for SHBG by cyproterone acetate than for drostanolone propionate. Figure 6 shows the electrophoretic pattern of a cutaneous metastasis, which was assayed for oestrogen and androgen receptors. This specimen was derived from a patient with metastatic breast cancer who showed remission after treatment with drostanolone propionate. This medicament

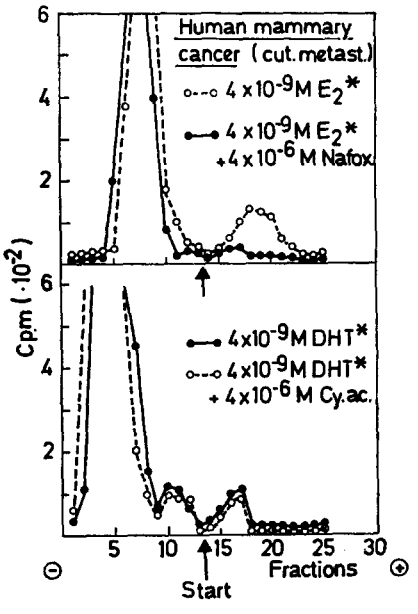

Fig. 6. Determination of oestradiol and DHT-binding in human mammary cancer. Because of cutaneous metastases the patient was treated with drostanolone propionate before excision of metastatic tissue for receptor assay (for further details see text). Protein content of the cytosol was 5·6 mg/ml.

was given (3 × 100 mg/week) over a period of 17 months up to 7 days prior to excision of the tumor. This tumor possesses oestrogen receptors (34 fmole/mg protein), but no DHT-binding is obtained (lower panel). This effect may be due to a primary lack of androgen receptors or it is due to a depletion of spare DHT-receptor sites by the abundance of circulating drostanolone. Kinetic studies [15] have shown, that after a single injection of drostanolone propionate the maximal concentration of free drostanolone in serum remains at a constant level between day 4 and 12 after administration.

DISCUSSION

This investigation was undertaken to obtain some more insight into the mechanism by

which drostanolone propionate exerts its growth inhibitory effect on a variety of mammary tumours. The data presented clearly indicate a competition for androgen binding sites in the cytosols of uterus as well as human breast cancer tissue. In contrast to these findings drostanolone propionate does not affect the oestrogen binding capacity of these target tissues.

Because of these findings it is unlikely that drostanolone propionate exerts its growth inhibitory effect in mammary tumors by competition for specific oestradiol receptor proteins. This is contradictory to the findings of Deshpande *et al.* [6] who noticed a pronounced reduction of 17β-oestradiol in breast cancer tissue after pretreatment with the androgenic compound. Discussing this point it must surely be stressed that the experimental conditions of the studies mentioned were different. Deshpande *et al.* and Braunsberg *et al.* measured the uptake of ^{3}H-oestradiol, which was applicated *in vivo* after pretreatment of the patient with drostanolone propionate. Heise and Görlich and our group determined the specific binding of ^{3}H-oestradiol *in vitro* adding the androgenic compound simultaneously with the labelled steroid. That means that the results obtained by Deshpande *et al.* and Braunsberg *et al.* are comparable regarding the experimental procedures. But in contrast to Deshpande's work the infusion experiments of Braunsberg *et al.* as well as the *in vitro* studies of Heise and Görlich support our results. Both groups likewise could not demonstrate an effect of drostanolone propionate on oestrogen binding in their assay systems.

Recent studies [15] have shown, that drostanolone propionate itself is the active growth inhibitory compound and not a metabolite.

Based on studies on DMBA-induced mammary tumors in rat Van Der Gugten [18] and Hagen *et al.* [19] have suggested that the effect of drostanolone propionate is mediated through a decrease of prolactin secretion by the pituitary gland. Our investigations did not deal with this possibility and it is still open whether the results of these authors are true for human breast cancer.

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REFERENCES

1. Ch. M. Blackburn and D. S. Childs, Use of 2a-methyl-androstan-17β-ol-3-one (2α-methyldihydrotestosterone) in the treatment of advanced cancer of the breast. *Proc. Mayo Clin.* **34,** 113 (1959).
2. A. N. Thomas, G. S. Gordan, L. Goldman and R. Lowe, Antitumor efficacy of 2α-methyldihydrotestosterone propionate in advanced breast cancer. *Cancer (Philad.)* **15,** 176 (1962).
3. J. S. Goldenberg and M. A. Hayes, Hormonal therapy of metastatic female breast carcinoma—II. 2α-methyldihydrotestosterone propionate. *Cancer (Philad.)* **14,** 705 (1961).
4. Cooperative Breast Cancer Group, Progress Report: results of studies by the Cooperative Breast Cancer Group 1956–60. *Cancer Chemother. Rep.* **11,** 119 (1961).
5. R. J. Dorfman, S. Baba, O. Abe, T. Harada and W. H. Rooks, The influence of drostanolone on a transplantable rat mammary fibroadenoma and carcinogen-induced adeno-carcinomas. In: *The Treatment of Carcinoma of the Breast.* (Edited by A. S. Jarett), p. 15. Exerpta Medica, Amsterdam (1968).
6. N. Deshpande, V. Jensen and R. D. Bulbrook, Accumulation of tritiated oestradiol by human breast tissue. *Steroids* **10,** 219 (1967).
7. L. Simpson-Herren and D. P. Griswold, Studies of the cell population kinetics of induced and transplanted mammary adenocarcinoma in rats. *Cancer Res.* **33,** 2415 (1973).
8. R. K. Wagner, Characterization and assay of steroid hormone receptors and steroid binding serum proteins by agar gel electrophoresis at low temperature. *Hoppe Seylers Z. physiol. Chem.* **353,** 1235 (1972).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193,** 265 (1951).
10. P. W. Jungblut, S. F. Hughes, L. Görlich, U. Gowers and R. K. Wagner, Simultaneous occurrence of individual estrogen- and androgen-receptors in female and male target organs. *Hoppe Seylers Z., physiol. Chem.* **352,** 1603 (1971).

11. R. K. WAGNER and P. W. JUNGBLUT, Estradiol- and dihydrotestosterone receptors in normal and neoplastic human mammary tissue. *Acta endocr. (Kbh.)* **82,** 105 (1976).
12. G. TRAMS and H. MAASS, Nachweis von Androgen-Rezeptoren in menschlichen Mammakarzinomen. *Arch. Gynäk.* **219,** 172 (1975).
13. L. RAITH, A. WIRTZ and H. J. KARL, Beziehungen zwischen Testosteronstoffwechsel und Dihydrotestosteron-Rezeptoren in menschlichen Mammatumoren. *Klin. Wschr.* **52,** 299 (1974).
14. K. B. HORWITZ, M. E. COSTLOW and W. L. MCGUIRE, MCF-7: A human breast cancer cell line with estrogen, androgen, progesterone, and glucocorticoid receptors. *Steroids* **26,** 785 (1975).
15. CHEMIE GRÜNENTHAL GMBH, Stolberg, Personal communication. Unpublished data (1972).
16. H. BRAUNSBERG, W. T. IRVINE and V. H. T. JAMES, Radioactivity in human breast tumours after infusion of ^{3}H-oestradiol: effect of androgen treatment. *Steroids* **15,** 669 (1970).
17. E. HEISE and M. GÖRLICH, The influence of some hormone-therapeutic substances on oestradiol receptor analysis in various organs. 6th Intern. symp. on the biological characterization of human tumors. Copenhagen (1975).
18. A. A. VAN DER GUGTEN, The effect of 1-(Morpholinomethyl)-4-phtalimidopiperidindione-2,6 and drostanolone propionate on the plasma prolactin concentration of oestrone-treated orchidectomized R-Amsterdam rats. *Europ. J. Cancer* **7,** 581 (1971).
19. E. HAGEN, W. WITTKOWSKI and PH. A. STÖHR, Morphologische Veränderungen an Hypophyse und Nebenniere der Ratte nach Gabe hormonell wirksamer Substanzen zur Behandlung von DMBA-induzierten Mammatumoren. *Z. Krebsforsch.* **76,** 97 (1971).