Failure of Progestins to Induce Estradiol Dehydrogenase Activity in Endometrial Carcinoma, in Vitro

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ABSTRACT

Estradiol dehydrogenase (E2DH) is a well-known progesterone-dependent enzyme in human endometrium, and its induction has been proposed as a means to test hormonal sensitivity of endometrial carcinoma. While administration of progestins to some patients with endometrial carcinoma resulted in increased endometrial E2DH activity, efforts to induce this enzyme, in vitro, in these tumors have been unsuccessful. The reasons for such failure were investigated in the present study. Progesterone receptor (PR) concentrations and E2DH activities were simultaneously measured in proliferative and malignant endometria under organ culture conditions. Cytoplasmic PR concentrations were determined by Scatchard plot analysis of [3H]progesterone binding in fresh samples and in tissue explants incubated in nutrient medium at 37°C in a humidified 5% CO2 atmosphere for various periods of time. Parallel incubations of explants with and without 500 ng medroxyprogesterone acetate per ml were carried out for monitoring E2DH induction. In proliferative endometrium, the progesterone-specific binding sites remained stable during the culture periods, and the E2DH activities were stimulated severalfold by medroxyprogesterone acetate. In contrast, the PR concentrations in carcinoma explants were undetectable after a 24-hr period, and this was associated with a lack of increase in E2DH activity. These findings provide evidence that progestin-induced endometrial E2DH activity is a receptor-mediated phenomenon. In addition, these results demonstrate clearly that the ineffectiveness of progestin to induce E2DH in endometrial cancer specimens, in vitro, is related to the instability of PR under culture conditions. It is suggested that any experiment designed to follow effects of steroids on target tissues must take into account the stability of steroid receptors under in vitro conditions.

INTRODUCTION

Activity of the enzyme E2DH is increased severalfold in the human endometrium during the luteal phase of the menstrual cycle (15, 20). Results from both in vitro and in vivo studies have shown that progesterone regulates the activity of E2DH in the human endometrium (8, 9, 21). Administration of progestins to patients with endometrial adenocarcinoma also results in the enhancement of E2DH activity in some of these tumors (5, 14). Extension of these studies to cultured explants of endometrial carcinoma was attempted to develop a simple in vitro test for predicting responsiveness of endometrial tumors to progestin therapy. While in normal proliferative endometrium there was a consistent induction of E2DH activity with progestin, neoplastic tissue maintained under identical conditions persistently failed to respond to this agent. Since all cancer specimens used in this study initially contained PR, it was reasoned that the lack of response of this tissue to added progestin may be due to (a) the instability of PR under culture conditions or (b) a defect in the receptor mechanism in the tumor explants, e.g., translocation of PR to the nucleus, interaction of the steroid:receptor complex with the chromosomal machinery, etc. The first possibility was tested by measuring PR concentrations in cultured explants of both normal and neoplastic tissue at various time intervals. Simultaneous measurements of E2DH activity were also carried out in explants of these tissues cultured in parallel for 2 days in the absence or presence of the progestin, MPA. Our results indicate that the inability of progestin to induce E2DH activity in endometrial carcinoma is due to the instability of PR in these tissue explants. In addition, the close correlation between the maintenance of PR concentrations and response to progestin, in vitro, provides additional evidence for receptor mediation in progestin induction of E2DH activity in human endometrium.

MATERIALS AND METHODS

Reagents. The radioactive steroids, [1,2,6,7-3H]progesterone (90 Ci/mmol), 17β[1,2-3H]estra diol (50 Ci/mmol), and [4-14C]estrone (50 mCi/mmol), were purchased from New England Nuclear and used for PR measurements and enzyme assays after verification of radiochemical purity. Crystalline steroids, estrone, estradiol, and progesterone were obtained from Steraloids, Wilton, N. H., and NAD+ was from Sigma Chemical Co.; MPA was a gift from The Upjohn Company, Kalamazoo, Mich.

Tissue. Endometrial carcinoma specimens were obtained from postmenopausal women undergoing dilatation and curettage or hysterectomy. Endometria were also obtained from premenopausal women undergoing dilatation and curettage or curettage for various disorders. Only those tissues that were histologically determined to be from proliferative stage of the menstrual cycle were included in these studies.

Organ Culture. Endometrial curettings were transported to the laboratory in sterile, ice-cold Ham’s F-10 medium (Flow Laboratories) containing 10% fetal calf serum (Flow), 1% antibiotic-antimotic mixture (Grand Island Biological Co.), insulin (10 μg/ml) (Eli Lilly and Co.), 17β-estradiol (2.5 ng/ml), and d-glucose (final concentration, 5 mg/ml). All preparative procedures for culture studies were carried out aseptically in sterile hoods. The organ culture of the cuttings were washed free of blood clots, cut into 1-cm pieces, and placed over filters on stainless steel grids in 60- x 15-mm culture dishes (Falcon Plastics). The filter was kept moist and in contact with about 3 ml of culture broth.

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3 The abbreviations used are: E2DH, estradiol dehydrogenase; PR, progesterone receptor; MPA, medroxyprogesterone acetate; TED buffer, 20 mM Tris-HCl (pH 7.8) containing 3 mM EDTA, 1 mM dithiothreitol, and 0.01% sodium azide.

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1322 CANCER RESEARCH VOL. 42

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medium. The cultures were incubated for 2, 24, and 48 hr at 37°C in a humidified 95% air:5% CO₂ atmosphere in a Wedco CO₂ incubator when stability of PR was determined. When E₂DH induction was followed, parallel cultures of explants were maintained for 2 days with or without the addition of MPA (500 ng/ml) to the culture medium.

Assay of Cytosol PR in Human Endometrium. The PRs were measured in the cytosol preparations of fresh endometrial tissue and in cultured explants according to the procedure of Bayard et al. (1) with the following modifications: (a) the receptor concentrations were estimated by Scatchard analysis of specific binding data (17) instead of single-point assays; and (b) the removal of free and loosely bound [³H]progesterone was affected by a 5-min instead of a 30-min incubation of the reaction mixture at 0°C with Dextran-coated charcoal (0.05% dextran and 0.5% charcoal in TED buffer).

All procedures were carried out at 0°C. The fresh endometrial tissue and cultured explants were washed twice in 10 ml TED buffer and homogenized in 20 volumes of the same buffer using a motor-driven glass-glass homogenizer with 15-sec bursts at 1000 rpm for 5 times, each separated by a 30-sec interval. The receptor assay was carried out in cytosol fractions of these homogenates after a 105,000 x g centrifugation for 1 hr. For determination of total bound [³H]progesterone, aliquots (200 μl) of cytosol were incubated with increasing concentrations of [³H]progesterone (0.5 to 5 nM) and 100-fold excess concentrations of cortisol for 3 hr. Identical samples containing 100 times excess unlabeled progesterone were used for determination of nonspecifically bound [³H]progesterone. Free and loosely bound radioactive ligand were removed from the cytosol preparations by the addition of 100 μl ice-cold TED buffer and 300 μl chilled TED buffer containing Dextran-Coated Charcoal followed by a 5-min incubation at 0°C. After centrifugation at 2000 rpm for 5 min, an aliquot of the supernatant was counted for radioactivity using 4 ml Dimiscint (National Diagnostics, Inc., Somerville, N. J.) in minivials in a Beckman Model 7500 liquid scintillation spectrometer with an automatic quench correction feature and counting efficiencies of 48% for tritium.

Specific binding was obtained by subtracting nonspecific binding from total binding at each concentration of the [³H]-steroid. Results were subjected to Scatchard plot analysis providing the concentration of nonspecifically bound [³H]progesterone. Free and loosely bound radioactive ligand were removed from the cytosol preparations by the addition of 100 μl ice-cold TED buffer and 300 μl chilled TED buffer containing Dextran-Coated Charcoal followed by a 5-min incubation at 0°C. After centrifugation at 2000 rpm for 5 min, an aliquot of the supernatant was counted for radioactivity using 4 ml Dimiscint (National Diagnostics, Inc., Somerville, N. J.) in minivials in a Beckman Model 7500 liquid scintillation spectrometer with an automatic quench correction feature and counting efficiencies of 48% for tritium.

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The reasons for the instability of PR in cancer tissue are unclear. Release of proteolytic activity by various tumors has been reported. Secretion of considerable amounts of plasminogen activator activity by endometrial carcinoma specimens has also been shown (19). We have also observed that, while carcinoma explants elaborate high plasminogen activator activity concentrations, the culture fluids of normal endometrium contain negligible amounts of this enzyme. Notides et al. (13) showed that human uterus contains a serine protease which accounted for the multiple molecular species of estradiol receptors. Most of this activity, however, was localized to the myometrium. Fluctuations in protease activity as a function of the menstrual cycle were, unfortunately, not followed by these investigators. The remarkable stability of PR in cultured proliferative endometrium, noted in the present study, suggests that either the uterine protease activity is specific to estrogen receptor or that there is negligible protease activity during the proliferative phase of the menstrual cycle.

Although the role of progesterone in the induction of E$_2$DH activity in the human endometrium has been accepted widely, receptor mediation in this process has not been established. The existence of a correlation between PR levels and E$_2$DH activity in the human endometrium during the menstrual cycle and the response of endometrial carcinoma to progestin administration, as measured by increased E$_2$DH activity in these tissues, were reported (18). The present findings on the close relationship between PR stability and induction of E$_2$DH activity by progestin, in vitro, provide additional support for the receptor mechanism in this system. Unequivocal establishment of PR mediation in this phenomenon, however, would require the demonstration of a temporal sequence, namely, cytosol PR $\rightarrow$ nuclear PR $\rightarrow$ induction of E$_2$DH, in progestin-exposed endometrium.

In conclusion, we have identified the dramatic decrease in PR concentration in explants of endometrial carcinoma as the reason underlying the repeated failure to induce E$_2$DH activity in vitro. Identification of agents which may prevent receptor degradation within carcinoma tissue and their addition to these cultures may be expected to render these tissues responsive to progestin. The intimate relationship between PR stability under culture condition and induction of E$_2$DH in these explants has provided further evidence for the receptor mediation in progestin effect. Finally, it is suggested that any experiment designed to follow the effects of steroids on target tissues must take into account the stability of steroid receptors under in vitro conditions.

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Endometrial Carcinoma and E2DH Induction in Vitro


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