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Membrane 5α-pregnane-3,20-dione (5αP) receptors in MCF-7 and MCF-10A breast cancer cells are up-regulated by estradiol and 5αP and down-regulated by the progesterone metabolites, 3α-dihydroprogesterone and 20α-dihydroprogesterone, with associated changes in cell proliferation and detachment

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Abstract

Previous studies have shown that the progesterone metabolite, 5α-pregnane-3,20-dione (5αP), exhibits mitogenic and metastatic activity in breast cell lines and that specific, high affinity receptors for 5αP are located in the plasma membrane fractions of tumorigenic (ER/PR-positive) MCF-7 cells. The aim of this study was to determine the effects of the mitogenic (estradiol; 5αP) and anti-mitogenic (3α-hydroxy-4-pregnen-20-one, 3αHP; 20α-hydroxy-4-pregnen-3-one, 20αHP) endogenous steroid hormones on 5αP receptor (5αP-R) numbers and on cell proliferation and adhesion of MCF-7 and MCF-10A cells. Exposure of MCF-7 cells for 24 h to estradiol or 5αP resulted in significant ($p < 0.05$–0.001) dose-dependent increases in 5αP-R levels. Conversely, treatment with 3αHP or 20αHP resulted in significant ($p < 0.05$–0.01) dose-dependent decreases in 5αP-R levels. Treatment with one mitogenic and one anti-mitogenic hormone resulted in inhibition of the mitogen-induced increases, whereas treatment with two mitogenic or two anti-mitogenic hormones resulted in additive effects on 5αP-R numbers. Treatments with cycloheximide and actinomycin D indicate that changes in 5αP-R levels depend upon transcription and translation. The non-tumorigenic breast cell line, MCF-10A, was also shown to posses specific, high affinity plasma membrane receptors for 5αP that were up-regulated by estradiol and 5αP and down-regulated by 3αHP. Estradiol binding was demonstrated in MCF-10A cell membrane fractions and may explain the estradiol action in these cells that lack intracellular ER. In both MCF-7 and MCF-10A cells, the increases in 5αP-R due to estradiol or 5αP, and decreases due to 3αHP or 20αHP correlate with respective increases and decreases in cell proliferation as well as detachment. These results show distribution of 5αP-R in several cell types and they provide further evidence of the significance of progesterone metabolites and their novel membrane-associated receptors in breast cancer stimulation and control. The findings that 3αHP and 20αHP down-regulate 5αP-R and suppress mitogenic and metastatic activity suggest that these endogenous anti-mitogenic progesterone metabolites deserve considerations in designing new breast cancer therapeutic agents.
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Keywords: Breast cancer; Progesterone metabolites; Membrane receptor; 5α-Dihydroprogesterone; 3α-Dihydroprogesterone; 5αP; 3αHP; MCF-7 cells; MCF-10A cells; Cell proliferation; Cell adhesion; Receptor regulation

1. Introduction

Recent studies indicate that progesterone metabolites produced in breast tissue may play significant roles in suppressing or promoting breast cancer. Evidence from tissue

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metabolism studies shows elevated 5α-reductase activity and depressed 3α-hydroxysteroid oxidoreductase (3α-HSO) and 20α-HSO activities in tumorous as compared to normal (non-tumorous) breast tissue [1]. Thus, tumorous breast tissue produces significantly higher levels of 5α-pregnanes, especially 5α-pregnane-3,20-dione (5αP), whereas normal breast tissue produces more 4-pregnenes, especially 3α-hydroxy-4-pregnen-20-one (3αHP) and 20α-hydroxy-4-pregnen-3-one (20αHP) [1]. The changes in progesterone metabolizing enzyme activities in breast tumor tissue are correlated with increases in expression of 5α-reductase (SRDA1/2) mRNA [2] and decreases in expression of 3α-HSO (AKR1C2/3) and 20α-HSO (AKR1C1) mRNAs [2,3]. Similarly, tumorigenic breast cell lines (MCF-7, MDA-MB-231, T-47D) have significantly higher progesterone 5α-reductase and lower 3α-HSO and 20α-HSO activities than non-tumorigenic (MCF-10A) cells [4] and these differences in activity correlate with differences in expression of the respective enzyme genes [4]. The shift in ratio of 5α-pregnanes:4-pregnenes is viewed as significant for breast cancer because in vitro studies with breast cell lines have shown that exposure to 5αP (and other 5α-pregnanes) results in changes associated with neoplasia, including increased proliferation [1], decreased attachment to the substratum and cell-to-cell contact, depolymerization of F-actin, and decreases in adhesion plaque-associated vinculin [5]. On the other hand, exposure to 3αHP results, in general, in opposite (anti-cancer-like) effects on breast cell lines [1,5].

The action of the metabolites is not mediated via the known cytosolic/nuclear estrogen, progesterone, androgen or corticosteroid receptors. High affinity saturable binding sites, specific for either [^{3}H]3αHP or [^{3}H]5αP, have been demonstrated in the plasma membrane, but not the nuclear or cytosolic, fraction of MCF-7 cells [6]. Numerous other steroids (including estradiol, androgens, corticosteroids, progesterone and its other metabolites) failed to displace the ligands at 200–500-fold excess [6], demonstrating stereospecificity. Recent findings [7 and unpublished results] show that 5αP activates the Ras/Raf MAP kinase pathway within 5–10 min, leading to increased cell proliferation and detachment. These 5αP-induced changes are abrogated by specific inhibitors of the MAP kinase cascade, strongly linking the physiological responses to the 5αP membrane-based binding sites. The 5αP binding sites thus meet the attributes of high affinity, specificity, saturability, reversibility and biological function that constitute criteria for receptor designation [8,9]. Preliminary evidence [6,7] indicated that estradiol and 3αHP may play a role in the regulation of 5αP receptor (5αP-R) numbers. Because of the potential importance of 5αP in promoting breast cancer via the binding to its membrane-based receptors, we explored the role of mitogenic (estradiol, 5αP) and anti-mitogenic (3αHP, 20αHP) endogenous steroids on 5αP-R levels in a tumorigenic (MCF-7; ER/PR-positive) and a non-tumorigenic (MCF-10A; presumptive ER/PR-negative) breast cell line. The findings presented here show for the first time that 5αP-R levels can be modulated in at least two diverse breast cell lines by steroid hormones, and the altered levels correlate with changes in cell proliferation and adhesion.

2. Materials and methods

2.1. Chemicals

Radiolabelled [^{3}H]5αP was synthesized by performing an oxidation of [9,11,12-^{3}H]5α-pregan-3α-ol-20-one as previously described [6]. Unlabelled 3αHP was synthesized and purified according to our published procedures [10,11]. Radiolabelled thymidine (methyl-^{3}H-thymidine; 48.0 Ci/mmol) was obtained from Amersham Biosciences (Piscataway, NJ). [9,11,12-^{3}H]5α-Pregan-3α-ol-20-one (65 Ci/mmol), [2,4,6,7-^{3}H]progesterone (102 Ci/mmol) and [2,4,6,7-^{3}H]-estradiol-17β (71 Ci/mmol) were purchased from Perkin-Elmer Life Sciences (New England Nuclear; Woodbridge, Ont.). The other unlabelled steroids as well as bacitracin, leupeptin, Tris–HCl and Bradford Reagent were obtained from Sigma Chemical Co. (Oakville, Ont.). Sucrose and EDTA were purchased from BDH (Toronto, Ont.).

2.2. Cell culture

MCF-7 cells (passage 102) came from Dr. R. Shiu through the courtesy of Dr. J. Wimalasena. The cells were grown as previously described [6] in Dulbecco's Modified Essential Medium (DMEM):F12 HAM in a 1:1 ratio (Sigma Chemical Co.). The medium was supplemented with 5% calf serum (Gibco BRL, Burlington, Ont.), penicillin (100 units/ml), streptomycin (100 ng/ml), insulin (10 μg/ml) and sodium bicarbonate (1.2 mg/ml). Cells were grown in T-75 flasks (Sarstedt Inc.), maintained in a humidified incubator at 37 °C, with a 5% CO_2 atmosphere. About 2×10^6 cells were seeded per T-75 flask and allowed to reach about 80–90% confluence before experimental treatments.

MCF-10A cells (passage 116) were obtained from the Barbara Ann Karmanos Cancer Institute (Detroit, MI) and were cultured in T-75 flasks in DMEM:F12 HAM in a 1:1 ratio (Sigma Chemical Co.). The medium was supplemented with 5% horse serum, penicillin (100 units/ml), streptomycin (100 μg/ml), insulin (10 μg/ml), sodium bicarbonate (1.2 mg/ml), epidermal growth factor (0.02 μg/ml), hydrocortisone (0.5 mg/l) and cholera toxin (0.1 μg/ml) (Sigma Chemical Co.). Cells were grown in humidified conditions with 5% CO_2 at 37 °C. About 2×10^6 cells were seeded per T-75 flask and allowed to reach about 80–90% confluence before experimental treatments.

2.3. Cell treatments

Medium was removed and cells were rinsed with BSS. Medium containing dextran-coated charcoal-stripped serum (5%) and steroids (final concentrations of 10^{-10}–10^{-5} M) was added and cells were incubated for 24 h. The steroids

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