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Induction of aromatase in stromal vascular cells from human breast adipose tissue depends on cortisol and growth factors

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Abstract

The regulation of aromatase (estrogen synthase) activity of cultured stromal vascular cells from human breast adipose tissue by cortisol, db-cAMP and growth factors was studied in a serum-free culture system. While PDGF-BB alone inhibited the effect of db-cAMP on aromatase induction, it stimulated aromatase activity in the presence of cortisol with or without db-cAMP. In the presence of 1 μM insulin consistently higher aromatase activities were found as compared to 1 nM insulin. In contrast to PDGF-BB, bFGF led to an increase of aromatase activity only in the presence of both cortisol and db-cAMP.

Key words: Aromatase; Cortisol; PDGF; bFGF; Breast adipose tissue (human)

1. Introduction

Stromal vascular cells from human adipose tissue, due to their high aromatase activity, are able to produce large amounts of estrogens by aromatization of androgens [1–3]. They can be kept in culture and may be used to study the regulation of extraovarian estrogen formation. In these cells synthetic analogues of cyclic AMP are inducers of aromatase activity, however they have been reported to act only under serum-free culture conditions [4]. Phorbol esters potentiate the action of db-cAMP, whereas tumor necrosis factor, transforming growth factors and , interleukin-1, epidermal growth factor and PDGF have been reported to be strong inhibitors [5]. Glucocorticoids also are powerful inducers of aromatase. Their action seems to depend on the presence of as yet unidentified factors present in serum, as they are ineffective in its absence [6]. As the functional integrity of the epithelial cells of the human breast depends on the presence of estrogens, the ability of the stromal cells from mammary adipose tissue to produce estrogens due to their aromatase activity (for review see [5]) could account for at least some part of their estrogen supply.

Significantly higher estradiol concentrations were observed in malignant as compared to normal breast tissues in pre- and postmenopausal women [7]. In addition despite of large differences in peripheral plasma estrogen concentrations in the pre- versus the postmenopausal state, similar estradiol concentrations were found in malignant breast tissue from pre- as well as from postmenopausal women. These observations support the idea, that there exist local sources of estrogens within the human breast which might play an important role for the development of breast cancer [8–10]. The finding of elevated aromatase activity in the adipose tissue localized near breast tumors [11,12] implicate, that tumor cells produce factors, which act as inducers of aromatase activity. A large variety of growth factors, including PDGF and bFGF, is released by breast cancer cells (for review see [13,14]), often without any known effect on the cancer cells themselves. To test whether there exists a paracrine mechanism for aromatase induction based on growth factors, we studied the regulation of aromatase activity in stromal vascular cells of human breast adipose tissue by some of these factors. We show here, that under serum-free culture conditions the induction of aromatase activity by cortisol treatment of stromal vascular cells depends on the addition of either PDGF-BB or bFGF together with db-cAMP.

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Abbreviations: PDGF, platelet-derived growth factor; bFGF, basic fibroblast growth factor; cyclic AMP, adenosine 3′:5′-cyclic monophosphate; db-cAMP, N^6,2′-*O*-dibutyryladenosine 3′:5′-cyclic monophosphate; SF-medium, serum-free medium; FCS, fetal calf serum.

2. Materials and methods

2.1. Cells and cell culture

Human adipose tissue for the preparation of stromal vascular cells was obtained during plastic breast surgery from healthy women with an age of 25–33 years. The preparation of stromal vascular cells was performed essentially as described earlier [15,16]. Cells were seeded at

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a density of 10000 cells/cm^2 in 25cm^2 Falcon flasks after isolation and in 24-well plates after one passage for subcultivation. They were grown to confluence in medium 199 with Earle's salts containing penicillin (100 U/ml), streptomycin (0,1 mg/ml) and 10% fetal calf serum, which was replaced three times a week. For experiments under serum-free conditions, cells were pretreated with serum-free medium (SF-medium) consisting of Dulbecco's modified Eagle's medium and Ham's F12 medium in a ratio of 3:1 (both without Phenol red) supplemented with penicillin (100 U/ml), streptomycin (0.1 mg/ml), transferrin (2 μg/ml), pantothenate (17 μM), biotin (1 μM). Insulin was added in a concentration of either 1 nM or 1 μM as indicated. Pretreatment was done by changing media three times within 48 h to get total depletion of serum components. During culture cells were kept in a humidified atmosphere with 5% CO_2 at a temperature of 37°C.

2.2. Induction of aromatase and assay of aromatase activity

If not indicated otherwise confluent cells were kept in SF-medium for 2 days prior to the induction of aromatase activity. After that period, growth factors in concentrations as indicated were added with or without cortisol (1 μM) and db-cAMP (1 mM) respectively. 18 h later [1β,2β-^{3}H]testosterone was added to the cells in a concentration of 150 nM (3 μCi/well). After 6 h the incorporation of ^{3}H-label in H_2O was measured essentially as described [3,16]. Aromatase activity is given as pmol testosterone used/ 6 h × mg protein calculated according to [17]. Cellular protein was determined by an improved protocol of the method of Bradford [18], described by Peterson [19].

2.3. Materials

Cell culture media were from Biochrom, Berlin, Biotin, Norit A, Dextran and Serva Blue G-250 from Serva, Heidelberg, and [1β,2β-^{3}H]testosterone from DuPont, Dreieich. Insulin was a generous gift from Dr. Brocks, Farbwerke Hoechst AG, Frankfurt. Recombinant PDGF-BB and bFGF were obtained from BTS, St.Leon-Rot. All other chemicals were of analytical grade and purchased from Sigma, Munich or Merck AG, Darmstadt.

2.4. Statistical analysis

Analysis of aromatase activities induced by different agents was performed using the *t*-test for comparison of means. Differences were considered statistically significant at $P < 0.05$.

3. Results

As has been shown previously, stromal vascular cells from subcutaneous abdominal and breast adipose tissue respectively cultured in the presence of 10% FCS respond to cortisol with an approximately 5- to 7-fold increase of the specific aromatase activity [6,16] (Fig. 1, panel a). As the use of a serum-free medium is necessary for studies of the influence of growth factors on the induction of aromatase activity, the influence of pretreatment of stromal vascular cells with serum-free medium on aromatase induction was studied. Stromal vascular cells grown to confluence in medium 199 containing 10% FCS were pretreated for 48 h by incubation in SF-medium containing either 1 nM or 1 μM insulin. When, during the subsequent aromatase induction and assay, medium 199 supplemented with 10% FCS was used, no significant difference to cells without pretreatment under serum-free conditions could be observed (Fig. 1, panels a,b,d). db-cAMP had no effect on aromatase induction when added alone or in combination with cortisol.

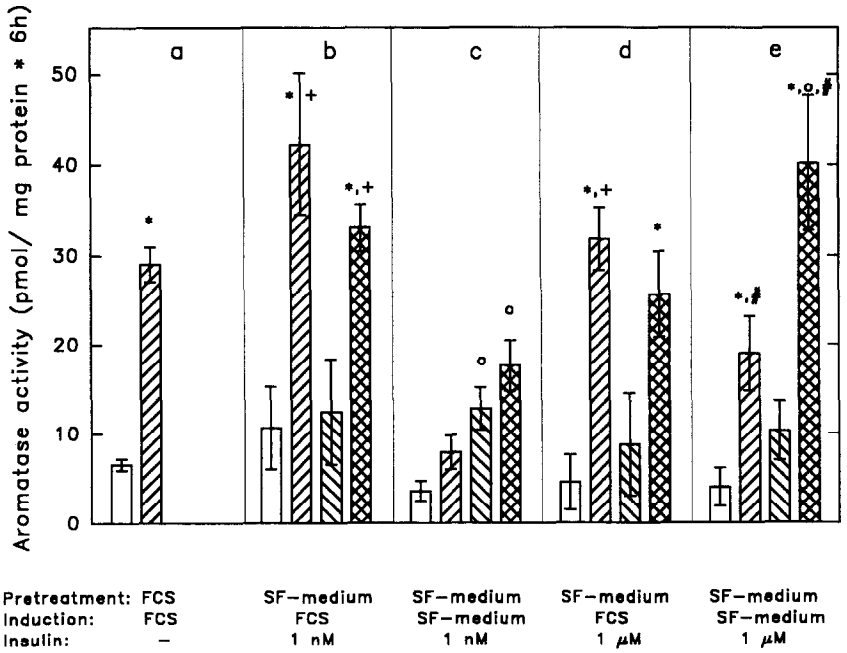

Fig. 1. Influence of serum deprivation on the induction of aromatase activity. Human breast adipose tissue stromal cells were grown to confluence in the presence of FCS and pretreated for 48 h prior to aromatase induction with medium containing 10% FCS (a) or with SF-medium containing either 1 nM (b,c) or 1 μM (d,e) insulin. The subsequent aromatase induction was carried out in the presence of either 10% FCS (a,b,d) or SF-medium containing the same insulin concentrations as during the pretreatment phase (c,e) by addition of 1 μM cortisol (▨), 1 mM db-cAMP (▩) or cortisol together with db-cAMP (▩); (□) induction without additions. Results are means of duplicate assays from at least 4 independent experiments ± S.E.M. Differences significant ($P < 0.05$) to the respective values without cortisol (*), without db-cAMP (○), without FCS during the induction period (+) or to the values obtained in the presence of 1 nM insulin (#).

When however serum-free conditions were maintained during the test period, in the presence of insulin in a physiological concentration the effect of cortisol on aromatase activity was nearly completely abolished (Fig. 1, panel c). In the presence of 1 μM insulin cortisol was able to induce aromatase activity, but to a significantly lower extent than in the presence of FCS (Fig. 1, panel e). In addition, db-cAMP led to a moderate increase of aromatase activity, which was statistically significant in cultures treated with 1 nM but not with 1 μM insulin (Fig. 1, panels c,e). Cortisol and db-cAMP given simultaneously to cultures under serum-free conditions were able to increase aromatase activity to values exceeding the activities found when cortisol or db-cAMP were added alone. This rise in activity was not significant when the effects of db-cAMP and db-cAMP together with cortisol in the presence of 1 nM insulin were compared. In the presence of 1 μM insulin the combination of db-cAMP together with cortisol was equally effective as cortisol in the presence of serum.

In a serum-free culture system aromatase activity of stromal vascular cells increased after the addition of cortisol, when recombinant PDGF-BB was added to the medium. The effect of PDGF was dose dependent with a half maximal effective concentration of about 10^{-10} M (Fig. 2, panels a,b).

The increase in aromatase activity due to treatment with PDGF-BB together with cortisol was dependent on the concentration of insulin. At a supraphysiological concentration (1 μM) significantly higher aromatase activities were obtained as compared to cultures treated with physiological insulin concentrations (1 nM) or without insulin (data not shown).

The effect of db-cAMP in the presence of various concentrations of PDGF is strongly affected by cortisol: in the absence of cortisol aromatase induction was suppressed by PDGF in a dose dependent manner (statistically significant in the presence of 1 nM insulin), the concentration of half maximal inhibition of db-cAMP induced aromatase activity was about 10^{-10} M. This inhibitory effect of PDGF on db-cAMP mediated aromatase induction was reversed in the presence of cortisol. The values obtained by the combination of cortisol, PDGF and db-cAMP always were significantly higher than those obtained in the presence of cortisol and PDGF for all PDGF concentrations tested at 1 nM and 1 μM insulin.

Supraphysiological insulin concentrations resulted in significantly elevated aromatase activities when the values measured after induction with PDGF and cortisol with and without db-cAMP were compared with the respective values at physiological insulin concentrations.

bFGF in the same concentration range as PDGF-BB had no effect on aromatase activity of stromal vascular

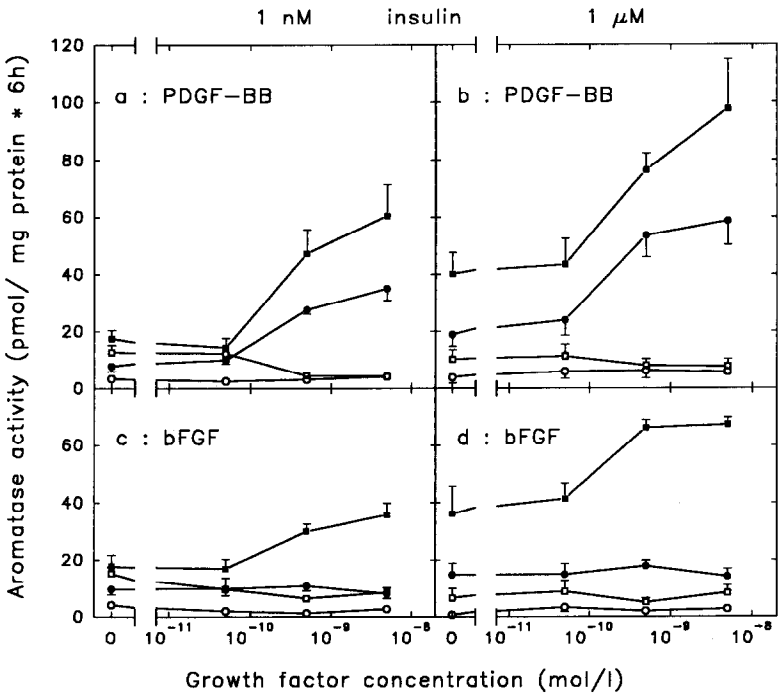

Fig. 2. Influence of PDGF-BB and bFGF on aromatase induction. Confluent human breast adipose tissue stromal vascular cells were pretreated in SF-medium and aromatase activity was induced under serumfree conditions as described in section 2. The experiments were done in the presence of physiological (1 nM, panels a,c) or supraphysiological (1 μM, panels b,d) insulin concentrations using either recombinant human PDGF-BB (panels a,b) or bFGF (panels c,d) as growth factors. Aromatase activity was determined without further additions (○) or in the presence of 1 μM cortisol (●), 1 mM db-cAMP (□) or cortisol together with db-cAMP (■). The results represent the means of duplicate assays from 4 independent experiments ± S.E.M. Information regarding the significance of differences are given in the text.

cells, neither alone nor together with cortisol. However the failure of bFGF to increase aromatase activity together with cortisol could be overcome by addition of db-cAMP (Fig. 2, panels c,d). Again treatment of cultures with 1 μM insulin resulted in higher aromatase activities as compared to treatment with 1 nM. An inhibitory effect of bFGF on db-cAMP induced aromatase activity was observed only when 1 nM insulin was present.

4. Discussion

To our knowledge we present in this paper the first data on the regulation of aromatase activity in breast adipose tissue stromal cells. Like in subcutaneous stromal vascular cells kept under serum-free culture conditions [20], growth factors inhibit, in the absence of cortisol and at physiological concentrations of insulin, aromatase induction by db-cAMP. In serum-free cultures, an effect of cortisol on aromatase activity was observed when PDGF-BB was added. This might indicate that PDGF or a substance with a similar mode of action could be the serum component necessary for the cortisol effect. At the maximal effective concentration of PDGF-BB even higher aromatase activities were obtained as compared to incubations with 10% FCS. Addition of db-cAMP together with cortisol at all PDGF-BB concentrations used consistently led to higher aromatase activities as compared to cultures kept without db-cAMP.

With respect to the receptor type used and the mode of action, PDGF and bFGF share some similarities [21]. Despite this fact, bFGF was not able to support the cortisol effect on aromatase activity in a similar way as PDGF or serum. At all FGF-concentrations tested, cortisol together with db-cAMP was necessary to get an increase of aromatase activity in stromal vascular cells.

These results indicate that aromatase activity of stromal vascular cells from breast adipose tissue is under multifactorial control. Glucocorticoids alone are not effective, although glucocorticoid responsive elements have been detected in the aromatase gene [22]. The fact that PDGF, which alone, like cortisol, has no influence on aromatase activity, together with cortisol leads to an increase of aromatase activity indicates, that a tyrosine kinase linked pathway is involved in aromatase activation. When bFGF is used instead of PDGF-BB, db-cAMP together with cortisol has to be added to cultures in order to get an aromatase activation. This is suggestive for an additional participation of cyclic AMP. The difference in the action of PDGF and FGF respectively could be explained by the speculation, that stromal vascular cells respond to PDGF, but not to FGF with an increase of cyclic AMP. Alternatively cAMP-analogues like db-cAMP might lead to an activation of the same downstream signalling molecules as PDGF does in the presence of cortisol, whereas bFGF would lack the possibility of activating this signalling pathway.

The complexity of regulation of aromatase activity in adipose tissue stromal cells is obvious, when the effect of growth factors and db-cAMP at physiological insulin concentrations in the absence and in the presence of cortisol are compared. The presence of glucocorticoid seems to be a prerequisite for a switch in the mode of action of growth factors, as their inhibitory effect in the absence of cortisol turns into a strong stimulatory effect in its presence.

The fact finally, that in the presence of micromolar insulin concentrations consistently higher aromatase activities were observed as compared to cultures at physiological insulin concentrations indicates, that IGF-I could be involved in the regulation of aromatase activity. Lueprasitsakul et al. [23] recently were able to demonstrate a small increase of aromatase activity of subcutaneous stromal vascular cells by high concentrations of IGF-I in the presence of serum and dexamethasone. Mahendroo et al have shown very recently that different promotors for the aromatase gene exist in stromal vascular cells from various sources and that the usage of the promotors depends on the hormonal treatment of the cells [24]. By RACE-PCR techniques they could demonstrate that the 5′-untranslated regions of aromatase gene transcripts in stromal vascular cells differ, when RNA from cells treated with dexamethasone and serum was compared with RNA from db-cAMP treated cells kept in the absence of serum. One could speculate from our findings that PDGF is important for the choice of the cortisol-dependent promotor.

The results presented here strongly support the hypothesis, that tumor cells are able to induce elevated aromatase activity in surrounding adipose tissue by a paracrine mechanism. Whether PDGF is the inducing agent provided by tumors in vivo has to be demonstrated. However, there might exist several still unknown factors, which are able to replace PDGF [16]. In view of our results one can speculate, that PDGF might be (the or one of) the serum component(s), responsible for glucocorticoid induction of aromatase. The increase of aromatase activity in adipose tissue with age of patients observed by Cleland et al. [25] could then be explained as a result of an increase in PDGF-receptor numbers on stromal cells or of higher PDGF concentrations available in the tissue.

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