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Expression and Regulation of Aromatase and 17β-Hydroxysteroid Dehydrogenase Type 4 in Human THP 1 Leukemia Cells

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Estradiol is active in proliferation and differentiation of sex-related tissues like ovary and breast. Glandular steroid metabolism was for a long time believed to dominate the estrogenic milieu around any cell of the organism. Recent reports verified the expression of estrogen receptors in "non-target" tissues as well as the extraglandular expression of steroid metabolizing enzymes. Extraglandular steroid metabolism proved to be important in the brain, skin and in stromal cells of hormone responsive tumors. Aromatase converts testosterone into estradiol and androstenedione into estrone, thereby activating estrogen precursors. The group of 17β-hydroxysteroid dehydrogenases catalyzes the oxidation and/or reduction of the forementioned compounds, e.g. estradiol/estrone, thereby either activating or inactivating estradiol. Aromatase is expressed and regulated in the human THP 1 myeloid leukemia cell line after vitamin D/GMCSF-propagated differentiation. Aromatase expression is stimulated by dexamethasone, phorbolesters and granulocyte/macrophage stimulating factor (GMCSF). Exons I.2 and I.4 are expressed in PMA-stimulated cells only, exon I.3 in both PMA- and dexamethasone-stimulated cells. Vitamin D-differentiated THP 1 cells produce a net excess of estradiol in culture supernatants, if testosterone is given as aromatase substrate. In contrast, the 17β-hydroxysteroid dehydrogenase type 4 (17β-HSD 4) is abundantly expressed in unstimulated THP 1 cells and is further stimulated by glucocorticoids (2-fold). The expression is unchanged after vitamin D/GMCSF-propagated differentiation. 17β-HSD 4 expression is not altered by phorbolester treatment in undifferentiated cells but is abolished after vitamin D-propagated differentiation along with downregulation of β-actin. Protein kinase C activation therefore appears to dissociate the expression of aromatase and 17β-HSD 4 in this differentiation stage along the monocyte/phagocyte pathway of THP 1 myeloid cells. The expression of steroid metabolizing enzymes in myeloid cells is able to create a microenvironment which is uncoupled from dominating systemic estrogens. These findings may be relevant in the autocrine, paracrine or iuxtacrine cellular crosstalk of myeloid cells in their respective states of terminal differentiation, e.g. in bone metabolism and inflammation.

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INTRODUCTION

Estrogens are active in cell proliferation and differentiation. Like other steroids they do exert genomic effects via intracellular estrogen receptors (ER) and extragenomic actions via membrane effects. Estrogen receptor proteins have primarily been characterized as specific binding proteins for estradiol [1, 2] and in the

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Abbreviations: DHEA, dehydroepiandrosterone; ER, estrogen receptor; FCS, fetal calf serum; GAPDH, glyceraldehyde phosphate dehydrogenase; GMCSF, granulocyte/macrophage colony stimulating factor; 17β-HSD 4, 17β-hydroxysteroid dehydrogenase type 4; PMA, phorbol-myristate-acetate; SCAD, short chain alcohol dehydrogenase; T3, trijodothyronine.

following three decades were found to be hormone-mediated transcription factors belonging to the steroid hormone receptor family [3–6]. Sex steroids are modified within endocrine glands like ovary and testes and active hormones are episodically or constantly secreted into the circulation, e.g. throughout the menstrual cycle. Steroid hormone metabolism is carried out by enzymes like members of the cytochrome *P*450 family [7–10] and the short chain alcohol dehydrogenase family [11–19]. The main target tissues for estrogens are sex-related tissues like endometrium, ovary, breast, testis and prostate. Estradiol secreted by glandular tissues was for a long time believed to be the sole source of active estrogens, dominating any responsive cell of the organism. In recent years evidence has accumulated for not only the ER to be expressed in many so called "non-target" tissues like for example malignant melanoma, cells of hematopoetic and lymphatic origin, bone tissues and brain [20–37], but also for the extraglandular expression of steroid-modifying enzymes [38–45]. The skin for example was shown to express the complete set of enzymes required for extraglandular synthesis of estradiol as well as dihydrotestosterone from systemic dihydroepiandrosterone(sulfate) [39, 44 and references therein, 45]. Extraglandular aromatization of testosterone in certain brain areas was demonstrated to be important for mating behaviour [37, 42]. Aromatase activity of stromal cells from mammary tumor tissue proved to propagate tumor progression in the presence of estradiol precursors [41].

The expression of estrogen receptors in "non-target" tissues has been a matter of debate over a decade. The demonstration of expression proved to be very difficult for reasons of low abundance of receptor molecules and the relevance of those phenomena has been doubted. Now the accumulated data speak in favour of ER expression in non-target tissues and there are some tissues where these phenomena appear to be relevant [for review see 22]. One major argument against biological relevance of low abundant ER has always been the lack of evident effects in women throughout the menstrual cycle. The demonstration of extraglandular expression of hormone-modifying enzymes in recent years gives way to new concepts of local regulatory systems creating their own microenvironment and thereby uncoupling local from systemic events.

Aromatase (cytochrome $P450_{arom}$) expression was shown to be regulated by biologically active compounds like glucocorticoids and cAMP mediated signal transduction in several systems. Stimulus-specific aromatase regulation was in addition shown to be dependent on the stage of differentiation, e.g. cAMP proved to either stimulate, suppress or to show no effect on aromatase expression during follicle development [38, 39, 43, 46–52]. Extraglandular expression in adipose tissue was a very early model for studies of aromatase regulation [38]. Aromatase in human systems comprises multiple untranslated exons I and is regulated by different promoters. The differential expression of different exons I in fetal tissues has recently been reported [40]. Thus in the human (in contrast to the rat) aromatase regulation appears to be very complex and the accessibility of different promoters possibly allows rapid switching from stimulation to suppression through identical compounds in different stages of differentiation. The gene product aromatizes androstenedione into estrone and testosterone into estradiol, thereby activating estradiol precursor steroids.

The activation of estradiol precursors into estradiol is not only performed by aromatase but also by 17β-hydroxysteroid dehydrogenases (17β-HSD). They are capable of catalyzing reversible conversions of estradiol to estrone depending on the enzyme, cofactor and the substrate/product concentrations [11–19]. Thus in terms of biological activity on genomic estradiol actions these enzymes are potentially ambiguous. Their potential in cancer propagation is confirmed by reports about aromatization activity in stromal cells of hormone responsive tumors as well as by clinical data about the anticancer efficiency of aromatase inhibitors in postmenopausal women. To date four different human 17β-HSDs have been described. Whereas types 1, 2 and 3 seem to participate in the steroid synthesis, the type 4 enzyme preferentially inactivates estradiol into estrone (360-fold preference) and was found to interact with the actin. Its further unusual property is that its mRNA and gene product comprises three domains which display sequence similarities with short chain alcohol dehydrogenase family, enzymes for peroxisomal fatty acid β-oxidation and sterol carrier proteins, respectively [17, 18, 53–59].

Human myeloid precursor cells differentiate into two main pathways of differentiation: the granulocyte pathway and the monocyte/phagocyte pathway. From the promyelocyte stage of differentiation, the two pathways diverge into the two directions. *In vitro* and *in vivo* retinoic acid and calcitriol are potent compounds to mediate irreversible commitment for the respective differentiation pathway. These compounds can be substituted by several others, which are redundant in terms of signal transduction, as for example the activation of protein kinase C through phorbol esters can partly substitute for calcitriol [60–65]. The monocyte/phagocyte system is a system of considerable plasticity and a great number of monocytoid cells and specific tissue-macrophages can develop therefrom [66–68]. In terms of estradiol metabolism and sensitivity the monocyte/phagocyte system is more important. Monocytes have been reported to be estradiol target cells, several parameters of immune response are mediated by sex steroids, and autoimmunity in animals can be prevented by testosterone [26, 28, 29, 69]. T-cells and monocytes are the relevant cells to mediate these effects, the molecular mechanisms of which

remain to be elucidated. Of all the forms of macrophages, the osteoclasts are the most important estrogen target cells. They have been reported to express ER and several target genes like, for example, lysozyme have been identified. Their bone resorbing activity has been reported to be suppressed by estradiol [33–36]. The local regulation of estradiol generation may therefore be of interest in the determination of bone resorption activity in an individuum throughout life and may contribute to the pathogenesis of bone disorders like osteoporosis.

We have previously reported on the characterization of porcine endometrium estrogen receptor and on the expression of ER in non-target tissues derived from the lymphatic and hematopoetic system [31, 70–72] and on aromatase expression in vitamin D-differentiated myeloid cells [43, 65]. We report here the expression and regulation of aromatase CYP *P*450 and 17β-HSD 4 in a differentiation system of THP 1 human myeloid leukemia cells.

EXPERIMENTAL

Materials

THP 1 (monocytic) human myeloid leukaemia cell lines were from ATCC (Rockville, MD, U.S.A.). 1,25-dihydroxycholecalciferol was from Duphar (Netherlands). Estradiol, estrone, testosterone, androstenedione, 4-OH-androstenedione, androstenediol, dehydroepiandrosterone (DHEA), dexamethasone, triamcinolone, hydrocortisone, retinoic acid, trijodothyronine (T3), clofibrate (2-(*p*-chlorophenoxy)-2-methylpropionic acid etyl ester), cyclic adenosine-monophosphate (cAMP), forskolin, phorbol-myristate-acetate (PMA) and granulocyte/macrophage colony-stimulating factor (GMCSF) were from Sigma (München, Germany). Interleukin 1α and specific monoclonal antibodies against IL 1α and β, RPMI medium, glutamine, fetal calf serum and Random-priming Kit were from Gibco (Gaithersburg, U.S.A.). γ^{32}P-ATP and α^{32}P-ATP/CTP were from Amersham (Braunschweig, Germany). Gene-Screen Plus membranes were from Dupont, Boston, U.S.A. For CD 14 Northern analysis a 25 base antisense oligonucleotide (bases 1358–1333) [62] was used. A complete human aromatase cDNA (full length cDNA, 3030 b hA-24 clone from human placenta) was donated by Dr Harada (Aichi, Japan). A cDNA-fragment of this complete cDNA was amplified by PCR ranging from base 700–1353 using the respective 24 b-framing oligonucleotides according to the published sequence. Either the complete cDNA or this fragment were used for hybridization experiments.

For 17β-HSD 4 hybridization a cDNA fragment ranging from bases −48–999 was used, which was obtained from a human placenta library [59].

Radioimmunoassay for estradiol was from Biermann (Bad Nauheim, Germany). All other chemicals were grade A from Merck (Darmstadt), Boehringer (Mannheim) and Serva (Heidelberg).

Cell culture

Cell lines were cultured at 37°C in a humid atmosphere (5% CO_2) in RPMI medium with 5% fetal calf serum (FCS). Polypeptides were dissolved in RPMI medium. Steroids (10^{-3} mol/l) were dissolved in pure

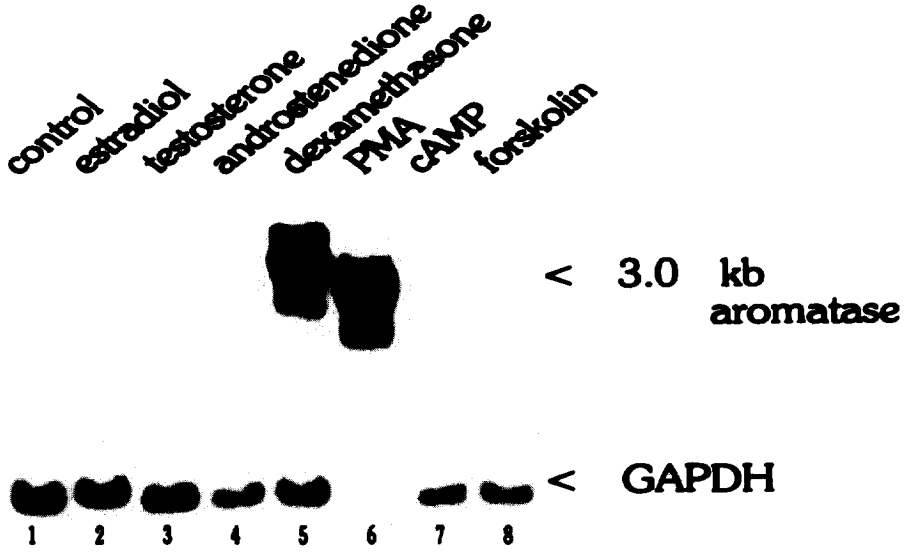

Fig. 1. Regulation of expression of aromatase cytochrome *P*450 mRNA in undifferentiated human THP 1 leukemia cells. Very low levels of basal 3.0 kb aromatase mRNA could be detected when membranes were overexposed. In contrast to vitamin D-differentiated cells [43] no stimulation through sex steroids was observed. Phorbolester markedly stimulated this mRNA species, dexamethasone stimulated a different species of approx. 4.0 kb and a second one of approx. 4.8 kb in this experiment, but the marked shift to higher molecular weight mRNA species was not consistently observed (see also Fig. 2). cAMP and forskolin had no effect on aromatase mRNA expression.

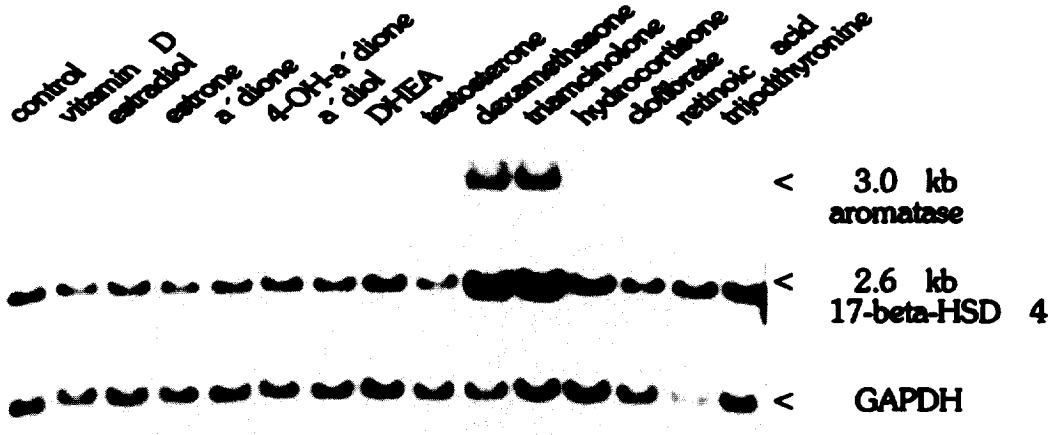

Fig. 2. Expression of aromatase cytochrome *P*450 and 17β-HSD 4 and regulation by (seco)steroidal compounds, trijodothyronine (T3) and clofibrate in undifferentiated human THP 1 myeloid leukemia cells. Basal expression of aromatase appeared to be very low to undetectable, whereas 17β-HSD 4 mRNA was expressed in comparably high levels. Both enzymes were stimulated 2–5-fold by the synthetic glucocorticoids dexamethasone and triamcinolone when compared to controls and normalized to GAPDH expression. All other compounds tested did not significantly stimulate nor inhibit the mRNA expression of both enzymes in undifferentiated cells.

ethanol and directly added to the culture dishes. Final ethanol concentration did not exceed 0.01% (v/v).

(m)RNA preparations

Extraction of total RNA was performed with the single-step guanidinium thiocyanate procedure described by Chomczynski and Sacchi [73]. mRNA was prepared from this total RNA with oligo (dT) beads from Diagen using buffers and working instructions from the supplier.

Labelling procedures and hybridization

Random primed labelling and oligonucleotide end-labelling was performed according to Sambrook *et al.* [74] and instructions by the supplier (Gibco (Gaithersburg, U.S.A.)). Hybridization was done at 42°C in 25% formamide (v/v), 1% sodium dodecylsulfate (w/v), 1 mol/l sodiumchloride, 10% v/v dextranesulphate. Hybridized filters were consecutively washed in 2 × SSC, 1% sodium dodecylsulfate at increasing stringency (58 and/or 60°C) for all hybridization procedures described [74].

Densitometry

Densitometry was performed on a Froebel Video-Densitometer, (Froebel, Germany). Video pictures of autoradiographic films from rehybridized membranes were processed and bands quantified in relative optical density units normalized to the amount of β-actin mRNA (software "biprofile", distributed by Froebel, Germany).

Differentiation of THP 1 cells

For differentiation stimulation THP 1 cells were seeded at a density of 1×10^7 cells/40 ml RPMI/5% FCS and cultured in the presence of vitamin D (10^{-7} mol/l). Medium was changed every 3 days. Concentrations of substances for regulation experiments were: 10^{-7} mol/l for 1,25-dihydroxycholecalciferol, estradiol, estrone, testosterone, androstenedione, 4-OH-androstenedione, androstenediol, DHEA, dexamethasone, triamcinolone, hydrocortisone, T3, clofibrate; cAMP was added 10^{-3} mol/l, retinoic acid and forskolin were added 10^{-5} mol/l; PMA final concentration was 6 ng/ml.

RESULTS

THP 1 human myeloid leukemia cells in their undifferentiated state expressed very low or undetectable levels of aromatase mRNA. Aromatase mRNA expression could be markedly stimulated by dexamethasone and phorbolester in undifferentiated cells (Fig. 1). The main 3.0 kb message stimulated by PMA was shifted towards higher molecular weight compounds by dexamethasone, probably due to alternative splicing events. This effect was persistent after 72 h of vitamin D treatment [shown in ref. 43], but was not consistently observed to the same extent (see Fig. 2) and was less prominent in cells differentiated with vitamin D/GMCSF for 10 days (see Fig. 3).

A series of other (seco)steroidal compounds, clofibrate and trijodothyronine did not modulate aromatase mRNA expression in wild-type cells as shown in Fig. 2. We have already reported on the stimulation of aromatase mRNA expression and aromatase activity by estradiol, androstenedione, testosterone, dexamethasone and PMA after differentiation of cells with vitamin D for 72 h [43]. If we cultured cells in the presence of vitamin D and GMCSF from day 4–10 a very similar pattern of stimulation could be shown compared to day 4 of differentiation (Fig. 3), except that estradiol did

not significantly stimulate aromatase mRNA. GMCSF itself yielded a significant 5-fold increase in aromatase mRNA expression on day 3 of differentiation (not shown).

The aromatization capacity of cells was already reported to mirror mRNA expression when testosterone was added to the culture system and the generation of estradiol was measured in culture supernatants [43].

The expression of β-actin as a housekeeping gene was obviously regulated after induction of differentiation in this cell system when compared to the expression of glyceraldehyde phosphate-dehydrogenase (GAPDH) or the 18 S bands in ethidium bromide stained RNA agarose gels. Especially the addition of PMA appeared to downregulate β-actin expression (Fig. 3) after cell differentiation, which we already speculated on when we reported our experiments with cells differentiated for 72 h with vitamin D [43]. No significant effect of PMA on β-actin regulation was seen in wild-type cells (not shown).

17β-HSD 4 mRNA was expressed in undifferentiated THP 1 cells in comparably high levels. From a series of (seco)steroidal compounds, clofibrate and trijodothyronin, only dexamethasone and triamcinolone added to the already high basal expression by approximately doubling the mRNA levels as measured by densitometry (Fig. 2). Hydrocortisone may slightly but not significantly elevate 17β-HSD 4 mRNA levels. Sex steroids and their precursors did not modulate basal 17β-HSD 4 mRNA expression.

In contrast to the lack of PMA effects in undifferentiated cells on 17β-HSD 4 mRNA and β-actin mRNA expression, PMA abolished 17β-HSD 4 mRNA expression after calcitriol propagated cell differentiation for 72 h (not shown) and by day 10 of differentiation (Fig. 3). This effect was coincident with the downregulation of β-actin mRNA levels by PMA, which was certified by the unchanged expression of GAPDH as a second housekeeping gene. When taking GAPDH as the relevant parameter of normalization there was no significant effect on 17β-HSD type 4 mRNA upon the addition of estradiol, cAMP and forskolin. No significant effect occurred upon the addition of testosterone, androstenedione and dexamethasone in this stage of differentiation.

In undifferentiated THP 1 myeloid leukemia cells there appears to be a prevailing "antiestrogenic" situation, since 17β-HSD 4 mRNA is expressed in comparably high levels. In situations of stimulation of aromatase mRNA there is either coincident expression

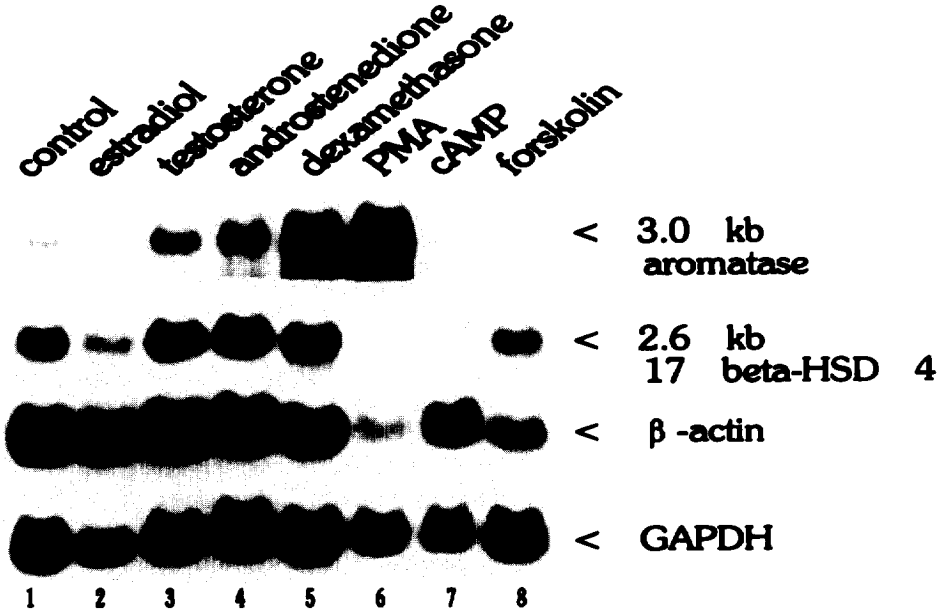

Fig. 3. Expression of aromatase cytochrome *P*450 and 17β-HSD 4 and regulation by sex steroids, dexamethasone, phorbolester and cyclic AMP in human THP 1 myeloid leukemia cells differentiated by vitamin D/GMCSF for 10 days. Cells were treated with 1,25 dihydroxycholecalciferol (10^{-7} mol/l) throughout, hormone was added once every 72 h with medium change. Granulocyte/macrophage-stimulating factor (GMCSF 0.5 ng/ml) was added from days 5–10 with medium renewal. Aromatase cytochrome *P*450 mRNA but not 17β-HSD 4 mRNA was stimulated (approx. 2-fold) by testosterone and androstenedione. No significant effect on either enzyme was seen upon estradiol treatment. Aromatase mRNA was stimulated 15–45-fold, respectively, by dexamethasone and PMA. 17β-HSD 4 mRNA was again stimulated 2-fold by dexamethasone but was completely abolished by PMA in contrast to undifferentiated cells (not shown). Along with 17β-HSD 4 downregulation β-actin expression was markedly inhibited when compared to a second housekeeping gene GAPDH. cAMP and forskolin again did not show any significant effect.

of both enzymes or a parallel stimulation. Only in the situation of PMA-stimulation of cells is there an absolutely "estrogenic" situation, since 17β-HSD 4 mRNA is completely abolished along with down-regulation of β-actin.

DISCUSSION

Estradiol metabolism has become very complex. It is very intensely linked with androgen metabolism, since both testosterone and androstenedione can be converted into the most active estrogen, estradiol. Estradiol can be activated from testosterone by aromatization through aromatase cytochrome *P*450 and from estrone by reduction through 17β-HSDs. The inactivation of estradiol to estrone is carried out by several 17β-HSDs. Type 1 and 2 reversibly oxidize estradiol or reduce estrone, depending on the coenzyme, pH and substrate and/or product concentration [13, 15, 16]. 17β-HSD type 3 almost exclusively converts estrone to estradiol and appears to be specific for testis [12]. Type 4 enzyme preferentially converts estradiol into estrone [17, 18, 53–59]. A fifth enzyme was very recently cloned, which also reveals 17β-HSD activity [75]. This enzyme shows very similar substrate and cofactor specificity to type 2 17β-HSD and from sequence similarities was reported to belong to the aldo-ketose reductase family rather than to the SCAD-family. The widespread expression in peripheral tissues of aromatase and 17β-HSD 4 points to the importance of extraglandular hormone modification.

In addition to placenta, ovary and testis, aromatase expression has so far been described in brain, adipose tissue, adrenals and the stromal cells of human mammary tumor tissue and was recently demonstrated in myeloid cells [38–43]. In human systems several differentially expressed exons I and their supposed different promoters have been characterized. According to these possibilities of pleiotropic regulation, aromatase expression is differentially regulated depending on the stimulus and the stage of differentiation. The most important biologically active agents are phorbolesters, cAMP (and all events driven by ligands, which act via cAMP like growth factors), dexamethasone, sex steroids and interleukin 1 [46–52]. In part these compounds exert ambiguous effects on aromatase expression, depending as well on the actual stage of differentiation. We have recently described aromatase expression in cells of myeloid origin (THP 1 human monocytoid leukemia). We found different species of mRNA, which in part appeared to be due to so far unknown splicing events. Exons I.2 and I.4 were expressed in PMA-stimulated cells only, whereas no product was seen in the dexamethasone-stimulated ones. Strong signals for exon I.3 were found in both the dexamethasone- and PMA-stimulated cells. Aromatization of testerone as substrate mimicked changes in mRNA expression [43]. In HL 60 cells we found identical results for aromatase mRNA expression but did not detect aromatization activity in the assay applied (our unpublished observation). Thus aromatase cytochrome *P*450 is expressed and regulated in these cells of myeloid origin in their states of monocyte/phagocyte differentiation. Expression and regulation persists in long term cultures along this differentiation pathway.

Human and porcine 17β-HSD 4 were characterized as almost exclusively oxidizing enzymes [54, 55, 59]. 17β-HSD 4 exhibits some unique properties in that its mRNA codes for a 80 kDa protein comprising three domains: 17β-estradiol dehydrogenase; a central domain similar to some enzymes of the β-oxidation pathway of fatty acids; and a C-terminal domain similar to a sterol carrier protein 2 [17]. A minor amount of the extractable protein was shown to be covalently linked to actin via a ε(γ-glutamyl)-lysine bond [57]. The enzyme was shown to be packed in peroxisomes [56]. The actin anchor of the protein was suggested to be involved in the positioning of the peroxisomes according to metabolic requirements.

We show here, that 17β-HSD 4 mRNA is expressed in THP 1 cells, derived from the myeloid lineage. In undifferentiated cells the level of 17β-HSD mRNA appeared to be abundant. There was a single band at ~2.6 kb, which was merely regulated in this stage of differentiation. The only regulating steroidal compounds appeared to be synthetic glucocorticoids, which also upregulate aromatase expression. Whether these effects are extragenomically or genomically mediated effects is unclear at present. There is no significant effect of cAMP and sex steroids on 17β-HSD 4 regulation. The basal level of expression might however be too high to demonstrate stimulatory effects and this basal expression might already be stimulated by compounds derived from fetal calf serum, most probably progesterone. Further experiments exploiting steroid-free medium are in progress.

In undifferentiated THP 1 cells, PMA does not exert a significant influence on 17β-HSD 4 and β-actin expression. After vitamin D-stimulated cell differentiation however both compounds are down-regulated by PMA-treatment. This phenomenon persists with cell differentiation along the phagocyte pathway, e.g. GMCSF-stimulation. The coincident down-regulation of β-actin expression and 17β-HSD 4 expression can only speculatively be interpreted. It is interesting with respect to the reported covalent linkage of β-actin and 17β-HSD 4 and the speculated mechanism of organelle positioning. If β-actin is downregulated, the specialized organelles possibly can not be positioned and topologically uncontrolled estradiol oxidation might not be warranted. In addition in this situation the marked stimulation of aromatase expression by phorbolesters creates an absolutely pro-estradiol situation. When we tested the aromatization capacity of THP 1 cells we did however not realize a significant difference

between the PMA stimulated and the dexamethasone-stimulated cells, which should have been expected with respect to the comparably high levels of 17β-HSD 4 expression in the dexamethasone-stimulated cells in comparison with that stimulated with PMA. A possible explanation would be that the abundant number of estradiol molecules generated by aromatase activity are not shuttled into the compartmentalized region of 17β-HSD 4 activity. The question of the accessibility or the route to the peroxisome vesicles is so far unresolved. If the theory of compartmentalized "post-nucleus" estradiol oxidation is correct, estradiol would not be reshuttled under normal conditions. The phorbolester-stimulation of these cells might be a special situation, where estrogens are essential and may be reused or produced for the cells in the neighbourhood. A second possibility of interpretation might be the short time of downregulation of 17β-HSD 4 mRNA (24 h), which possibly does not exceed the protein turnover of presynthesized 17β-HSD 4, so that there is not yet a difference in steroid metabolism when stimulation by dexamethasone and PMA are compared.

The physiological relevance of the expression of both ER and estradiol modifying enzymes in myeloid cells and their respective counterparts in inflammation and bone metabolism, e.g. lymphoid cells and as well osteoblasts appears to be obvious. Although the level of expression of these enzymes in the exact stages of terminal differentiation is not yet clear, the estrogenic milieu created by osteoclast precursors would be as important as the expression in active osteoclasts. Deficient or intact local rather than systemic delivery of estrogens might for example be an important condition for the development of osteoporosis, since only approximately one third of women develop osteoporosis in spite of the general systemic loss of estradiol in menopause. The expression and regulation of the two counteracting enzymes aromatase and 17β-HSD type 4 might well determine the estrogenic microenvironment and may be important in terms of intracrine and paracrine effects in bone metabolism and inflammation.

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