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Role of 17β-Hydroxysteroid Dehydrogenase Type 1 in Endocrine and Intracrine Estradiol Biosynthesis

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Enzymes with 17β-hydroxysteroid dehydrogenase (17β-HSD) activity catalyse reactions between the low-active female sex steroid, estrone, and the more potent estradiol, for example. 17β-HSD activity is essential for glandular (endocrine) sex hormone biosynthesis, but it is also present in several extra-gonadal tissues. Hence, 17β-HSD enzymes also take part in local (intracrine) estradiol production in the target tissues of estrogen action. Four distinct 17β-HSD isozymes have been characterized so far, and the data strongly suggests that different 17β-HSD isozymes have distinct roles in endocrine and intracrine metabolism of sex steroids. Current data suggest that 17β-HSD type 1 is the principal isoenzyme involved in glandular estradiol production both in humans and rodents. During ovarian follicular development and luteinization, rat 17β-HSD type 1 is regulated by gonadotropins, and the effects of gonadotropins are modulated by steroid hormones and paracrine growth factors. Human 17β-HSD type 1 favors the reduction reaction, thereby converting estrone to estradiol both *in vitro* and in cultured cells. Hence, the enzymatic properties of the enzyme are also in line with its suggested role in estradiol biosynthesis. Interestingly, 17β-HSD type 1 is also expressed in certain target tissues of estrogen action such as normal and malignant human breast and endometrium. Hence, 17β-HSD type 1 could be one of the factors leading to a relatively high tissue/plasma ratio of estradiol in breast cancer tissues of postmenopausal women. We conclude that 17β-HSD type 1 has a central role in regulating the circulating estradiol concentration as well as its local production in estrogen target cells.

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INTRODUCTION

17β-Hydroxysteroid dehydrogenase (17β-HSD) activity is responsible for the interconversion of 17-ketosteroids and 17β-hydroxysteroids. Thus, enzymes with 17β-HSD activity catalyse the reactions between the low-active female sex steroid, estrone, and the more potent estradiol, for example. 17β-HSD activity is essential for glandular (endocrine) sex hormone biosynthesis, but the enzyme activity is also present in several extra-gonadal tissues. Hence, it is also evidently responsible for local (intracrine) estradiol production from the circulating low active prehormones in the target tissues of estrogen action [1].

Four distinct 17β-HSD isozymes have been characterized so far [2–5]. The enzymes belong to the short-chain alcohol dehydrogenase (SCAD) family, and use nicotinamide adenine dinucleotide [NAD(H)] and/or its phosphate [NADP(H)] as cofactors. Members of the family include prokaryotic and eukaryotic enzymes involved in nitrogen fixation, metabolism of sugars, steroids, aromatic hydrocarbons and prostaglandins, as well as the synthesis of antibiotics [6, 7]. It is apparent that the various human 17β-HSD enzymes have different substrate and cofactor specificities, tissue distributions and subcellular localizations, and they also possibly catalyze predominantly opposite reactions *in vivo* [2–5, 8–11]. This strongly suggests that different 17β-HSD isozymes have distinct physiological functions, and the role of each of the enzymes in endocrine and intracrine metabolism of sex steroids is currently being intensively investigated. The present review is focused on the recent findings clarifying the role of 17β-HSD type 1 in these metabolic pathways.

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PRIMARY STRUCTURE AND BIOCHEMICAL PROPERTIES OF 17β-HSD TYPE 1

The cDNA for the human 17β-HSD type 1 has been isolated independently in three laboratories [2, 10, 12], and in addition we have recently clarified the primary structure of the rat type 1 isoform [13]. The open reading frame of the human cDNA encodes a polypeptide of 327 amino acids with a predicted molecular weight of 34,853 Da, while the rat enzyme extends 17 amino acids beyond the human sequence at the carboxy terminus. The most abundant form of 17β-HSD type 1 is a dimer consisting of two identical subunits [8, 14]. The dimer has one substrate-binding site and one cofactor-binding site per monomer [15, 16]. It has been shown that the monomers in the enzyme dimer are not covalently bound by disulfide bridges [8, 16]. Thus dimerization is dependent on the concentration and affinity of the monomers, but whether the enzyme is catalytically active in the monomeric form is not known. The enzyme dimer has been shown to exist in 3–5 different forms with different isoelectric points [15], but the reason(s) for microheterogeneity remain(s) to be clarified. Furthermore, two genetic variants (Ser312<->Gly) with equal frequencies have been detected for human 17β-HSD type 1 in all the populations studied so far [17, 18], and in addition, a rare substitution of alanine by valine at position 237 has been identified [18]. The catalytic properties of the enzyme variants are, however, identical [11].

17β-HSD type 1 contains 5 conserved domains (A–D) common to the members of the SCAD-family [6, 7]. The importance of the conserved domains for enzyme activity is supported by the fact that in these regions, consisting of 86 amino acids altogether, the identity and similarity between the rat and human enzymes are 93 and 98%, respectively [13]. The three-dimensional structure of one of the members of the SCAD-family (3α,20β-HSD), together with the current model of the common reaction mechanism of the enzymes, indicates that of these highly conserved amino acids at least serine^{142}, tyrosine^{155} and lysine^{159} are located at the catalytic site of human 17β-HSD type 1 [6, 7, 19]. Studies with site-directed mutagenesis have further confirmed the critical role of the tyrosine residue for activity of several of the SCAD-family enzymes including 17β-HSD type 1 [11, 7 and refs therein]. In contrast to the amino acids mentioned above, the three histidines (His) in human 17β-HSD type 1 (His^{210}, His^{213}, His^{221}), which have been suggested to participate in substrate-binding of 17β-HSD type 1 [20], are poorly conserved in the corresponding rat enzyme. Furthermore, mutations of two of these histidines, His^{210} and His^{213}, either separately or simultaneously to alanine, did not considerably decrease the catalytic activity. Hence, it is unlikely that these amino acids participate in the catalytic reaction of 17β-HSD type 1. However, a notable reduction in the catalytic activity of the enzyme was observed when His^{221} was mutated to alanine [11]. This is in line with previous findings [20] suggesting that although His^{221} does not directly participate in hydrogen transfer between the cofactor and the substrate, it might play a role in substrate-binding or orientation in 17β-HSD type 1 [11]. We have recently studied the role of seven different amino acids in the catalytic activity of human 17β-HSD type 1, and the results are summarized in Table 1.

Table 1. Mutated human 17β-HSD type 1 enzymes expressed in SF-9 insect cells: effects of the mutations on the catalytic activity

Mutation	Catalytic activity
Cofactor binding domain	
Cys^{54}Ala	no effect
Substrate binding domain	
Tyr^{155}Ala	strongly decreased
His^{210}Ala	no effect
His^{213}Ala	no effect
His^{210}AlaHis^{213}Ala	moderately decreased
His^{221}Ala	strongly decreased
Polymorphisms detected	
Ala^{237}Val	no effect
Ser^{312}Gly	no effect

Kinetic data on catalytic activity have shown that the reaction catalyzed by purified human 17β-HSD type 1 is reversible and specific for estrogenic substrates. The K_m/V_{max}-value for the reductive activity from estrone into estradiol is, however, about 2-fold higher than that for the oxidative activity converting estradiol into estrone [11]. In cultured cells transiently or stably expressing human 17β-HSD type 1, the reductive activity predominates even more significantly, suggesting that the intracellular environment of the cells favors estradiol production by 17β-HSD type 1 [8; Fig. 1]. In cultured cells the enzyme favors estrone over androstenedione (A-dione) as substrate as also observed with the purified enzyme. However, compared with the results from cell-free extracts, a relatively high reductive activity converting A-dione to testosterone is observed [8]. This suggests that in addition to substrate availability, the intracellular environment and possibly cofactor concentrations in the cells may also limit the reaction rate in cultured cells and *in vivo*. Interestingly, both human and rat 17β-HSD type 1 contain a putative site (Ser^{134}) for cAMP-dependent phosphorylation [2, 13]. Furthermore, the human enzyme, produced as a glutathione *S*-transferase fusion protein, has been shown to be phosphorylated exclusively on serine by protein kinase A *in vitro* [21], but the physiological importance of phosphorylation remains to be clarified.

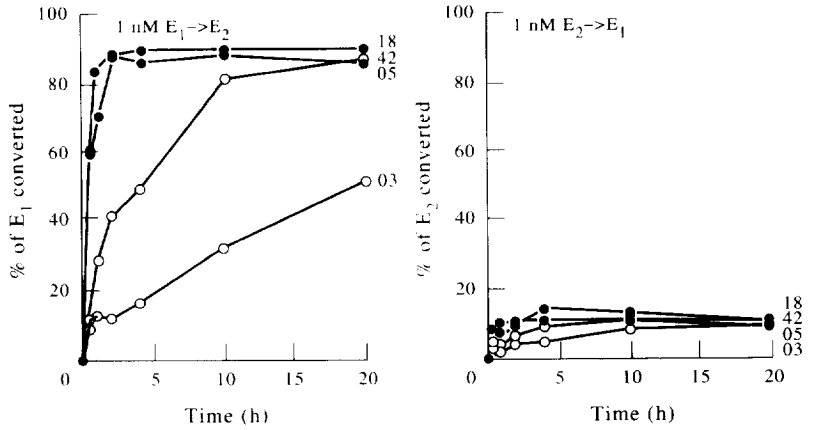

Fig. 1. Reductive (E1->E2) and oxidative (E2->E1) 17β-HSD-activity in T-47D breast cancer cells stably transfected with PSG5 expression vector only (03, 05) or with PSG5-17β-HSD type 1 (18, 42). 400,000 cells/well in 6-well plates were attached overnight. The enzyme activities were then measured in cells cultured in serum-free medium. 1 nM E1 or E2 was used as substrate and the reactions were followed for 20 h. The reductive reaction in the cultured cells strongly predominated and was related to the 17β-HSD type 1 enzyme concentration in the cells.

STRUCTURE OF 17βHSD TYPE 1 GENE AND mRNAs

The total length of the gene encoding human 17β-HSD type 1 (gene EDH17B2) is 3.2 kb, containing six exons and five introns [22, 23]. The EDH17B2 gene is located at the chromosomal loci 17q11–21 [10, 24] in tandem with the gene EDH17BI. The genes share an 89% overall similarity, but in the 5′-region of EDH17B genes, upstream from the cap site for the 1.3 kb 17β-HSD type 1 mRNA, the similarity is exceptionally high (98%). In that region one of the rare differences between the EDH17B genes is a transversion of adenine (EDH17B2) to cytosine (EDH17B1) at position −27 in the putative TATA-box [24]. Interestingly, a similar A to C alteration was also detected in the EDH17B2 gene of a few individuals analyzed. The mutation, when introduced into a reporter gene construct was found to decrease EDH17B2 promoter activity significantly *in vitro* [25], but its effects on 17β-HSD type 1 expression and estradiol production *in vivo*, are not known. The results suggest that the adenine at position −27 is needed for efficient transcription of the EDH17B genes.

Human EDH17B genes are surrounded by Alu-sequences which have been generally proposed as sites of recombination events responsible for the duplication and evolution of new genes [26]. Whether rat 17β-HSD type 1 genes are duplicated in a similar manner remains to be seen. Using a reverse transcriptase–PCR technique, transcripts corresponding to the EDH17B1 gene have also been detected [27], but their physiological significance is not known at present. As a result of the marked dissimilarities between the EDH17B1 and the EDH17B2 genes, e.g. the premature in-frame stop codon in the EDH17B1 gene [22, 23], the function of the EDH17B1 gene product, if any, most likely differs from that of 17β-HSD type 1.

Two transcription start points in the EDH17B2 gene result in two major 17β-HSD type 1 mRNAs, 1.3 and 2.3 kb in size. There is a clear difference in the relative expression of the two human 17β-HSD type 1 transcripts. A low concentration of the 2.3 kb mRNA for the human enzyme is expressed in all the tissues and cell types so far analyzed, including cells with no measurable 17β-HSD type 1 protein [22, 28, 29]. In contrast, the 1.3 kb mRNA, being principally expressed in steroidogenic cells of ovarian and placental origin and in several breast cancer cells, is related to the enzyme concentration [29, 30]. Thus, activation of the promoter which is responsible for expression of the 1.3 kb mRNA, with a shortened 5′-untranslated sequence, is suggested to be the key regulatory event controlling 17β-HSD type 1 concentration. In addition to the TATA-box, a GC-rich area and an inverted CAAT element are located within 100 nucleotides upstream from the transcription start site for human 1.3 kb 17β-HSD type 1 mRNA, suggesting that this region most likely serves as a basal promoter for the 1.3 kb mRNA. On the other hand the promoter for 2.3 kb mRNA is more difficult to define, but the transcription start point lies within an Alu element [22, 23], and typical promoter elements have not been found in this region.

The low basal expression of the 2.3 kb mRNA in a wide variety of cell types and tissues is in line with the finding that promoters without TATA- and CAAT-boxes are typical of house-keeping genes, but recently several other non-house-keeping genes have also been found to contain this kind of promoter [31 and refs therein]. The data also suggest different translational efficiencies for the 1.3 and 2.3 kb mRNAs, and this should be taken into account when 17β-HSD type 1 protein concentration and enzyme activity are predicted based on mRNA measurements. The 2.3 kb

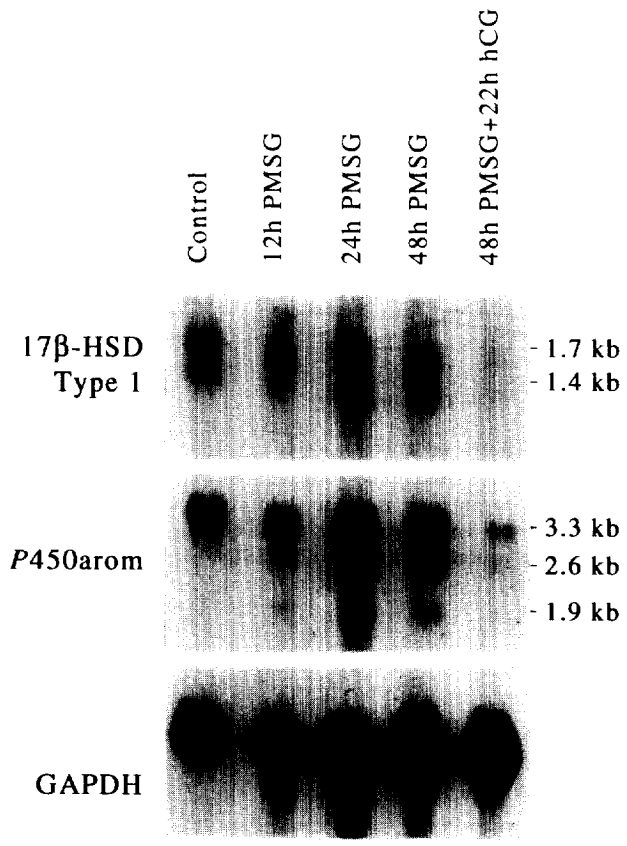

Fig. 2. Northern analysis of 17β-HSD type 1 and *P*450arom expression in ovaries of immature rats treated with PMSG (45 IU/day) followed by hCG (25 IU). 10 μg poly-(A)$^+$ RNA was used. Both 17β-HSD type 1 and *P*450arom expressions were increased by PMSG treatment and down-regulated during luteinization of the follicles brought about by the hCG treatment [data partially from Ref. 34].

mRNA, having an unusually long 5′-untranslated sequence, could contain hairpin structures reducing the translation of the mRNA. In contrast to human 17β-HSD, the two rat 17β-HSD type 1 mRNAs (1.3 and 1.7 kb) are regulated in parallel in all the experimental models so far used [13, 32, 33; Fig. 2]. This suggests, but does not prove, that the rat mRNAs have a common promoter, while the difference in the transcripts could lie at the 3′-non-coding region.

REGULATION OF 17β-HSD TYPE 1 IN STEROIDOGENIC CELLS

Analysis of the tissue distribution of human 17β-HSD type 1 along with type 2 and type 3 mRNAs has shown that of these isozymes, 17β-HSD type 1 is the only form expressed both in human ovary and placenta [2–4, 10, 34]. This, together with the catalytic properties of the enzymes, suggests that 17β-HSD type 1 is the principal isozyme involved in glandular estradiol biosynthesis in females.

In ovarian granulosa cells 17β-HSD type 1 and *P*450 aromatase (*P*450arom) catalyze the final steps in estradiol biosynthesis from theca cell-derived androgens, mainly androstenedione [13, 33–37]. In recent studies directed at regulation of 17β-HSD type 1 in the ovary, we have applied models which have been used previously in analyzing the regulation of *P*450arom expression. Results obtained from studies carried out both *in vivo* and *in vitro* suggest that rat 17β-HSD type 1 and *P*450arom are regulated by interaction of steroid hormones, gonadotropins and paracrine factors such as growth factors, but the enzymes are, at least partially, regulated by distinct mechanisms. The enzymes were, however, expressed in parallel during several hormonal treatments. The studies showed for example that the expression of 17β-HSD type 1 and *P*450arom in rat ovary were induced in parallel by PMSG-treatment and were similarly decreased after hCG-treatment (Fig. 2). Immunohistochemical analysis confirmed that 17β-HSD type 1 expression is low in antral follicles, up-regulated during follicular maturation, and is highest in Graafian follicles. Thereafter, the expression decreases during luteinization and the enzyme was almost undetectable in corpora lutea [32]. A good correlation between human 17β-HSD type 1 and *P*450arom expression was also found in small and large follicles obtained from patients undergoing an *in vitro* fertilization program [34]. The most marked difference found in the regulation of these enzymes is that estrogen (diethylstilbestrol, DES) treatment of immature hypophysectomized animals, resulting in the formation of a homogeneous population of developing follicles, also results in strong expression of 17β-HSD type 1 in the follicles (Fig. 3), while *P*450arom was still non-detectable [13]. This effect of estrogens on 17β-HSD type 1 may contribute to maintaining the increase in estradiol production in developing follicles. Interestingly, in cultured rat granulosa cells *in vitro*, estrogens alone do not affect the expression of the enzyme in DES-primed cells [33]. This suggests that the estrogen-dependent induction of the enzyme *in vivo* is mediated via paracrine factors, which remain to be identified.

Treatment of rats with FSH alone resulted in induction of the enzyme, while in the presence of estrogen (DES), FSH resulted in luteinization of the follicles and a simultaneous down-regulation of 17β-HSD type 1 enzyme [13]. The distinct effects of FSH are likely to be related to the presence of estrogens which have been shown to modulate FSH-action. Furthermore, in the model systems used, hCG-treatment *in vivo* always resulted in the suppression of 17β-HSD type 1 expression, which was related to luteinization or atresia of the follicles. Furthermore, with some of the treatments, 17β-HSD type 1 was more efficiently down-regulated than *P*450arom and could possibly limit estradiol biosynthesis [13]. In contrast to the situation found in rats, 17β-HSD type 1 is expressed in human corpora lutea as well as in granulosa-luteal cells [34, 37]. This difference between the regulation of human and rat enzymes is probably related to the fact that, in contrast to the

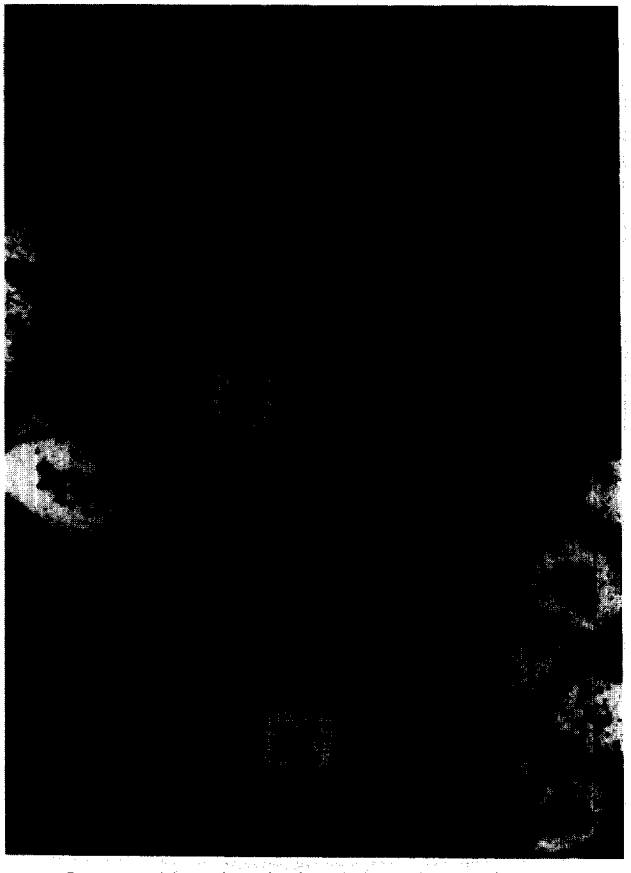

Fig. 3. Immunohistochemical staining of rat 17β-HSD type 1 in the granulosa cells of ovarian follicles after 5 days of diethylstilbestrol (DES) treatment of hypophysectomized immature rats. DES-treatment resulted in formation of a large number of homogeneous developing follicles (DF) strongly expressing 17β-HSD type 1 [see Ref. 13].

corpora lutea of cycling rat, human corpora lutea produce significant amounts of estradiol.

In all the steroidogenic cells so far studied, 17β-HSD type 1 is regulated by a cAMP-dependent pathway. Despite this, opposite effects have been observed in different cell types. In short-term cultured human granulosa-luteal cells, enzyme expression was decreased by Br-cAMP [28], while it was shown to be up-regulated by cAMP-analogs in cultured rat granulosa cells [33], normal human trophoblasts and choriocarcinoma cells [28, 38, 39]. Therefore, it is likely that the effect of cAMP is dependent on the cell type and, in the case of granulosa cells, also on the differentiation stage of the cells. In addition in JEG-3 choriocarcinoma cells a synergistic response with cAMP-analogs and phorbol esters on 17β-HSD type 1 expression has been detected [38, 39]. This suggests that both protein kinase-A- and protein kinase-C-dependent signalling pathways are involved in the regulation of 17β-HSD type 1 in these cells. Recent data suggest that in steroidogenic cells, 17β-HSD type 1 expression is also regulated by growth factors. Epidermal growth factor has been found to decrease the effect of FSH on 17β-HSD type 1 expression in rat granulosa cells, similarly to the effect previously found for *P*450arom [33]. In addition, several growth factors, including fibroblast growth factor, epidermal growth factor and transforming growth factor alpha, stimulate expression of the 17β-HSD type 1 enzyme in choriocarcinoma cells, and these growth factors may hence have a stimulatory role in estradiol production in the placenta [40].

ROLE OF 17β-HSD TYPE 1 IN ESTRADIOL BIOSYNTHESIS IN ESTROGEN TARGET TISSUES

Besides the cAMP- and protein kinase-C-mediated regulation of 17β-HSD type 1 expression in steroidogenic cells, the results of immunohistochemical studies have shown that the enzyme is expressed in a progestin-dependent manner in human endometrial epithelial cells [41, 42]. In these studies, immunostaining of 17β-HSD type 1 was found to be modulated during normal menstrual cycles. Staining was slight or absent in specimens taken during the follicular phase, and the enzyme appeared in the early secretory phase. Staining intensity reached a maximum in the mid-secretory phase and thereafter it disappeared towards the end of the luteal phase [41]. The progestin-dependent mechanism of 17β-HSD type 1 induction was further supported by findings showing that an antiprogestin (RU486), given during the early luteal phase, blocked the induction of 17β-HSD type 1, whereas an antiestrogen, tamoxifen, was ineffective [42]. Recently it has been shown that 17β-HSD type 2 is the principal isozyme in the endometrium, and similarly to 17β-HSD type 1, the type 2 enzyme is also induced during the luteal phase of the menstrual cycle [9]. This is in harmony with the hypothesis suggesting that the increased oxidative activity during the luteal phase of the cycle is partially responsible for the down-regulation of estrogen action in the endometrium [43]. The physiological relevance of the simultaneous expression of several isoforms in the same tissue remains to be clarified.

In addition to its presence in normal endometrial epithelial cells, the type 1 enzyme is expressed in about half of the endometrial cancer specimens analyzed. In malignant endometrial epithelial cells, enzyme expression was not related to serum progesterone concentrations, suggesting that paracrine factors may also be involved in regulation of the enzyme, at least in malignant endometrium [44].

Highly variable concentrations of 17β-HSD type 1 protein have been detected in the cytosolic fractions of breast cancer tissues and cell lines [45], while no data are available on the expression of other 17β-HSD isoenzymes in breast tissue. In immunohistochemical staining, 17β-HSD type 1 protein was detected in about 50% of the breast tumor specimens, and the protein was exclusively localized in the epithelial cells

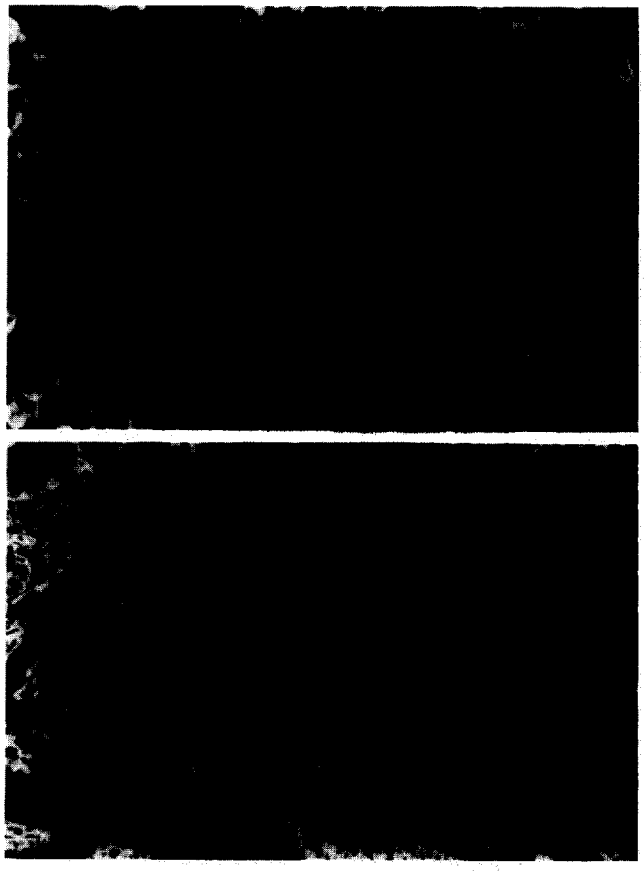

Fig. 4. Immunohistochemical staining of 17β-HSD type 1 in malignant human breast tissue. (A) In a tumor specimen containing a high 17β-HSD type 1 concentration (160 ng/mg protein), strong staining for the enzyme was detected in malignant epithelial cells forming gland-like structures (750×). (B) In some tumors, strong staining of the enzyme in the malignant epithelial cells was associated with strong proliferation of the stromal cells (300×).

of benign and malignant breast tissues [45; Fig. 4]. In some of the tissue specimens, strong 17β-HSD type 1 expression was associated with strong stromal proliferation, suggesting the possibility that stromal-derived paracrine factors could be responsible for the enzyme expression in these tumors [Fig. 4(B)]. In another recent report, 50% of breast tumors were found to contain a high affinity and highly estrogen-specific form of 17β-HSD responsible for estradiol formation [46]. Our immunohistochemical results, together with the catalytic properties of 17β-HSD type 1, suggest that the 80 kDa high affinity form of 17β-HSD expressed in breast tissue is identical to the 17β-HSD type 1 dimer. According to this hypothesis, 17β-HSD type 1 could be responsible for the relatively high estradiol concentrations found in breast tumor tissues in postmenopausal women [47, 48], leading to a supply of estradiol from estrone for receptor-binding. Thus, 17β-HSD type 1 may regulate the biological response to a hormone in target tissues; for instance the estradiol-responsive growth in normal and malignant breast tissue. This hypothesis should, however, be confirmed *in vivo*.

The absence of concentration-dependent correlations between 17β-HSD type 1, progestin receptor (PR) and estrogen receptor (ER) in breast tissues is in harmony with the results of previous studies in which 17β-HSD activity was measured [49]. Even if no concentration-dependent correlation was detected in our study, the expression of PR was characteristic of samples presenting moderate or strong immunohistochemical 17β-HSD type 1 staining [45]. This suggests that these tissues could respond to progestin and is in line with the previously-reported progestin-dependent regulation of 17β-HSD activity in breast cancer tissue

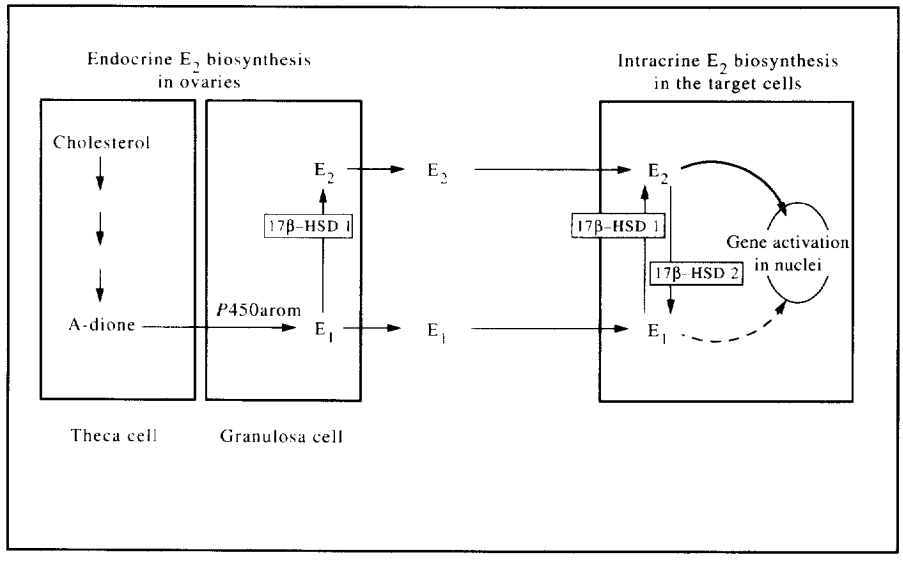

Fig. 5. Role of 17β-HSD type 1 in estradiol biosynthesis. In ovarian granulosa cells, 17β-HSD type 1 predominantly converts estrone into estradiol. In estrogen target cells, 17β-HSD type 1 has a similar enzyme activity, while 17β-HSD type 2 mainly catalyzes the opposite reaction.

in vivo [49]. Progestin-dependent induction of 17β-HSD type 1 was detected in T-47D breast cancer cells and thus this cell line could be used as a model for analyzing the progestin-dependent regulation of 17β-HSD type 1 in malignant breast epithelial cells. The induction in this cell line was progestin-specific, but the relatively long time (5 days) needed for maximal stimulation suggests the possibility of an indirect effect of the hormone [50]. Whether the measurement of 17β-HSD type 1 could give additional information about the response of a tumor to endocrine therapy remains to be clarified. Since high 17β-HSD type 1 expression was detected in some PR-negative cancer specimens from postmenopausal women, it is suggested that, in addition to the reported regulation by progestins, autocrine and/or paracrine factors are also involved in the regulation of 17β-HSD type 1 in breast tissues [43]. Based on results using cell line models, there are multiple candidates for these factors, including IGF-I, IGF-II and IL-6 [51, 52]. Autocrine effects could also be responsible for the high basal expression of the enzyme in BT-20 breast cancer cells.

In conclusion (Fig. 5), it is suggested that 17β-HSD type 1 is involved in the endocrine and intracrine production of estradiol in females, in classical steroidogenic cells and in some peripheral, estrogen target tissues, respectively. This suggests a central role for the enzyme in regulating the circulating estradiol concentration as well as its local production in estrogen target cells. The mechanisms involved in the regulation of enzyme expression are not totally understood. Our recent data, however, suggest that in the ovaries, 17β-HSD type 1 is regulated by a complex interaction of steroid hormones, gonadotropins and paracrine factors such as growth factors, in a similar manner to several other steroidogenic enzymes. It is evident that in the estrogen target tissues such as malignant breast epithelial cells, the regulatory pathways for the enzyme are at least partially distinct from those present in the ovaries. Furthermore, the relative concentrations of 17β-HSD type 1 (converting estrone into estradiol) and type 2 (converting estradiol into estrone), and possibly other isozymes as well, are critical determinants of the reaction direction in each cell type.

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