REVIEW

Intracrinology: role of the family of 17β-hydroxysteroid dehydrogenases in human physiology and disease

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ABSTRACT

In women and men, an important proportion of estrogens and androgens are synthesized locally at their site of action in peripheral target tissues. This new field of endocrinology has been called intracrinology. In postmenopausal women, 100% of active sex steroids are synthesized in peripheral target tissues from inactive steroid precursors while, in adult men, approximately 50% of androgens are made locally in intracrine target tissues. The last and key step in the formation of all estrogens and androgens is catalyzed by members of the family of 17β-hydroxysteroid dehydrogenases (17β-HSDs) while different 17β-HSDs inactivate these steroids in the same cell where synthesis takes place. To date, seven human 17β-HSDs have been cloned, sequenced and characterized. The 17β-HSDs provide each cell with the means of precisely controlling the intracellular concentration of each sex steroid according to local needs.

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INTRACRINOLOGY

An important finding in the field of sex steroids is that a large proportion of androgens and estrogens in men and women are synthesized locally in peripheral target tissues from the inactive adrenal precursors dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEA-S) (Fig. 1). In fact, in postmenopausal women, 100% of sex steroids are synthesized in peripheral tissues from precursors of adrenal origin except for a small contribution from ovarian and/or adrenal testosterone and androstenedione. Thus, in postmenopausal women, all active sex steroids are made in target tissues by an intracrine mechanism.

The secretion of DHEA and DHEA-S by the adrenals increases during the adrenarche in children at the age of 6–8 years and elevated values of circulating DHEA and DHEA-S are maintained throughout adulthood, thus providing the high level of substrates required for conversion into potent androgens and estrogens in peripheral tissues. In fact, plasma DHEA-S levels in adult men and women are 100 to 500 times higher than those of testosterone and 1000 to 10 000 times higher than those of estradiol, thus providing a large reservoir of substrate for conversion into androgens and/or estrogens in peripheral intracrine tissues.

The term intracrinology was coined in 1988 by Labrie *et al*. to focus our attention on the synthesis of active steroids in peripheral target tissues where steroid action is exerted in the same cells where synthesis takes place without release of the active hormones in the extracellular space and in the general circulation (Labrie 1991) (Fig. 2). The rate
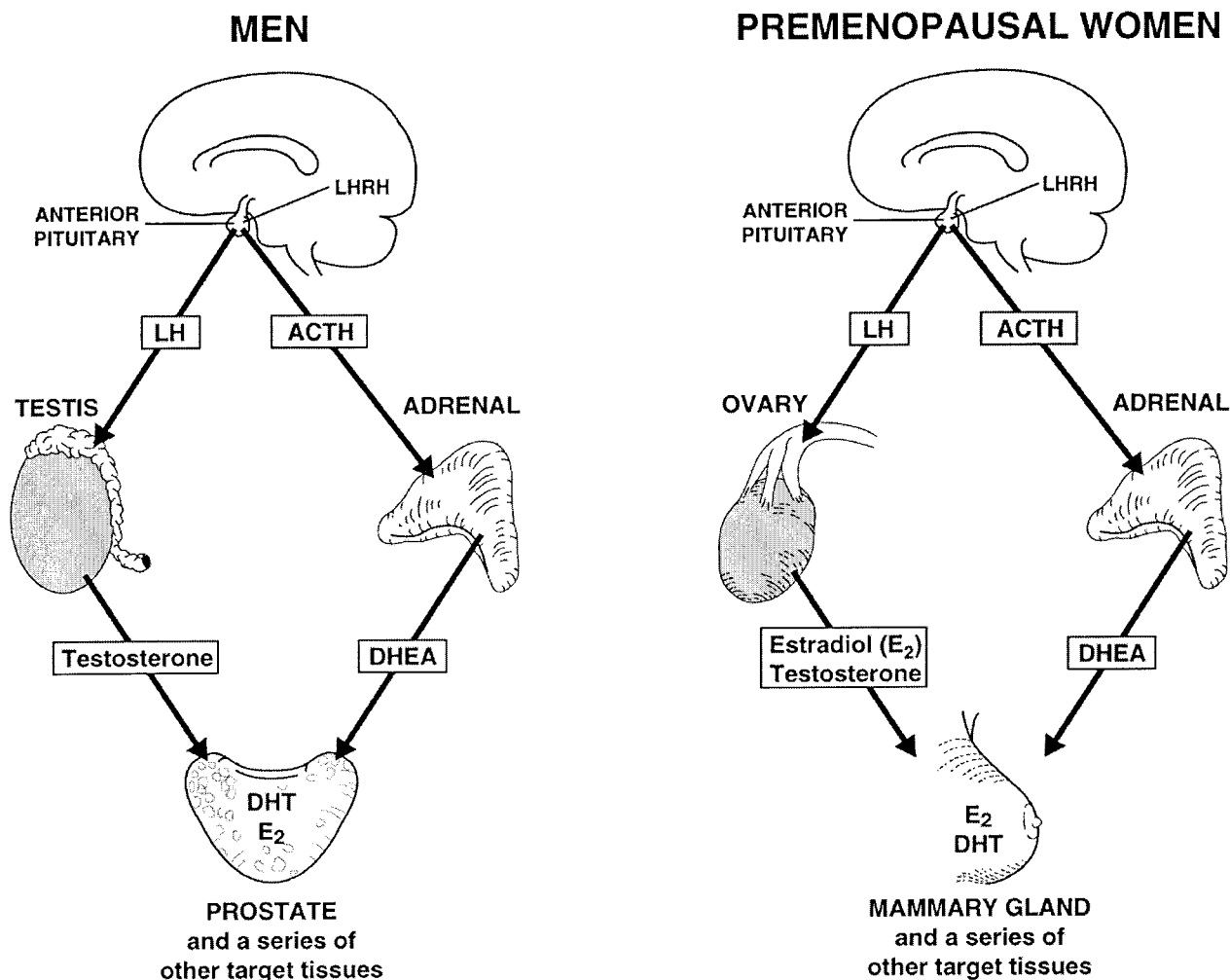
MEN

PREMENOPAUSAL WOMEN

FIGURE 1. Schematic representation of the role of gonadal (testicular and ovarian) and adrenal sources of sex steroids in men and premenopausal women. After menopause, the secretion of estradiol by the ovaries ceases and 100% of sex steroids are made locally in peripheral target intracrine tissues. LH, luteinizing hormone; ACTH, adrenocorticotropin.

of formation of each sex steroid thus depends upon the level of expression of the specific androgen- and estrogen-synthesizing enzymes in each cell of each tissue (Labrie *et al*. 1985*a*, Labrie 1991, Stewart & Sheppard 1992, Hobkirk 1993).

It is thus remarkable that humans, in addition to possessing a highly sophisticated endocrine system, have largely vested sex steroid formation in peripheral tissues. In fact, while the ovaries and testes are the exclusive sources of androgens and estrogens in the lower mammals, the situation is very different in higher primates, where active sex steroids are to a much larger degree or wholly synthesized locally in peripheral tissues; this provides autonomous control to target tissues which

are thus able to adjust the formation and metabolism of sex steroids according to local requirements (Labrie 1991). The situation of a high secretion rate of adrenal precursor sex steroids in men and women is thus completely different from the animal models used in the laboratory, namely the rat, mouse, guinea pig and all others (except monkeys) where the secretion of sex steroids takes place exclusively in the gonads (Cutler *et al*. 1978, Labrie *et al*. 1985*a*, Bélanger *et al*. 1989, Labrie *et al*. 1992*b*, Labrie 1993, and references therein). In these lower animal species, no significant amounts of androgens or estrogens are made outside the testes or ovaries and no sex steroids are left after castration.
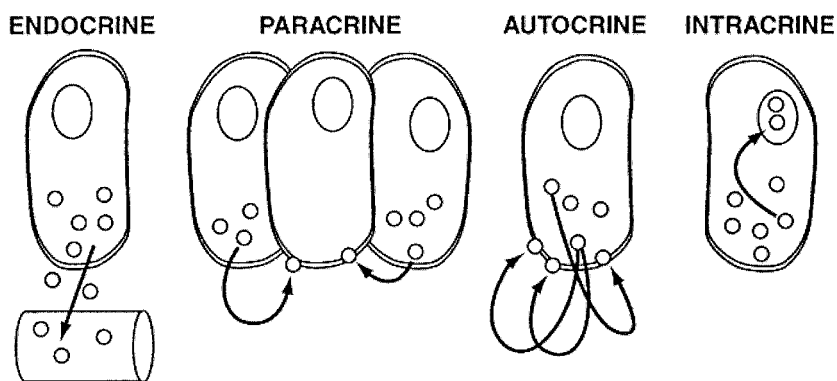
FIGURE 2.   Schematic representation of endocrine, paracrine, autocrine and intracrine secretion. Classically, endocrine activity includes the hormones secreted in specialized glands, called endocrine glands, for release into the general circulation and transport to distant target cells. On the other hand, hormones released from one cell can influence neighboring cells (paracrine activity) or can exert a positive or negative action on the cell of origin (autocrine activity). Intracrine activity describes the formation of active hormones which exert their action in the same cells where synthesis took place without release into the pericellular compartment. Reproduced with permission from Labrie (1991).

HOW THE ROLE OF ADRENAL DHEA AS A SOURCE OF ANDROGENS WAS DISCOVERED

In the course of studies on the hormonal therapy of prostate cancer, a surprising observation was that approximately 50% of DHT was left in the prostate in men who had their testicles removed or had complete blockade of testicular androgen secretion following treatment with a luteinizing hormone-releasing hormone (LHRH) agonist (see Labrie *et al*. 1985*b*, 1996*a* for reviews). Thus, while blood levels of testosterone were reduced by 90–95% following castration, the intraprostatic concentration of dihydrotestosterone (DHT), the active intracellular androgen, was decreased by only 50%, thus suggesting that, in the absence of testicles, another source continued to provide androgens to the prostate.

This crucial observation led to the development of combined androgen blockade which uses a pure antiandrogen added to medical or surgical castration. The objective is to block simultaneously the androgens of both testicular and adrenal origin at the start of treatment of prostate cancer (Labrie *et al*. 1982, 1985*a*). Combined androgen blockade was thus the first treatment demonstrated in prospective and randomized trials to prolong life in prostate cancer (Crawford *et al*. 1989, Caubet *et al*. 1997, Bennett *et al*. 1999, Prostate Cancer Triallists' Collaborative Group 2000). Recent meta-analyses of all the randomized studies using the pure antiandrogens, flutamide and nilutamide, show that the risk of death from any cause is reduced by 8% to 20% in men with advanced prostate cancer who received combined androgen blockade compared with castration alone (Caubet *et al*. 1997, Bennett *et al*. 1999, Prostate Cancer Triallists' Collaborative Group 2000). In analogy with any type of cancer, where treatment of early disease is known to be much more efficient than treatment of advanced cancer, the same combined androgen blockade applied at the localized stage of prostate cancer can even cure the disease in a large proportion of patients (Labrie *et al*. 1999, Labrie 2000).

On the other hand, proof of the role of estrogen formation in peripheral intracrine tissues is well illustrated in women by the important benefits on breast cancer observed in postmenopausal women treated by a series of aromatase inhibitors (Buzdar *et al*. 1996). Most convincingly, the recent observation that postmenopausal women who received the antiestrogen, raloxifene, for only 3 years had a 76% decrease in the incidence of breast cancer (Cummings *et al*. 1999) is a clear demonstration of the role of extra-ovarian estrogens in the development and growth of breast cancer.

THE 17β-HYDROXYSTEROID DEHYDROGENASES, KEY ENZYMES ACTING AT THE LAST STEP OF ANDROGEN AND ESTROGEN FORMATION

The synthesis from DHEA of the most potent natural androgen, DHT and of the most potent

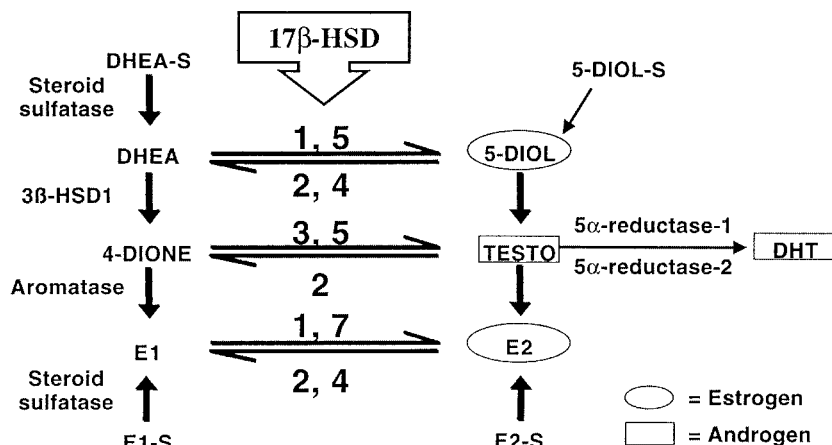# Human steroidogenic enzymes in peripheral intracrine tissues

FIGURE 3.   Main biosynthetic and inactivating pathways of androgens and estrogens in the human. The various types of 17β-HSDs are indicated as numbers; arrows indicate the direction of their enzymatic activity. TESTO, testosterone.

natural estrogen, 17β-estradiol (E_2) involves several enzymes, namely 3β-hydroxysteroid dehydrogenase/ Δ5-Δ4 isomerase (3β-HSD), 17β-HSD, 5α-reductase and/or aromatase (Fig. 3).

As illustrated in Fig. 3, the enzymes of the 17β-HSD gene family are responsible for the interconversion of DHEA and androst-5-ene-3β, 17β-diol (5-diol), androstenedione (4-dione) and testosterone, as well as estrone (E_1) and E_2. The interconversion of androstanedione and DHT as well as androsterone (ADT) and androstane 3α,17β-diol is controlled by the same enzymes. The 17β-HSDs are therefore required for the synthesis of all active androgens and all active estrogens as well as for their inactivation.

Since the molecular structure of the key non-P450-dependent enzymes involved in sex steroid formation had not been elucidated and knowing that local formation of sex steroids plays a major role in both normal and tumoral hormone-sensitive tissues (Labrie *et al*. 1985*a*), an important proportion of the research program of many groups, including ours, has been devoted to this exciting and therapeutically promising area (for reviews see Labrie *et al*. 1992*b*, 1993, 1996*b*). It is important to mention that 40% of all cancers, namely breast, prostate, ovarian and uterine cancers, are sex steroid-sensitive and are thus prime candidates for approaches based upon control of intracrine activity.

That 17β-HSD activity is widely distributed is illustrated by the finding that in a study of 25 tissues

in the monkey, both estrogenic and androgenic 17β-HSD activities were found in all tissues examined, thus indicating the generalized role of 17β-HSDs in the formation of androgens and estrogens in peripheral target intracrine tissues (Martel *et al*. 1994). Estrogenic 17β-HSD-type 1 mRNA (expression) and activity have been found in the fifteen human tissues examined (Martel *et al*. 1992). The highest rates of estrogenic 17β-HSD activity were found in the placenta, liver, ovary, endometrium, prostate, testis and adipose tissue (Martel *et al*. 1992).

Type 1 17β-HSD

The molecular structure of a human type 1 17β-HSD cDNA and its corresponding gene, which encodes a predicted protein of 327 amino acids, was the first to be elucidated (Peltoketo *et al*. 1988, Luu-The *et al*. 1989, 1990, Peltoketo *et al*. 1992). This enzyme is a member of the short-chain alcohol dehydrogenase superfamily. The type 1 17β-HSD gene consists of 6 exons and 5 introns within a genomic DNA fragment of 3·2 kbp (Fig. 4). The type 1 17β-HSD gene was assigned by *in situ* hybridization to the 17q11-q21 region (Luu-The *et al*. 1989). The type 1 17β-HSD enzyme is a cytosolic protein that exists in a homodimeric form that catalyses predominantly the interconversion of E_1 to E_2 using NAD(H) or NADP(H) as cofactor (Dumont *et al*. 1992, Lin *et al*. 1992).
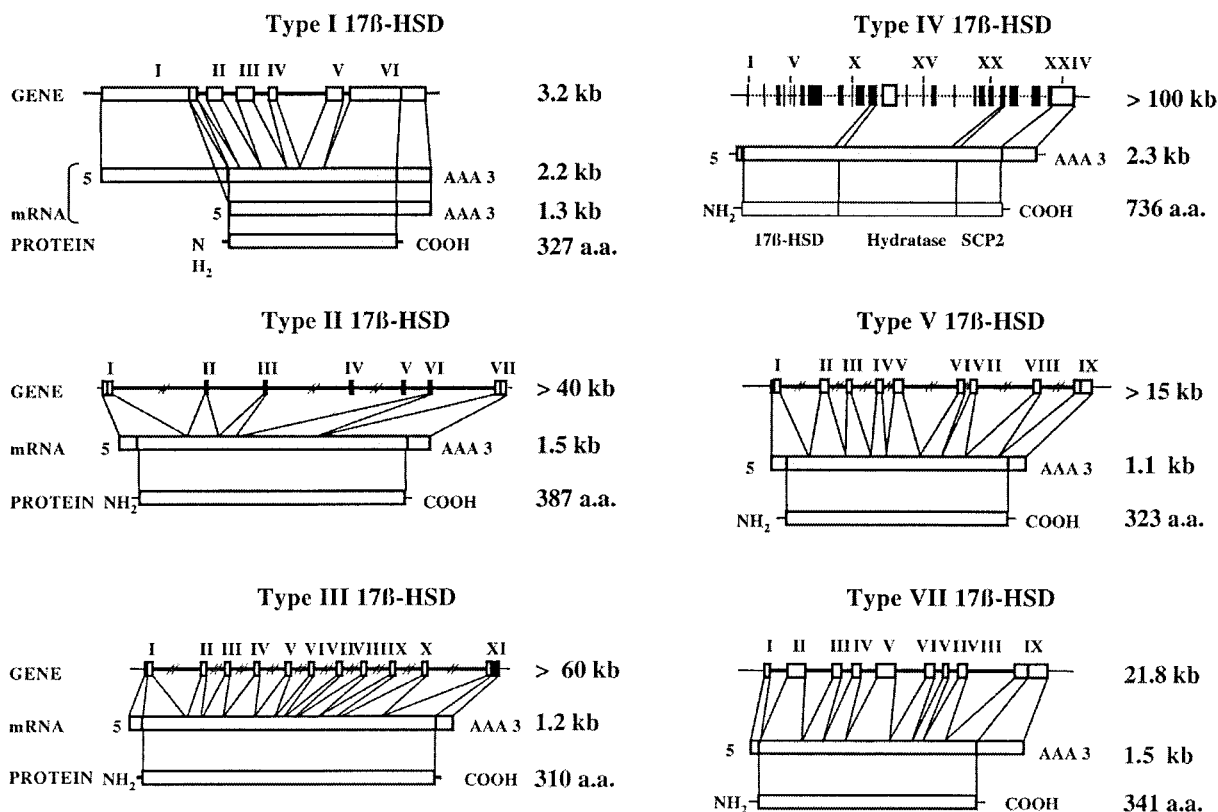
FIGURE 4. Structure of human types 1, 2, 3, 4, 5 and 7 17β-HSD genes, mRNAs and corresponding proteins. a.a., amino acids.

In order to perform the structure–function analysis of type 1 17β-HSD, the protein was rapidly purified, thus yielding a highly active preparation, and over-expressed in baculovirus and crystallized (Lin *et al*. 1992, Zhu *et al*. 1993, Breton *et al*. 1994). This work has led to the elucidation of its three-dimensional structure (Ghosh *et al*. 1995), thus achieving the first X-ray structure determination of a mammalian steroidogenic enzyme. The structure of type 1 17β-HSD from human placenta was determined at 2·2 Å resolution by a combination of isomorphous replacement (with a single mercury derivative) and molecular replacement techniques. The core of the structure is the seven-stranded parallel β-sheet (βA to βG), surrounded by six parallel α-helices (αB to αG), three on each side of the β-sheet (Fig. 5). The first structure of type 1 of 17β-HSD has shown that the enzyme has a folding characteristic of short-chain dehydrogenase with Tyr155, Lys159 and Ser142 constituting the catalytic triangle of the enzyme.

After determination of the first 3-D structure of the enzyme, we have studied in detail the substrate specificity of the enzyme. In its complex with the most potent estrogen, namely estradiol, it has been observed that the specificity of the enzyme was determined, in part, by four hydrogen interactions which determine the substrate orientation, as well as by important hydrophobic interactions between the steroid and the hydrophobic surface in the binding site, thus contributing the most thermodynamic force for binding. The binding site is a narrow hydrophobic tunnel showing a high degree of complementarity to the substrate (Azzi *et al*. 1996, Lin *et al*. 1999).

To understand further the discrimination between various steroids by type 1 17β-HSD, we crystallized and determined the structures of type 1 17β-HSD complexes with DHEA and DHT, providing the first pictures of DHEA and DHT bound to a protein. Comparison of these structures with the enzyme–estradiol complex shows that relatively small changes in the shape of the steroid molecule can markedly affect the binding affinity and specificity. In fact, the K_m of estrone is more than 1000-fold lower than that of DHEA while the K_m of estradiol is about 10 times lower than that of DHT. The structures suggest that Leu149 is the
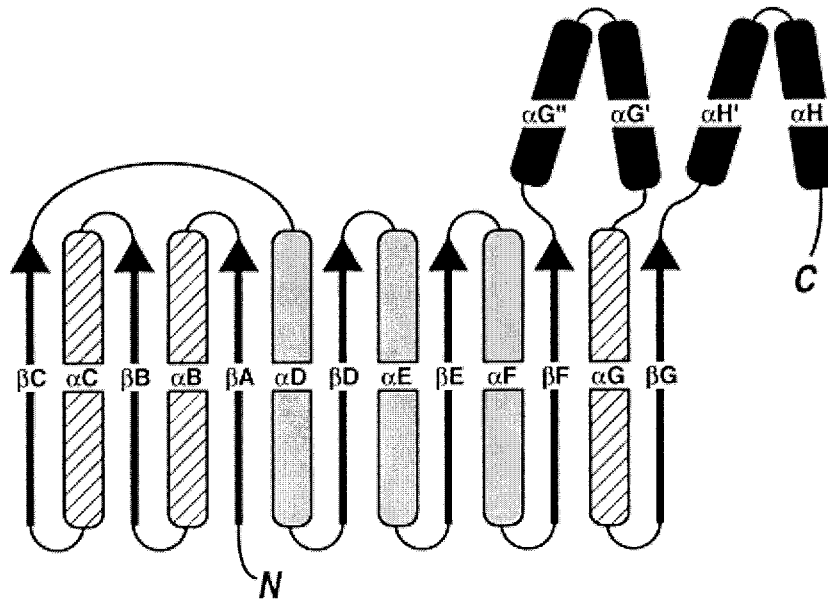
FIGURE 5.   Secondary structure pattern in 17β-HSD with β-strands represented by solid arrows and α-helix by tubes. The light gray α-helices are on one side of the β-sheet (towards the viewer) while the hatched α-helices are on the opposite side of the β-sheet (away from the viewer). Reproduced from Labrie *et al*. (1998) with permission.

primary contributor to the discrimination of C18/C19 steroids by type 1 17β-HSD. The critical role of Leu149 has been confirmed by site-directed mutagenesis. The Leu149Val variant showed a significantly decreased K_m for C19 steroids while losing discrimination between estrogens and androgens. The electron density of DHEA also revealed a distortion of its 17-ketone group towards a β-oriented form (Han *et al*. 2000).

To elucidate the structure–function relationships more completely and to facilitate the design of inhibitors for this critical steroidogenic enzyme, we have studied the dynamics of the estradiol binding process. This study was carried out using Monte-Carlo minimization with the crystallographic data as starting point. The results show that the movement of the steroid along the binding tunnel is accompanied by essential conformation rearrangements of the enzyme side chains, noticeable rotation of estradiol along its longitudinal axis, and some conformational changes of the steroid (Zhorov & Lin 2000).

Type 2 17β-HSD

The structure of a second type of 17β-HSD cDNA was then reported (Wu *et al*. 1993). This cDNA encodes a predicted protein of 387 amino acids with a molecular mass of 42 782 (Fig. 4); it is most likely associated with the membranes of the endoplasmic reticulum. This enzyme catalyzes the conversion of E_2 into estrone, testosterone into 4-dione and 5-diol into DHEA. This enzyme, chronologically designated type 2 17β-HSD, is also a member of the short-chain alcohol dehydrogenase super-family but it shares only about 20% sequence identity with the cytoplasmic enzyme encoded by the type 1 17β-HSD gene (Luu-The *et al*. 1989). This enzyme uses NAD(H) as a cofactor (Wu *et al*. 1993). It is less specific than type 1 17β-HSD since it uses both estrogens and androgens as substrates.

Type 3 17β-HSD

A third type of human 17β-HSD cDNA encoding a predicted protein of 310 amino acids with a molecular mass of 34 513 was then characterized (Geissler *et al*. 1994) (Fig. 4). Type 3 17β-HSD, a microsomal isozyme, uses NADP(H) as a cofactor, and is expressed predominantly in the testes, with an equilibrium of the reaction favoring testosterone production from 4-dione. This enzyme, which shares 23% sequence identity with the two other 17β-HSD enzymes, is the site of the mutations responsible for male pseudohermaphroditism from

17β-HSD deficiency (Geissler *et al*. 1994). The type 3 17β-HSD mRNA species was detected only in the testes out of the 16 tissues tested by Northern blot analysis (Geissler *et al*. 1994).

Type 4 17β-HSD

Human type 4 17β-HSD is a 736 amino acid protein of M_r 80 kDa (Fig. 4) which shares 84% identity with the corresponding porcine enzyme and transforms E_2 into E_1 and 5-diol into DHEA (Leenders *et al*. 1994, Adamski *et al*. 1995). The human type 4 17β-HSD mRNA is expressed in virtually all human tissues examined by Northern blot, including the liver, heart, prostate, testis, lung, skeletal muscle, kidney, pancreas, thymus, ovary, intestine, placenta, and several human breast cancer cell lines. Thus, this enzyme is likely to play an important role in the inactivation of estrogens in a large series of peripheral tissues.

Type 5 17β-HSD

While type 3 17β-HSD synthesizes testosterone from 4-dione in the Leydig cells of the testicles, thus providing approximately 50% of the androgens acting in the prostate and other androgen-sensitive tissues, the same enzymatic reaction is catalyzed in peripheral tissues by another enzyme, namely type 5 17β-HSD. This enzyme belongs to the aldo-keto reductase family. In fact, type 5 17β-HSD is highly homologous with types 1 and 3 3α-HSDs as well as 20α-HSD (Dufort *et al*. 1999).

To assess the importance of type 5 17β-HSD in peripheral tissues, especially in the prostate, we used an RNase protection assay to detect specifically the level of type 5 17β-HSD mRNA expression in the liver, adrenal, and prostate as well as in the prostatic carcinoma cell lines DU-145 and LNCaP and in the osteosarcoma cells MG-63. Type 5 17β-HSD mRNA was found in all the tissues mentioned above. However, the level of mRNA expression in the normal prostate and adrenal was lower than that in the liver, prostatic cancer, and osteocarcinoma cells.

We will now use the human prostate as an example of the potential role of type 5 17β-HSD as a local source of androgens. As mentioned earlier, approximately 50% of androgens in the human prostate are synthesized locally from the inactive adrenal precursors DHEA and DHEA-S of adrenal origin. In order to obtain more precise information about the localization and potential role of type 5 17β-HSD in this tissue, we have used two complementary approaches, namely *in situ* hybridization and immunocytochemistry, in order to properly identify the human prostatic cells which contain the type 5 17β-HSD mRNA and the corresponding enzyme. Localization of 3β-HSD and of the androgen receptor (AR) was also investigated by immunostaining in the same tissue.

The stratified epithelium lining the tube-alveoli of the human prostate is divided into two layers, namely the basal layer made of low cuboidal cells located underneath a layer of columnar secretory cells (luminal cells) (Fig. 6). In the glandular epithelium, the basal cells highly express the type 5 17β-HSD enzyme while luminal cells show a much lower and variable level of expression (Fig. 6A). Similar results are observed when the cellular distribution of 3β-HSD is investigated (Fig. 6B). The *in situ* hybridization results obtained with a [^{3}H]UTP-labeled type 5 17β-HSD riboprobe are in agreement with the immunostaining data obtained with a specific antibody to the enzyme (El-Alfy *et al*. 1999). The immunostaining results obtained from normal prostate tissue and benign prostatic hyperplasia (BPH) were found to be similar. Androgen receptor immunoreactivity, on the other hand, shows a different distribution since, in the prostatic epithelium, most of the nuclei of basal cells are negative while the majority of nuclei of the luminal cells show intense and positive staining (Fig. 6C).

In conclusion, human type 5 17β-HSD, as well as 3β-HSD are highly expressed in the basal epithelial cells of the human prostate. AR, on the other hand, is highly expressed in the luminal cells. The present data suggest that DHEA is transformed in the basal cells of the glandular epithelium into 4-dione by 3β-HSD and then into testosterone by type 5 17β-HSD, while DHT appears to be synthesized in both the basal and luminal cells by 5α-reductase (El-Alfy *et al*. 1999) (Fig. 7).

It is generally believed that prostatic stem cells are located in the basal cell compartment (Bonkhoff & Remberger 1998). The presence of type 5 17β-HSD, 3β-HSD and 5α-reductase isoenzymes in the basal cells suggests that this cell type is actively involved in androgen production and cannot be considered as being only a precursor of the luminal secretory cells.

It is clear from the data obtained that the androgen receptor is localized exclusively in the nuclei of the luminal cells. This finding is in agreement with the observation that androgen blockade in men treated for prostate cancer leads to an atrophy and even, after long-term androgen blockade, disappearance of the luminal cells by apoptosis. The basal cells, on the other hand, are not affected by androgen deprivation.

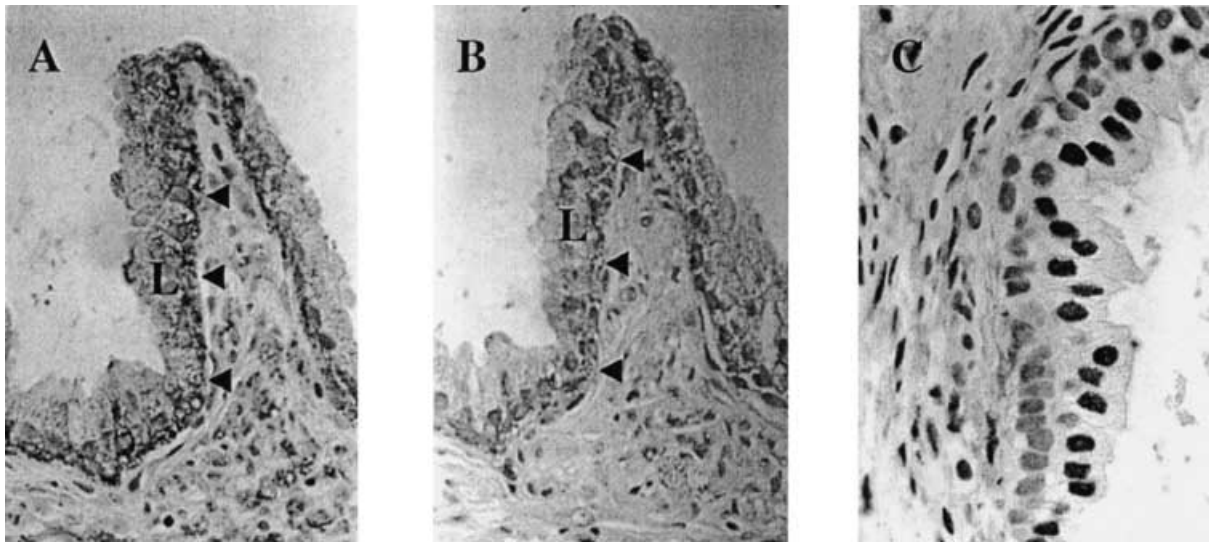Concerning the site of DHT synthesis, the presence of 5α-reductase in the basal cells suggests

FIGURE 6.   Paraffin sections of BPH tissue, immunostained with antibodies for (A) type 5 17β-HSD, (B) 3β-HSD and (C) AR (× 800). Consecutive sections were immunostained for type 5 17β-HSD (A) and 3β-HSD (B). Although the reaction for 3β-HSD is somewhat weaker than that obtained for type 5 17β-HSD, the distribution of the two enzymes in basal (arrowheads) and luminal cells (L) is similar. (C) AR immunoreactivity was found to be exclusively localized in the nuclei. In the epithelium, the reaction can be seen in the majority of the luminal cells (L) nuclei, but not in the nuclei of most basal cells (El-Alfy *et al*. 1999).

that the DHT made in basal cells is then transferred to luminal cells where interaction with the androgen receptor takes place. On the other hand, since type 5 17β-HSD and 3β-HSD are both highly expressed in basal cells but not in the luminal cells while the androgen receptor is mainly present in the luminal cells which also contain 5α-reductase, it is tempting to suggest that an undefined proportion of testosterone synthesized in the basal cells diffuses or is transported into the luminal cells where it is ultimately transformed into DHT by 5α-reductase (Fig. 7). DHT made in the luminal cells by the action of 5α-reductase exerts its action in the luminal cells themselves.

The involvement of two cell types in the biosynthesis of steroids has already been shown to occur in the ovary. In fact, in the ovary, C19 steroids (4-dione and testosterone) synthesized by theca interna cells are transferred to granulosa cells where they are aromatized into estrogens (McNatty *et al*. 1979). The present data strongly suggest the possibility of a similar two-cell mechanism of androgen formation in the human prostate: testosterone is first synthesized in the basal cells before diffusing into the luminal cells where transformation into DHT occurs. DHT is also made in basal cells. As mentioned above, the basal cells of the human prostate are themselves unresponsive to androgens.

Type 6 17-HSD

Using a rat prostate cDNA obtained by expression cloning, Biswas and Russell (1997) have isolated cDNA clones which metabolize 3α-diol. Among the many clones obtained, one type termed type 6 17β-HSD catalyzes selectively the oxidation of 3α-diol to androsterone. However, the transformation of other C19 steroids, namely DHT to androstanedione (A-dione) and testosterone to 4-dione also occurs but at an approximately 50- to 100-fold lower rate.

Type 6 17β-HSD shares 65% homology with rat type 1 retinol dehydrogenase (RoDH1) and thus belongs to the retinol dehydrogenase family. Retinol dehydrogenase is well recognized to catalyze the transformation of retinol to retinal (Chai *et al*. 1995). Many members of this family, such as rat RoDH1, human RoDH1 (Biswas & Russell 1997) and 9-cis-RoDH (Huang *et al*. 1998), also show oxidative 3α-HSD activity that transforms 3α-diol to DHT and ADT to A-dione. Northen blot analysis shows high expression of type 6 17β-HSD in rat liver and prostate. However, because of the relatively high homology with other members of the retinol dehydrogenase family, the high signal observed could also be due to the presence of other RoDH members. The size of the message is approximately 2 kb.
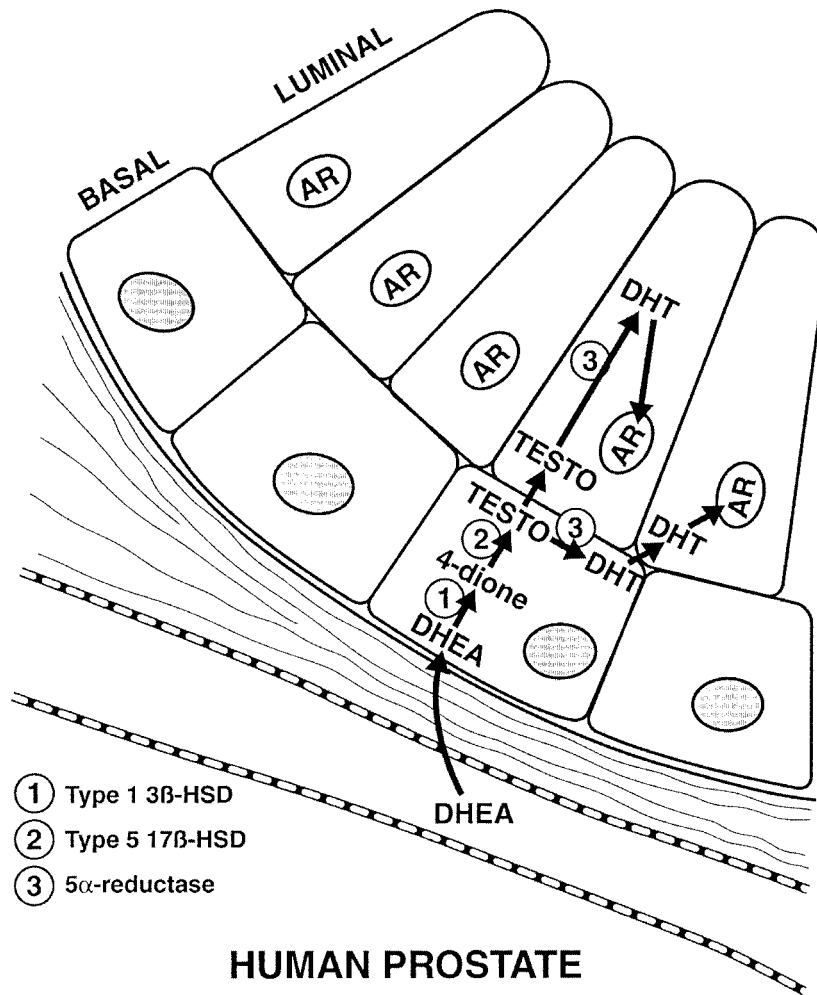
FIGURE 7. Schematic representation of the intracrine formation of androgens in the human prostate. In basal cells of the prostatic epithelium, the inactive precursor DHEA is transformed into androstenedione by 3β-HSD and then into the weak androgen, testosterone (TESTO), by type 5 17β-HSD. An undefined proportion of testosterone is then transformed into the most potent androgen, DHT, by 5α-reductase in basal cells. Another fraction of testosterone is transported into the luminal cells where 5α-reductase transforms testosterone into DHT. The androgen receptor (AR), on the other hand, is exclusively expressed in the nuclei of the luminal cells which are the site of prostate cancer development. Basal cells do not contain significant amounts of AR and are apparently unresponsive to androgens.

The human counterpart has not yet been described. Using rat type 6 17β-HSD cDNA as probe to screen human liver and prostate cDNA libraries, we have obtained many other members of the RoDH family. However, a human clone that possesses the characteristics of rat type 6 17β-HSD has not been found.

Type 7 17β-HSD

Type 7 17β-HSD was first cloned in a rat corpus luteum cDNA library and was identified as prolactin receptor associated protein (PRAP) (Duan *et al.* 1996). Using expression cloning of a mouse mammary epithelial (HC11) cell cDNA library, a clone

that shares 89% identity with rat PRAP, and catalyzes selectively the transformation of E_1 to E_2 has been isolated (Nokelainen *et al*. 1998). Following transfection into HEK-293 cells, Nokelainen *et al*. (1998) also found that rat PRAP catalyzes efficiently and selectively the transformation of E_1 to E_2 while the transformation of C19 steroids was much weaker.

Northern blot analysis shows mRNAs having multiple sizes, the strongest bands being seen at 4·6 and 4·3 kb in the mouse and rat respectively. The 1·8 kb band that corresponds to the cDNA length is much weaker. Such results suggest that other type 7 17β-HSD-like proteins could exist. Both rat and mouse 4·3 and 4·6 bands are abundantly expressed in the corpus luteum during the second half of pregnancy. On the other hand, only weak expression is observed in the non-pregnant ovary. The mRNA has also been detected in the placenta, mammary gland and kidney. Using RT-PCR, mRNA has also been observed in the mouse brain, testis and small intestine.

A human type 7 17β-HSD cDNA clone (Krazeisen *et al*. 1999) shows a length of 1·5 kb and encodes a protein of 37 kDa or 341 amino acids. Using RT-PCR, this enzyme is detected in the ovary, breast, placenta, testis, prostate and liver. It shows 74 and 78% amino acid identity with the mouse and rat type 7 17β-HSDs respectively. Comparison with other 17β-HSDs indicates that it shares less than 20% identity, a typical percentage for the other members of the 17β-HSD family. Only the human type 7 17β-HSD gene structure has been described (Krazeisen *et al*. 1999). This gene spans 21·8 kb and consists of nine exons and eight introns. The gene is assigned to human chromosome bands 10p11·2. It is noteworthy that type 5 17β-HSD is also mapped to human chromosome 10 (bands 10p15→14).

Type 8 17β-HSD

Type 8 17β-HSD is also known as the Ke6 gene product found in the human leucocyte antigen (HLA) region (Kikuti *et al*. 1997). This area is well known to contain genes encoding the human major histocompatibility complex (MHC). This complex is thought to be involved in polycystic kidney disease (PKD) since aberrant expression has been found in two different models of PKD mice (Aziz *et al*. 1993). Recently, Fomitcheva *et al*. (1998) have found that the overexpressed protein fused with GST catalyzes efficiently the transformation of E_2 to E_1. The transformation of testosterone to 4-dione is about 25% of that of E_2 into E_1. In a recent review, Peltoketo *et al*. (1999) suggest that this enzyme be named type 8 17β-HSD. In addition to

the kidney, the Ke6a protein is abundant in the mouse liver and gonads, while the Ke6b form is more specifically expressed in the spleen. Interestingly, in the mouse ovary, type 8 17β-HSD which catalyzes the reaction opposite to that of types 1 and 7 17β-HSD is expressed in cumulus cells but not in granulosa or luteal cells where types 1 and 7 17β-HSD are more abundantly expressed (Nokelainen *et al*. 2000).

Although the human Ke6 gene has been known for a few years (Kikuti *et al*. 1997), the cDNA clone and thus the expressed protein has not been isolated and its activity is unknown. The gene encodes a protein of 274 amino acids. Whether human Ke6 protein possesses 17β-HSD activity thus remains to be determined.

UNIDIRECTIONAL ACTIVITY OF 17β-HSDs

It is important to indicate, at this stage, that in intact cells, the activity catalyzed by each type of 17β-HSD is almost exclusively unidirectional: for example, types 1, 3, 5 and 7 17β-HSDs catalyze the reaction in a reductive way, while types 2 and 4 17β-HSDs catalyze the oxidative reaction. In estrogen-target tissues, such as in the placenta and breast, the presence of type 1 17β-HSD ensures a high level of estradiol formation. Similarly, in the testis, type 3 17β-HSD drives the conversion of 4-dione to the testosterone needed for the development and growth of the internal male reproductive structures (epididymis, seminal vesicles, and vas deferens) as well as for the development, growth and function of the secondary sex organs.

The cell-specific expression of the different 17β-HSDs implies that measurements of 17β-HSD activity in an intact and even more in an homogenized tissue, is the sum of the activities of the reductive and oxidative activities of the different enzymes expressed at various levels in the different cell types which constitute that tissue. As an example, when we studied the cell-specific expression of type 5 17β-HSD in the human prostate, we used *in situ* hybridization in order to assess the level of mRNA encoding the enzyme and we used immunocytochemistry to estimate the localization of the enzyme not only in each cell but also in different compartments of the cells.

Not only is each type of 17β-HSD specifically expressed in individual cell types but, as shown in the few studies already performed, 17β-HSD expression is modulated specifically in each cell by precise mechanisms in order to control the concentration of each sex steroid according to local needs.

LOCAL CONTROL OF ESTROGEN AND ANDROGEN FORMATION AND INACTIVATION BY 17β-HSD IN BREAST CANCER

Sex steroids are well recognized to play a predominant role in the regulation of cell growth and differentiation of normal mammary gland as well as in hormone-sensitive breast carcinomas. Estrogens stimulate proliferation of hormone-sensitive breast cancer cells (Poulin & Labrie 1986, Davidson & Lippman 1989, King 1991, Bernstein & Ross 1993), while androgens exert an antiproliferative action in these cells (Poulin & Labrie 1986, Poulin *et al*. 1988, 1989, Labrie *et al*. 1992*a*). There is a large body of evidence showing that in the human, the local intracrine formation of active estrogens and androgens from inactive steroids precursors DHEA and DHEA-S regulate growth and function of peripheral target tissues, including the breast (Adams 1985, Labrie 1991, Labrie *et al*. 1997*a,b*, Couillard *et al*. 1998). Thus, the various types of human steroidogenic enzymes, namely 17β-HSD, 3β-HSD, 5α-reductase and the alternative promoter usage of the aromatase gene, because of their tissue- and cell-specific expression and substrate specificity, provide each cell with the necessary mechanisms to control the level of intracellular active estrogens and androgens (Labrie 1991, Martel *et al*. 1994, Simard *et al*. 1996, Labrie *et al*. 1997*c*, Simpson *et al*. 1997).

In fact, the concentration of the potent estrogen E_2 is significantly higher in breast tumor than in normal breast tissue (Bonney *et al*. 1983, Van Landedgem *et al*. 1985, Vermeulen *et al*. 1986, Recchione *et al*. 1995), while the tumor E_2 concentration is much higher compared with that of E_1 (Van Landedgem *et al*. 1985, Vermeulen *et al*. 1986). Moreover, a higher level of conversion of E_1 to E_2 compared with the reverse reaction was measured by direct uptake of labeled E_1 or E_2 in breast cancer tissue in postmenopausal women (McNeil *et al*. 1986).

It is therefore quite possible that higher reductive 17β-HSD activity contributes to higher levels of E_2 in tumors, thus resulting in a progression of tumor growth. The 17β-HSD activity can therefore exert a key role in regulating the tissue concentration of active estrogens through control of interconversion of E_1 and E_2 as well as that of DHEA and 5-diol, the latter being a weak estrogen (Poulin & Labrie 1986). In fact, in many estrogen receptor-positive (ER+) human breast cancer cell lines (MCF-7, ZR-75–1, and T-47D), the reductive pathway leading to the formation of E_2 from E_1 is predominant (Adams *et al*. 1988*a*, Pasqualini *et al*. 1989, Poutanen *et al*.

1990, Couture *et al*. 1993), whereas in hormone-independent cell lines (MDA-MB-231 and MDA-MB-346), the oxidative pathway responsible for the inactivation of E_2 into E_1 predominates (Pasqualini *et al*. 1989). Numerous studies have reported the presence of multiple 17β-HSDs in human breast cancer cells (Tait *et al*. 1989, MacIndoe 1990, Poutanen *et al*. 1990, 1992, Martel *et al*. 1992).

Endocrine, paracrine as well as intracrine influences on the proliferation of human breast cancer cells can play a role , thus supporting the suggestion that breast tumor growth is modulated by the hormonal environment (Adams *et al*. 1988*b,c*, Dickson & Lippman 1996). The presence in breast tumors of considerable numbers of tumor-associated macrophages and tumor-infiltrating lymphocytes secreting a wide spectra of cytokines also suggests a key role for these factors in neoplastic cell activity (Kelly *et al*. 1988, Whitford *et al*. 1990). Moreover, it has been observed that Natural Killer cells isolated directly from ductal invasive breast tumors secrete important amounts of interferon-γ and interleukin (IL)-4 (Tamm *et al*. 1989). In support of the potential role of cytokines in breast cancer cells, IL-1α, IL-4, IL-6 and IL-13 inhibit the proliferation of ZR-75–1, T-47D and/or MCF-7 human breast cancer cells (Tamm *et al*. 1989, 1991, Toi *et al*. 1992, Blais *et al*. 1994, 1995, 1996, Serve *et al*. 1996, Douglas *et al*. 1997). Furthermore, it has already been reported that some cytokines can regulate the expression of several enzymes involved in sex steroid formation and inactivation in breast cells. For example, IL-6 regulates the expression of 17β-HSD (Adams *et al*. 1991, Reed & Purohit 1997, Turgeon *et al*. 1998), estrone sulfatase (Purohit *et al*. 1996) and P450 aromatase (Zhao *et al*. 1995) whereas IL-4 regulates 17β-HSD activity.

We first investigated the effect of exposure to IL-4 and IL-6 on reductive and oxidative 17β-HSD activities in both intact ZR-75–1 and T-47D human breast cancer cells. In ZR-75–1 cells, a 6-day exposure to IL-4 and IL-6 decreased E_2-induced cell proliferation, the half maximal inhibitory effect being exerted at 88 pM and 26 pM respectively. In parallel, incubation with IL-4 and IL-6 increased oxidative 17β-HSD activity by 4·4- and 1·9-fold respectively, this potent activity being observed at EC_{50} values of 22·8 and 11·3 pM respectively (Fig. 8). (Turgeon *et al*. 1998). Simultaneously, reductive 17β-HSD activity leading to E_2 formation was decreased by 70 and 40% by IL-4 and IL-6 respectively. Moreover, IL-4 and IL-6 exerted the same regulatory effects on 17β-HSD activities when testosterone and 4-dione were used as substrates,
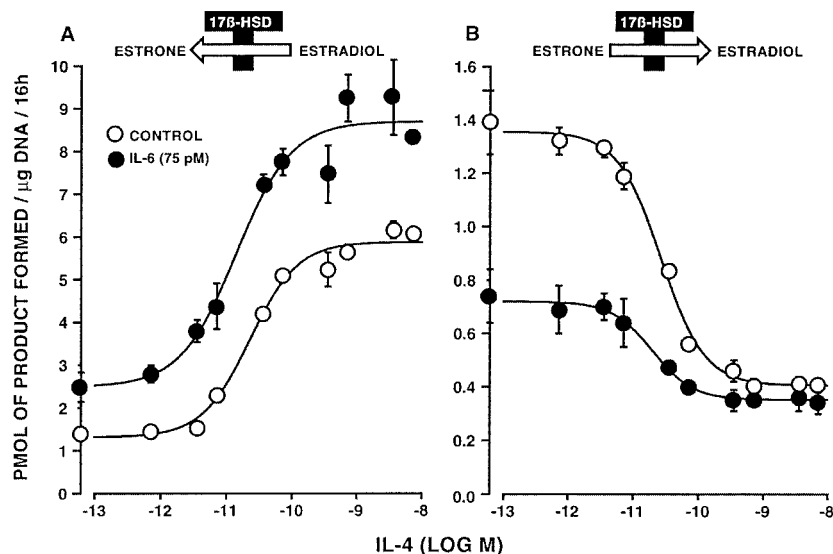
FIGURE 8.   Effect of increasing concentrations of IL-4 in the presence or absence of IL-6 on the oxidative (A) and reductive (B) 17β-HSD activities in ZR-75–1 human breast cancer cells. Three days after plating, cells were incubated for 10 days with the indicated concentrations of IL-4 in the presence or absence of 75 pM IL-6, with changes of media every 2 days, prior to a 16-h incubation with 10 nM [^{3}H]E$_2$ (A) or [^{3}H]E$_1$ (B). Data are expressed as means ± s.e.m. ($n=3$) (Turgeon *et al*. 1998).

thus strongly suggesting the expression of the type 2 17β-HSD in ZR-75–1 cells. In contrast, in T-47D cells, IL-4 increased the formation of E$_2$, whereas IL-6 exerted no effect on this parameter. It was found, however, that T-47D cells failed to convert testosterone efficiently into 4-dione, thus suggesting that there is little or no expression of type 2 17β-HSD in this cell line. The present findings demonstrate that the potent regulatory effects of IL-4 and IL-6 on 17β-HSD activities depend on the cell-specific gene expression of various types of 17β-HSD enzymes. We have also studied the effect of cytokines on the regulation of the 3β-HSD expression in both ZR-75–1 and T-47D human breast cancer cells. However, exposure to IL-4 caused a rapid and potent induction of 3β-HSD activity, whereas IL-6 failed to induce 3β-HSD expression. Such data thus demonstrate that cytokines may play a crucial role in sex steroid biosynthesis from inactive adrenal precursors in human breast cancer cells.

IMPLICATION OF 17β-HSD IN PHYSIOLOGY AND DISEASE

In the human, the continuous formation of sex steroids from DHEA in peripheral tissues is likely to play a major role in the maintenance of adequate functioning of most human tissues. Knowledge of this important role of intracrinology should be helpful in the design of better adapted therapies for the prevention and treatment of sex steroid-sensitive cancers, especially prostate and breast cancer which are the two most common cancers in men and women and the second greatest cause of cancer deaths. Intracrinology should also play an important role in the understanding, prevention and treatment of the important changes associated with menopause, namely osteoporosis and cardiovascular disease. Being at the final step in the formation of all active estrogens and androgens, it is clear that 17β-HSDs have a unique role to play in sex steroid physiology and hormone-sensitive disease.

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