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The Molecular Biology of Androgenic 17β-Hydroxysteroid Dehydrogenases

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The enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD) catalyzes the 17β-oxidation/reduction of C_{18}- and C_{19}-steroids in a variety of tissues. Three human genes encoding isozymes of 17β-HSD, designated 17β-HSD types 1, 2 and 3 have been cloned. 17β-HSD type 1 (also referred to as estradiol 17β-dehydrogenase) catalyzes the conversion of estrone to estradiol, primarily in the ovary and placenta. The 17β-HSD type 2 is expressed to high levels in the liver, secretory endometrium and placenta. The type 2 isozyme catalyzes the oxidation of androgens and estrogens equally efficiently. Also, the enzyme possesses 20α-HSD activity demonstrated by its ability to convert 20α-dihydroprogesterone to progesterone. Testicular 17β-HSD type 3 catalyzes the conversion of androstenedione to testosterone, dehydroepiandrosterone to 5-androstenediol and estrone to estradiol. The 17β-HSD3 gene is mutated in male pseudohermaphrodites with the genetic disease 17β-HSD deficiency.

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INTRODUCTION

The enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD) catalyzes a key step in the gonadal biosynthesis and extragonadal metabolism of steroid hormones (Fig. 1). Substrate and cofactor specificity, as well as intracellular localization of the different 17β-HSD activities suggested that multiple isozymes of 17β-HSD exist in human tissues. Firstly, in testis microsomes, the conversion of androstenedione to testosterone is catalyzed by a reductive 17β-HSD which utilizes NADPH as cofactor [1]. Secondly, in the placenta, a cytosolic, estrogen specific enzyme catalyzes the interconversion of estradiol and estrone and utilizes both NAD(H) and NADP(H) as cofactors [2, 3]. Thirdly, a microsomal 17β-HSD, also present in the placenta, catalyzes NAD^+ dependent oxidation of androgens and estrogens to their inactive 17-keto counterparts [4].

The breakthrough in cloning of 17β-HSD enzymes came when the human cDNA encoding the soluble placental estradiol 17β-dehydrogenase was cloned, herein designated 17β-HSD type 1, using antibody screening of a bacteriophage expression cDNA library [5]. The type 1 cDNA encodes a protein of 327 amino acids which preferentially catalyzes the reductive reaction in transfected cells, and shows higher affinity for estrogens than androgens [6]. A pseudogene, designated EDH17B1, and the functional gene, designated EDH17B2, were later cloned, both localized to the long arm of chromosome 17 [7, 8]. Northern blotting experiments show that the type 1 gene is expressed in a variety of male and female tissues, most highly in the placenta and ovary [7]. Immunohistochemical analyses have shown that the type 1 isozyme is expressed in the syncytiotrophoblast of the human placenta [9], the epithelial cells of the secretory endometrium [10], and the granulosa cells of the ovary [11].

CLONING OF 17β-HSD TYPE 2

We were interested in the 17β-HSD isozyme which inactivates androgens in the prostate. The expression cloning strategy of the cDNA responsible for the conversion of testosterone to androstenedione is outlined in Fig. 2. A size-fractionated and oriented cDNA library was prepared from human prostate mRNA in a pCMV expression vector. Two-hundred pools, each containing 10,000 clones, were transfected into human embryonic 293 cells. After 2 days, the 293 cells were assayed for 17β-HSD activity using [^{14}C]testosterone as substrate. One positive pool was identified, and

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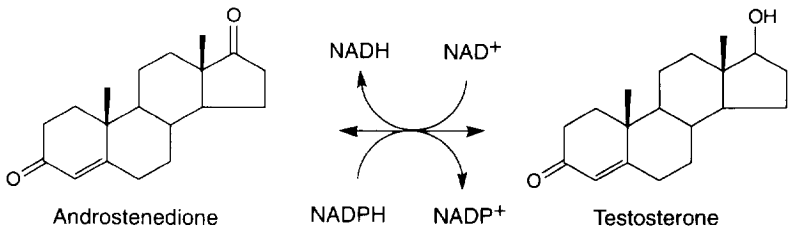

Fig. 1. The reaction catalyzed by 17β-hydroxysteriod dehydrogenase.

the cDNA responsible for the 17β-HSD activity was isolated by dilution cloning [12]. DNA sequence analysis revealed that the enzyme, designated 17β-HSD type 2, is a protein of 387 amino acids, containing an amino-terminal type II signal-anchor motif and a carboxy-terminal endoplasmic retention motif, which suggests that the enzyme is associated with the membranes of the endoplasmic reticulum. This suggestion is supported by experiments in which fractionation of cell extracts results in recovery of the recombinant enzyme in the membrane fraction. The most striking difference between the type 1 and 2 enzymes is the extended hydrophobic amino terminus of 17β-HSD type 2. Sequence alignment of the two isozymes show 23% sequence identity and 45% sequence similarity. The type 2 isozyme utilizes NAD^+ as the cofactor and preferentially catalyzes the oxidative reaction, i.e. the conversion of testosterone to androstenedione, dihydrotestosterone to androstanedione and estradiol to estrone. The enzyme also demonstrates 20α-HSD activity, i.e. the conversion of 20α-dihydroprogesterone to the more active progestin, progesterone. Together, these data suggest that the physiological role of the type 2 enzyme is to inactivate androgens and estrogens, and to activate progestins. This suggestion is supported by the tissue distribution, as revealed by Northern blotting analysis of the type 2 mRNA [13]. Very high levels are found in the placenta, liver and small intestine as compared to the prostate, kidney, colon and pancreas, whereas virtually undetectable levels are found in the testis, ovary, skeletal muscle, brain, heart, spleen and thymus. Interestingly, the secretory endometrium expresses relatively high levels of the type 2 mRNA in the mid- to late secretory phase of the ovarian cycle, in contrast to relatively low levels in the proliferative phase. In the endometrium, the type 1 mRNA level is very low relative to the type 2, suggesting that 17β-HSD type 2 is the major isozyme in this tissue. The 17β-HSD type 2 gene has been localized to chromosome 16q24 using fluorescence *in situ* hybridization analysis.

CLONING OF 17β-HSD TYPE 3

The tissue distribution, as well as the substrate and cofactor specificity of the type 2 enzyme, suggested that there must be a second androgenic isozyme of 17β-HSD expressed in the human testes. We recently expression cloned and characterized the cDNA encoding human testicular 17β-HSD, designated 17β-HSD type 3, using the expression cloning system described in the cloning of 17β-HSD type 2, with the following exceptions; the cDNA was prepared from human testes mRNA and [^{14}C]androstenedione was used as substrate in the assay rather than testosterone [14]. The type 3 isozyme is a hydrophobic protein of 310 amino acids that catalyzes NADPH-dependent 17β-reduction of androstenedione to testosterone, dehydroepiandrosterone to androstenediol and estrone to estradiol. Pairwise comparisons between the type 1, 2, and 3 proteins reveals a sequence identity of 23% and a sequence similarity of approx. 50%. Database searches also reveal that the three isozymes are members of

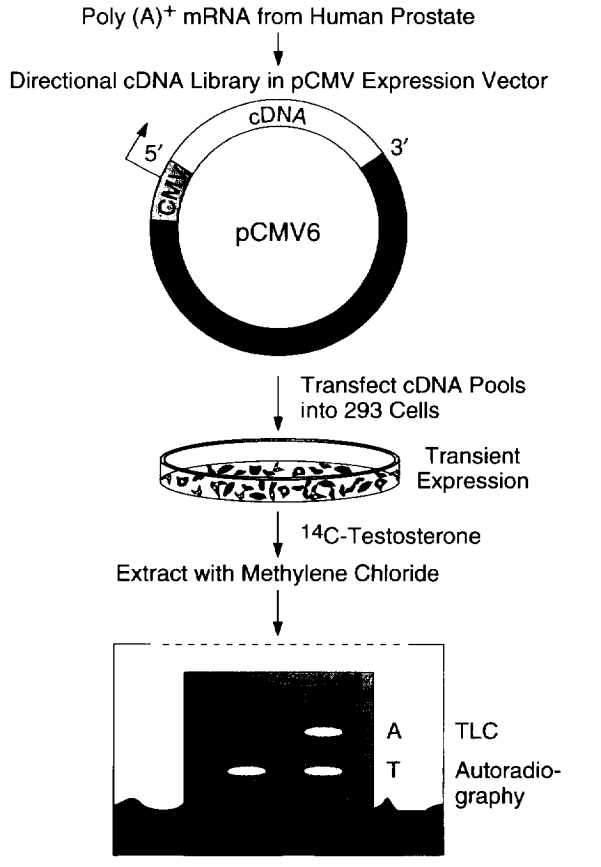

Fig. 2. Schematic representation of the expression cloning of 17β-HSD type 2.

the short-chain alcohol dehydrogenase superfamily [15]. The 17β-HSD 3 gene has been cloned and is localized to chromosome 9q22. The gene is predominantly expressed in the testes and molecular defects in the 17β-HSD type 3 enzyme underlie the genetic disease 17β-HSD deficiency (also termed 17β-hydroxysteroid oxidoreductase deficiency or 17-ketosteroid reductase deficiency). The disease is inherited in an autosomal recessive fashion with more than 40 cases described [16]. The phenotype of affected males is normal wolffian-duct derived internal genitalia and female external genitalia. Postpubertally, a biochemical hallmark of 17β-HSD deficiency is elevated plasma levels of androstenedione, luteinizing hormone and follicle stimulating hormone and subnormal to normal testosterone and dihydrotestosterone levels. To date, thirteen different mutations have been identified in the type 3 gene in twelve unrelated individuals with 17β-HSD deficiency. Nine are homozygotes, and three are compound heterozygotes. Three of the mutations are splice junction alterations, nine are substitution mutations, and one is a 7 bp deletion. The nine substitution mutations have been reconstituted by site-directed mutagenesis, and severely compromise enzyme activity. Interestingly, the substitution of a glutamine for an arginine at position 80, designated the Gaza allele, results in an enzyme with a 100-fold decreased affinity for the cofactor NADPH.

There are still some unsolved mysteries related to 17β-HSD deficiency. Firstly, why does testosterone-dependent differentiation of the wolffian ducts to the internal male reproductive structures (epididymis, vas deferens and seminal vesicles) occur in individuals with impaired testicular testosterone biosynthesis? Secondly, why do afflicted individuals virilize at puberty? It is conceivable that unidentified 17β-HSD isozyme(s) exist, alternatively, the type 1 enzyme may be capable of converting androstenedione to testosterone in peripheral tissues. In fact, in intact cells, the recombinant type 1 isozyme can catalyze the 17β-reduction of androstenedione [6], although twenty times slower than the conversion of estrone to estradiol (S. Andersson, unpublished observations). Furthermore, *in vivo*, 17β-HSD type 2 may catalyze the reductive reaction in certain cells. Another possibility is direct action by androstenedione.

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