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Review

Of marsupials and men: "Backdoor" dihydrotestosterone synthesis in male sexual differentiation

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ABSTRACT

Following development of the fetal bipotential gonad into a testis, male genital differentiation requires testicular androgens. Fetal Leydig cells produce testosterone that is converted to dihydrotestosterone in genital skin, resulting in labio-scrotal fusion. An alternative 'backdoor' pathway of dihydrotestosterone synthesis that bypasses testosterone has been described in marsupials, but its relevance to human biology has been uncertain. The classic and backdoor pathways share many enzymes, but a 3α-reductase, AKR1C2, is unique to the backdoor pathway. Human *AKR1C2* mutations cause disordered sexual differentiation, lending weight to the idea that both pathways are required for normal human male genital development. These observations indicate that fetal dihydrotestosterone acts both as a hormone and as a paracrine factor, substantially revising the classic paradigm for fetal male sexual development.

Contents

1. Role of androgens in fetal male differentiation

Following embryonic sex determination, in which the presence of a Y chromosome drives the differentiation of the primitive, bipotential gonad into a testis, androgens produced by the fetal testis mediate both the stabilization of structures derived from the Wolffian ducts and the closure of the urogenital sinus, resulting in the male phenotype. The indispensable role of androgens is demonstrated by the consequences of human androgen receptor deficiency and androgen-receptor knockout mice, which have female external genitalia despite the presence of testes and abun-

Abbreviations: AKR1C, AldoKeto Reductase; DHT, Dihydrotestosterone; DHEA, Dehydroepiandrosterone; HSD, Hydroxysteroid dehydrogenase; DSD, disorder of sex development; AMH, Anti Müllerian Hormone; StAR, steroid Acute Response protein; 17OH-Preg, 17-hydroxy pregnenolone; 17OHP, 17-hydroxy progesterone; NAD, Nicotinamide adenine nucleotide; NADPH, Nicotinamide adenine dinuclotide phosphate; mRNA, messanger ribonucleic acid; RT-PCR, reverse transcriptase-polymerase chain reaction; RoDH, Retinol Dehydrogenase.

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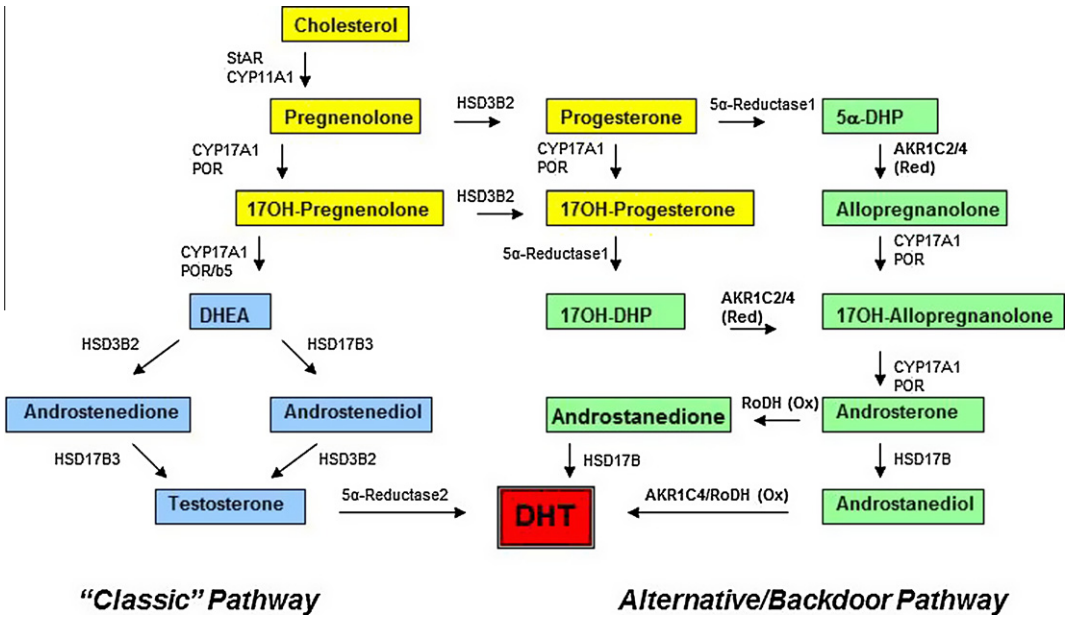

Fig. 1. The classic and alternative 'backdoor' pathways of androgen biosynthesis. The classic pathway proceeds from cholesterol via pregnenolone, 17OH-Preg and DHEA to androstenedione or androstenediol and then to testosterone in testicular Leydig cells (shown in blue). Hormonal testosterone from the circulation is then converted to DHT in genital skin. The backdoor pathway proceeds mainly from 17OH-Preg to 17OH-Prog, 17OH-DHP, 17OH-Allo, androsterone, androstanediol (3αDiol) (all shown in green) and thence to DHT (shown in red), all in the testis. The enzymes and proteins shown in the classic pathway are: CYP11A1 (P450scc, cholesterol side-chain cleavage enzyme), StAR (steroidogenic acute regulatory protein), CYP17A1 (P450c17, 17α-hydroxylase/17,20-lyase), HSD3B2 (3βHSD2, 3β-hydroxysteroid dehydrogenase, type 2), cytochrome b5, P450 oxidoreductase (POR), HSD17B3 (17βHSD3, 17β-hydroxysteroid dehydrogenase, type 3), and 5αRed2 (5α-reductase, type 2). The alternative pathway is characterized by the presence of three different enzymes: 5αRed1 (5α-reductase, type 1); reductive 3αHSD activity catalyzed by AKR1C2 and AKR1C4; and oxidative 3αHSD activity, apparently catalyzed by RoDH (also known as 17βHSD6). Steroid names include: 17OH-Pregnenolone, 17-hydroxypregnenolone; 17OH-Progesterone, 17-hydroxyprogesterone; 17OH-DHP, 17-hydroxydihydroprogesterone (5α-pregnan-3α,17α-ol-20-one); 5α-DHP, 5α-dihydroprogesterone (5α-pregnane-3,20-dione); 17OH-Allopregnanolone, 17-hydroxyallopregnanolone (5α-pregnan-3α,17α-diol-20-one); androstenediol, androsta-5-ene-3β,17β-diol.

dant androgens. Two androgens, testosterone and dihydrotestosterone (DHT), are needed to produce the human male phenotype (Wilson et al., 1981). Testosterone produced in the fetal testis reaches the genital skin of the fetal labioscrotal folds, where it is converted to DHT by 5α-reductase. The resulting DHT induces labio-scrotal fusion, the development of the phallic urethra and some phallic enlargement. Despite acting through a single androgen receptor, distinct roles for testosterone and DHT are shown by 46,XY males with 5α-reductase deficiency, who typically have an open urogenital sinus, a perineal urethral orifice, a small phallus and chordee (Wilson et al., 1994). In the "classic" pathway of androgen synthesis, DHEA and androstenedione or androstenediol are key intermediates in the synthesis of testosterone, and fetal DHT is produced in situ in its target organ, the genital skin, where it acts as a paracrine factor (Fig. 1). An alternative, or "backdoor" biosynthetic pathway leading to the production of DHT without going through testosterone has been described in marsupials (Renfree et al., 1995; Wilson et al., 2003, 2002) and in rodents (Eckstein et al., 1987; Mahendroo et al., 2004), but the potential relevance of this pathway to human genital masculinization has been unclear. Recent work has indicated this pathway as a player in human male sexual development. Patients with genetic lesions in AKR1C2, a 3α-hydroxysteroid dehydrogenase (3αHSD) that participates in the backdoor pathway but not in the classic pathway of testosterone biosynthesis, had a disorder of sexual development (DSD) (Flück et al., 2011). Several mutations were found, and the index family had combined partial disorders of two AKR1C enzymes, AKR1C2 and AKR1C4, whereas another patient had 46,XY DSD only with disordered AKR1C2 (Flück et al., 2011). This unique, newly described form of DSD supports the idea that the backdoor pathway is essential for normal male sexual development, dramatically revising former views of the mechanisms of normal and pathological masculinization.

2. Sexual development and sexual differentiation

Sexual determination and *sexual differentiation* refer to distinct processes. Sexual determination is the process by which the early bipotential gonad develops into a testis or an ovary, whereas sexual differentiation is the subsequent process by which gonadal hormones determine the anatomic phenotype (Biason-Lauber, 2010; Grumbach et al., 2003; MacLaughlin and Donahoe; 2004). The human bipotential gonad develops at about 4–6 weeks post conception, testicular development occurs soon thereafter, primarily between 6 and 8 weeks, while ovarian development occurs a bit later, at 10–14 weeks. Once developed, the fetal ovary is hormonally quiescent (Voutilainen and Miller, 1986), and ovarian function is not needed for the development of female genital organs; this is clearly evidenced by the normal female anatomy of newborns with 45,X gonadal dysgenesis (Turner Syndrome). By contrast, the fetal testis is active by 8 weeks, with the Sertoli cells producing Anti Müllerian Hormone (AMH) and the Leydig cells producing androgens, both of which are needed for normal male development. Fetal testicular androgens are required for male sexual differentiation, the process by which the labio-scrotal folds fuse, and the genital tubercle develops into the penis with the urethra traversing its inferior surface. Thus male sexual differentiation is crucially dependent on androgen biosynthesis and action.

3. The "classic" pathway of androgen biosynthesis

The 'classic' pathway of androgen biosynthesis is well-known (Fig. 1) (Miller and Auchus, 2011). After cholesterol reaches the inner mitochondrial membrane through the action of the steroidogenic acute regulatory protein (StAR), it is converted to pregnenolone in a three-step process catalyzed by the cholesterol side-chain