

Ovarian and Adrenal Androgen Biosynthesis and Metabolism

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SUMMARY

The pathways of adrenal and ovarian steroid biosynthesis use the same enzymes for the initial steps of steroidogenesis but express different enzymes that convert steroid precursors to the final active products. Both the adrenal and ovary produce dehydroepiandrosterone (DHEA), the principal precursor of androgens and estrogens. The key enzyme in DHEA production is P450c17, which catalyzes both 17 α -hydroxylation and 17,20-lyase activities. The 17,20-lyase activity of human P450c17 strongly favors 17-hydroxypregnenolone rather than 17-hydroxyprogesterone (17-OHP) as a substrate, producing abundant DHEA, so that most human androgens and estrogens derive from DHEA. Understanding the biochemistry of P450c17 is central to understanding the hyperandrogenism of polycystic ovary syndrome (PCOS). Rare genetic disorders of steroidogenesis provide human genetic knockout experiments of nature, yielding important information about the biosynthesis and physiological roles of steroids.

Key Words: Androgens; steroidogenesis; ovary; adrenal; 17,20-lyase; 17-hydroxylase; CYP21; P450c17; StAR; 3 β -hydroxysteroid dehydrogenase; 17 β -hydroxysteroid dehydrogenase; 5 α -reductase; 11 β -hydroxylase.

1. INTRODUCTION

The pathways of steroidogenesis employ a relatively small number of steroidogenic enzymes, but variations in their tissue specificity of expression and in the availability of substrates and cofactors result in the widely varying patterns of steroid production in each steroidogenic tissue (1). Although no cell type expresses all the steroidogenic enzymes, their interrelationships can be seen in the idealized integrated pathway shown in Fig. 1. Cholesterol is the precursor for all steroid hormones. The human adrenal and ovary can synthesize cholesterol *de novo* from acetate, but most cholesterol is provided by plasma low-density lipoproteins (LDLs) derived from dietary cholesterol. The presence of adequate LDL suppresses 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting step in cholesterol synthesis. HMG-CoA reductase, as well as LDL receptor number and uptake of LDL cholesterol, are stimulated by adrenocorticotrophic hormone (ACTH) in the adrenal and by follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the ovary. Steroidogenic cells take up LDL cholesterol esters by receptor-mediated endocytosis to be either stored or immediately converted to free cholesterol for use as substrate in steroidogenesis. Storage of cholesterol esters in lipid droplets is under the control of two opposing enzymes, cholesterol esterase (cholesterol ester hydrolase) and cholesterol synthetase. LH and ACTH stimulate esterase to increase the availability of free cholesterol for steroidogenesis while inhibiting synthetase.

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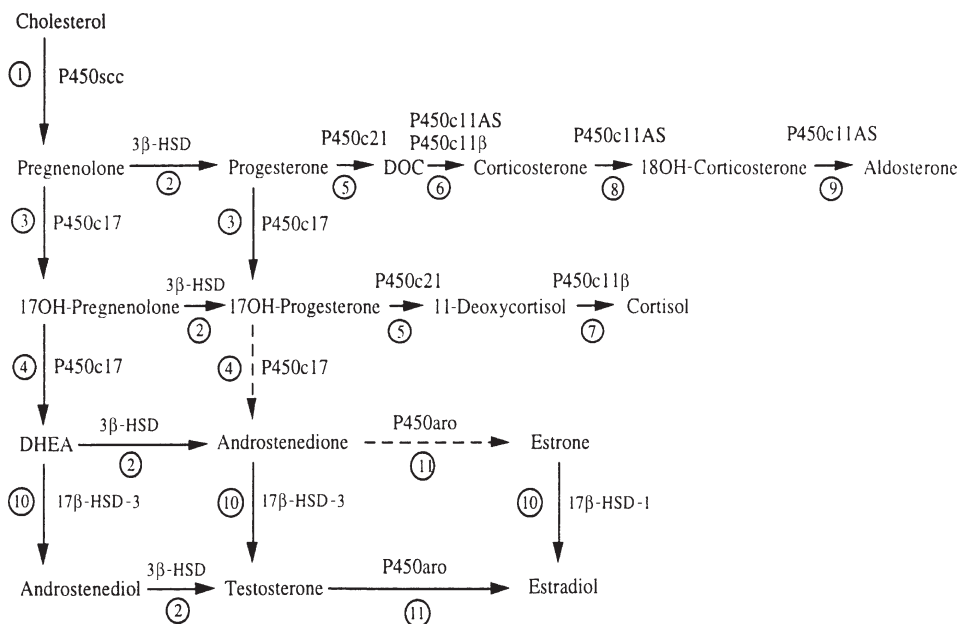


Fig. 1. Integrated view of human steroidogenesis showing adrenal and gonadal pathways. Reaction 1: P450scc converts cholesterol to pregnenolone. Reaction 2: 3β-Hydroxysteroid dehydrogenase (3β-HSD) converts Δ^5 steroids (pregnenolone, 17 α -hydroxypregnenolone, dehydroepiandrosterone [DHEA], androstenediol) to the corresponding Δ^4 steroids (progesterone, 17 α -hydroxyprogesterone, androstenedione, testosterone). Reaction 3: P450c17 catalyzes the 17 α -hydroxylation of pregnenolone and progesterone. Reaction 4: The 17,20-lyase activity of P450c17 converts 17 α -hydroxypregnenolone to DHEA; the conversion of 17 α -hydroxyprogesterone to androstenedione occurs in cattle and rodents, but human P450c17 cannot catalyze this reaction efficiently. Reaction 5: P450c21 catalyzes the 21-hydroxylation of progesterone and 17 α -hydroxyprogesterone. Reaction 6: Deoxycorticosterone (DOC) can be converted to corticosterone by either P450c11AS (in the adrenal zona glomerulosa) or P450c11β (in the adrenal zona fasciculata). Reaction 7: P450c11β converts 11-deoxycortisol to cortisol. Reactions 8 and 9: P450c11AS catalyzes 18 hydroxylase (reaction 8) and 18 methyl oxidase activities (reaction 9) to produce aldosterone in the adrenal zona glomerulosa. Reaction 10: Two isozymes of 17β-hydroxysteroid dehydrogenase (17β-HSD) activate sex steroids: 17β-HSD1 produces estradiol and 17β-HSD3 produces androgens. In peripheral tissues 17β-HSD5 has similar activity to 17β-HSD3, and 17β-HSD2 and 4 catalyze the “reverse” reactions to inactivate sex steroids. Reaction 11: P450aro aromatizes C19 androgenic steroids to C18 estrogens.

2. BACKGROUND

2.1. The Steroidogenic Enzymes

2.1.1. Early Steps: From Cholesterol to DHEA

2.1.1.1. THE STEROIDOGENIC ACUTE REGULATORY PROTEIN

Chronic regulation of steroidogenesis by LH or ACTH occurs at the level of gene transcription (1), whereas more acute regulation leading to steroid secretion following an LH surge is controlled by cholesterol access to the rate-limiting enzyme P450scc (2). This acute regulation is mediated by the steroidogenic acute regulatory protein (StAR), which facilitates the movement of cholesterol into the mitochondrion, where it becomes the substrate for the cholesterol side-chain cleavage enzyme, P450scc, the first steroid biosynthetic enzyme. StAR was first identified as short-lived 30- and 37-kDa phosphoproteins rapidly synthesized by steroidogenic cells in response to trophic hormone stimulation (2).

The central role of StAR was proven by two observations. First, robust steroid hormone synthesis follows co-transfection of StAR and the cholesterol side-chain cleavage system into nonsteroidogenic COS-1 cells (3,4). Second, patients with mutations of StAR have congenital lipid adrenal hyperplasia, in which all adrenal and gonadal steroidogenesis is disrupted (4,5). Thus, StAR is needed for the rapid flux of cholesterol from the outer to inner mitochondrial membrane to facilitate acute synthesis of aldosterone following angiotensin II stimulation, of cortisol following ACTH stimulation, and of gonadal sex steroids following LH stimulation.

StAR acts exclusively at the outer mitochondrial membrane, but its mechanism of action is not fully understood. Deletion of up to 62 of its N-terminal residues prevents StAR from entering the mitochondrion, yet it remains fully active (6). When attached to the outer mitochondrial membrane, StAR becomes constitutively active, but it is inactive when localized to the mitochondrial intramembranous space, and protein import studies show that its level of activity is directly related to the time it spends on the outer membrane (7). Conformational changes, apparently induced by the acidified outer mitochondrial membrane, are essential for StAR's activity (8–10). StAR is ultimately targeted to the mitochondrial matrix, where it is degraded more rapidly than other mitochondrial proteins. Thus, StAR is an unusual, perhaps unique protein that exerts its action in a cellular compartment other than that to which it is ultimately targeted.

However, some steroidogenesis, notably that in the placenta, is independent of StAR. Non-steroidogenic COS-1 cells transfected with the cholesterol side chain cleavage system convert cholesterol to pregnenolone at 14% of the rate achieved by co-transfection with the cholesterol side-chain cleavage system plus StAR, establishing the presence of StAR-independent steroidogenesis (4,5). The carboxyl half of a protein termed MLN64 is structurally related to StAR, exhibits StAR activity *in vitro*, and is cleaved from full-length MLN64 in the placenta, suggesting that it may play a role in placental steroidogenesis (11,12). The mitochondrial peripheral benzodiazepine receptor also plays a role in movement of cholesterol into mitochondria, but its precise role is less well understood (13).

2.1.1.2. CYTOCHROME P450

Steroidogenic enzymes fall into two broad categories: the cytochrome P450 enzymes and the hydroxysteroid dehydrogenases (1). Cytochrome P450 includes a large group of enzymes containing about 500 amino acids and a single heme group; their name derives from the characteristic absorption peak at 450 nm. There are two classes of P450 enzymes. Type I enzymes are found in mitochondria and include P450_{scc} and the two isozymes of P450_{c11}. Type II enzymes are found in the endoplasmic reticulum and include the steroidogenic enzymes P450_{c17}, P450_{c21}, and P450_{aro}. The human genome project has identified 57 P450 genes: 7 encode type I enzymes, all of which play key roles in sterol biosynthesis, and 50 encode type II enzymes. Of these 50 type II enzymes, about 20 participate in the biosynthesis of steroids, sterols, fatty acids, and eicosanoids, about 15 principally metabolize xenobiotic agents and drugs, and about 15 are “orphan” enzymes whose functions and activities remain unclear.

2.1.1.3. P450_{scc}

Conversion of cholesterol to pregnenolone by mitochondrial P450_{scc} is the initial, rate-limiting, and hormonally regulated step in steroid hormone biosynthesis (1). P450_{scc} catalyzes three sequential chemical reactions: 20 α -hydroxylation, 22-hydroxylation, and scission of the cholesterol side chain to yield pregnenolone and isocaproic acid. P450_{scc}, encoded by a single gene on chromosome 15 (14), possesses a single active site in contact with the hydrophobic lipid bilayer of the inner mitochondrial membrane. Deletion of the gene for P450_{scc} in rabbits eliminates all steroidogenesis (15), indicating that all steroid hormone biosynthesis is initiated through the action of this one enzyme. Haploinsufficiency of P450_{scc} as a result of *de novo* heterozygous mutation causes a late-onset form of congenital lipid adrenal hyperplasia (16,17).

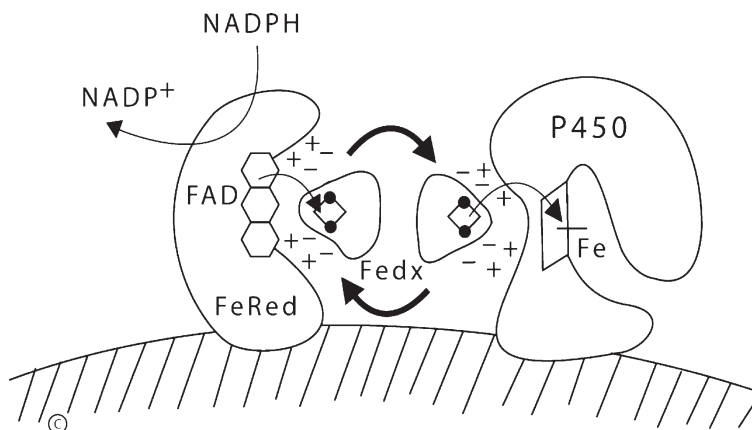


Fig. 2. Electron transfer by mitochondrial (type I) P450 enzymes. Nicotinamide adenine dinucleotide phosphate (NADPH) donates a pair of electrons to ferredoxin reductase, which is bound to the inner mitochondrial membrane. The flavin adenine dinucleotide (FAD) moiety of ferredoxin reductase passes the electrons to the iron/sulfur center of ferredoxin, depicted by a ball-and-stick diagram. Ferredoxin then dissociates from ferredoxin reductase, diffuses through the mitochondrial matrix, and interacts with the redox-partner binding-site of a type I P450. The electrons from the Fe_2S_2 center of ferredoxin then travel through an ill-described protein conduit in the P450 to reach the heme ring of the P450, which mediates catalysis.

Electrons are transported to P450scc by ferredoxin reductase and ferredoxin. All mitochondrial P450 enzymes function as the terminal oxidase in an electron transport chain (1,18) (Fig. 2). Electrons from reduced nicotinamide adenine-dinucleotide phosphate (NADPH) are accepted by a flavoprotein (ferredoxin reductase), loosely associated with the inner mitochondrial membrane (19). Ferredoxin reductase then transfers these electrons to an iron/sulfur protein (ferredoxin) located either in the mitochondrial matrix or in the inner mitochondrial membrane (20). In the final transfer, electrons are donated by ferredoxin to P450scc. No genetic disorders of these electron-transfer proteins have been described.

2.1.1.4. 3β -HYDROXYSTEROID DEHYDROGENASE/ $\Delta^5 \rightarrow \Delta^4$ ISOMERASE

Once cholesterol has been converted to pregnenolone, it may undergo 17α -hydroxylation by P450c17 to yield 17 -hydroxypregnenolone, or it may be converted to progesterone, the first biologically important steroid hormone in the pathway (Fig. 1). A single 42-kDa microsomal enzyme, 3β -hydroxysteroid dehydrogenase (3β -HSD), performs both the conversion of a hydroxyl to a ketone group and the subsequent isomerization of the double bond from the B ring (Δ^5 steroids) to the A ring (Δ^4 steroids) (21–23). A single enzyme therefore converts pregnenolone to progesterone, 17α -hydroxypregnenolone to 17α -hydroxyprogesterone (17 -OHP), dehydroepiandrosterone (DHEA) to androstenedione, as well as androstenediol to testosterone, all with the same catalytic efficiency. Therefore, this enzyme is essential for the synthesis of both sex steroids and corticoids. Characteristic of members of the hydroxysteroid dehydrogenase family, there are two isozymes for 3β -HSD, encoded by separate genes with nearly identical nucleotide sequence, but different tissue expression. The type II enzyme is found in the adrenals and gonads, and the type I enzyme, encoded by a closely linked gene with identical intron/exon organization, is found in placenta, breast, and other extraglandular tissues, such as skin. Mutations have been identified only in the 3β -HSD-II gene; mutations in the 3β -HSD-I gene would presumably prevent adequate placental production of progesterone, thereby precipitating spontaneous abortion.

2.1.1.5. P450c17

Both pregnenolone and progesterone may undergo 17 α -hydroxylation to 17 α -hydroxypregnenolone and 17-OHP, respectively. The 17 α -hydroxypregnenolone may also undergo scission of its C17,20 carbon bond to yield DHEA (Fig. 1). However, a small fraction of the 17-OHP is converted to androstenedione, although the human P450c17 enzyme catalyzes the Δ^4 substrate reaction at only 3% of the Δ^5 substrate rate (i.e., 17 α -hydroxypregnenolone to DHEA) (24). A single enzyme, P450c17, mediates all four reactions and also acts as a 16 α -hydroxylase. Whereas P450scc functions as the quantitative regulator in determining the amount of steroid hormone production, P450c17 serves as the qualitative regulator of steroidogenesis, apportioning cholesterol metabolites to the three principal classes of steroid hormone. If neither activity of P450c17 is present (e.g., in the adrenal zona glomerulosa), pregnenolone is converted to mineralocorticoids; if 17 α -hydroxylase activity is present in the absence of 17,20-lyase activity (e.g., in the adrenal zona fasciculata), pregnenolone is converted to the glucocorticoid cortisol. If both activities are present (e.g., in the gonads and the zona reticularis), pregnenolone is converted to precursors of sex steroids.

17 α -Hydroxylase and 17,20-lyase were once thought to be separate enzymes. The adrenals of prepubertal, preadrenarchal children synthesize ample cortisol but negligible DHEA, indicating the presence of 17 α -hydroxylase activity but not 17,20-lyase activity. During adrenarche the adrenal begins to produce DHEA and other C-19 steroids, suggesting that 17,20-lyase activity is turned on. Furthermore, some patients with apparently normal 17 α -hydroxylase activity yet nearly absent 17,20-lyase activity have been described. However, both 17 α -hydroxylase and 17,20-lyase activities reside in a single protein (25). P450c17 is encoded by a single gene residing on chromosome 10q24.3 that bears structural relation to the gene for P450c21 (26,27).

Thus, the distinction between 17 α -hydroxylase and 17,20-lyase is functional, not genetic or structural. Human P450c17 catalyzes the 17 α -hydroxylation of Δ^5 pregnenolone and Δ^4 progesterone with equal efficiency, but catalyzes the 17,20-lyase conversion of 17-OHP to Δ^4 androstenedione very poorly (24). Thus, most sex steroid synthesis proceeds through DHEA, and little proceeds through 17-OHP. This is evidenced by the large amounts of DHEA produced by both fetal and adult adrenal glands. Moreover, the 17 α -hydroxylase reaction is 20- to 25-fold more efficient (higher V_{\max}/K_m) than the 17,20-lyase reaction (24). The major factor regulating the 17,20-lyase reaction, and thus the production of all androgens and estrogens, is electron transfer.

All microsomal P450 enzymes (including P450c17, P450c21, and aromatase) receive electrons from a membrane-bound flavoprotein, P450 oxidoreductase (POR) (18). POR is an 82-kDa membrane-associated flavoprotein that has a bilobed structure (28). A pair of electrons from NADPH is accepted by the flavin adenine dinucleotide (FAD) moiety in one lobe; this elicits flexion of a hinge region permitting the FAD moiety to move close to the flavin mononucleotide (FMN) moiety in the second lobe. The electrons jump from the FAD to the FMN, and then the protein “unflexes” on its hinge, permitting the FMN moiety to interact with the redox-partner binding site of the P450. The FMN of POR then gives up the electrons, which migrate through multiple paths to the heme group of the P450, where they mediate catalysis (Fig. 3). The 17,20-lyase activity of P450c17 can be increased by increasing the molar ratio of POR to P450c17 or by factors that increase the affinity of POR for P450c17.

Two posttranslational mechanisms, the presence of cytochrome b_5 (24) and the serine phosphorylation of P450c17 (29), facilitate the interaction of P450c17 with POR to optimize electron transfer (30). The adrenal zona reticularis, the site of production of adrenal androgen precursors, contains abundant cytochrome b_5 , whereas the other adrenal zones have virtually none (31). Thus, cytochrome b_5 appears to play a major role in human adrenal androgen synthesis, but its potential role in the ovary has not been explored. Increasing the ratio of either P450 oxidoreductase or cytochrome b_5 to P450c17 in vitro or in vivo favors the 17,20-lyase activity and P450c17 mutations that interfere with electron receipt from POR cause isolated 17,20-lyase deficiency (32,33).

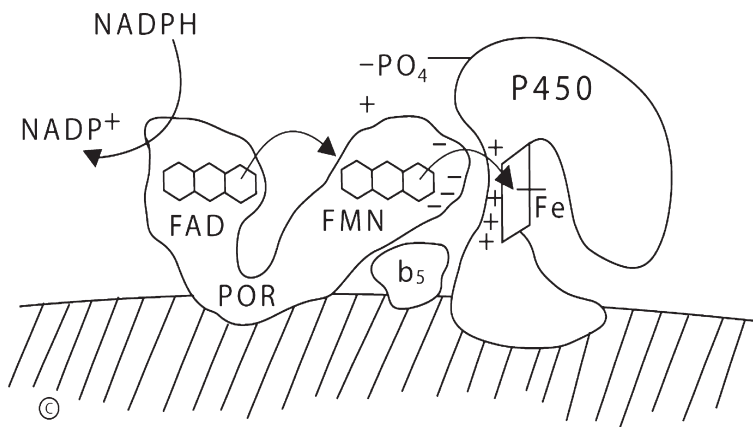


Fig. 3. Electron transfer by microsomal (type II) P450 enzymes. Nicotinamide adenine dinucleotide phosphate (NADPH) donates two electrons to the flavin adenine dinucleotide (FAD) moiety of P450 oxidoreductase (POR), bound to the endoplasmic reticulum. Electron receipt elicits a conformational change, permitting the FAD and flavin mononucleotide (FMN) moieties to come close together, so that the electrons pass from the FAD to the FMN. The protein then returns to its original orientation, and the FMN domain interacts with the redox-partner binding site of the P450, donating electrons to the heme group to achieve catalysis. The interaction of POR and the P450 is coordinated by negatively charged acidic residues on the surface of the FMN domain of POR and positively charged basic residues in the redox-partner binding site of the P450. In the case of human P450c17, this interaction is facilitated by the allosteric action of cytochrome b_5 and by the serine phosphorylation of P450c17.

There are three forms of cytochrome b_5 , encoded by two genes. The gene on chromosome 18q23 undergoes alternative splicing to yield two proteins: the widely expressed 134AA form bound to the endoplasmic reticulum (which interacts with P450c17) and a soluble 98AA form found in erythropoietic tissues. A second gene on chromosome 16q22.1 encodes a form of cytochrome b_5 termed OMB₃ associated with the outer mitochondrial membrane. OMB₃ can support the 17,20-lyase activity of P450c17 in vitro, but it is not clear whether it serves this function in vivo (18,30).

Although the kinase responsible for the serine/threonine phosphorylation of P450c17 has not yet been identified, it is clear that the balance achieved between this kinase and a counteracting phosphatase must regulate the level of P450c17 phosphorylation. Protein phosphatase 2A (PP2A), but not the closely related PP4 or PP6, will specifically dephosphorylate P450c17, and the level of PP2A activity in adrenal cells is regulated by a phosphoprotein termed SET (34). SET is of interest because it acts as a transcriptional regulator of P450c17 gene transcription in mouse Leydig MA-10 cells, although a similar action with the human gene has not yet been demonstrated. The acquisition of 17,20-lyase activity through serine phosphorylation may provide a direct link to the insulin resistance of PCOS (35), as girls who have increased 17,20-lyase activity and premature exaggerated adrenarche tend to develop PCOS as adults.

Although ablation of POR in mice results in embryonic lethality, numerous patients have been described as having POR mutations (36–40). Most of these patients came to medical attention because they had a severe skeletal malformation disorder termed Antley–Bixler syndrome in addition to having disordered adrenal and gonadal steroidogenesis with a pattern suggesting combined deficiencies of P450c17, P450c21, and P450aro. Careful analysis of the enzymology of all known mutants shows that all affect P450c17, with some affecting the 17,20-lyase activity to a greater degree than 17 α -hydroxylase activity (40). The altered steroidogenesis in these patients leads to undervirilization of males and partial virilization of females. One of the initial patients reported was an adult woman who

presented with PCOS (36); whether this is a rare event or indicates that POR may be more commonly associated with some forms of PCOS is not yet known.

2.1.2. Adrenal-Specific Enzymes

2.1.2.1. P450c21

Progesterone and 17-OHP may be hydroxylated at their C21 position to produce deoxycorticosterone (DOC) and 11-deoxycortisol, respectively (Fig. 1). The nature of the 21 α -hydroxylating step has been of intense interest because more than 90% of cases of congenital adrenal hyperplasia (CAH) are caused by defects in P450c21. The severe form of this common genetic disease can be fatal. Compromised cortisol and aldosterone synthesis can lead to hyponatremia, hyperkalemia, hypotension, and circulatory collapse, leading to death within the first month of life if not treated appropriately. Decreased cortisol production *in utero* results in a compensatory increase in ACTH, with consequent overstimulation of the adrenal steroid biosynthesis. 17-OHP levels rise in the face of impaired 21-hydroxylation and the limited ability of P450c17 to convert 17-OHP to androstenedione. However, continued ACTH-stimulated flux of cholesterol results in accumulation of 17-hydroxypregnenolone, which is then converted to DHEA, androstenedione, and ultimately testosterone, resulting in severe prenatal virilization of female fetuses (41). Mild, “nonclassical” variants of CAH are a common cause of adrenal (but not gonadal) hyperandrogenism.

The gene for P450c21 lies within the major histocompatibility locus on chromosome 6p21, and mutations in P450c21 are linked to specific human leukocyte antigen types. The genetics of this locus are complicated by its very high rate of genetic recombination. As a result, there can be one, two, three, or four copies of the P450c21 gene, but only one is functional, and its mutations arise from recombination with the nearby inactive pseudogene (41). Extra-adrenal 21 α -hydroxylase activity, observed in a variety of fetal and adult tissues remains poorly characterized, but is not mediated by the same P450c21 enzyme that is found in the adrenal glands (42).

2.1.2.2. P450c11 β AND P450c11AS

The final steps in the mineralocorticoid and glucocorticoid pathways are catalyzed by P450c11 β and P450c11AS (43,44) (Fig. 1). These two isozymes share 93% amino acid sequence identity and are encoded by tandemly duplicated genes located on chromosome 8q21-22. Both forms of P450c11 reside on the inner mitochondrial membrane and, like P450scc, utilize ferredoxin reductase and ferredoxin to receive electrons from NADPH. P450c11 β is abundantly expressed in the zona fasciculata, where it catalyzes the classic 11 β -hydroxylase reactions that convert 11-deoxycortisol to cortisol and DOC to corticosterone. The less abundant P450c11AS (aldosterone synthase) is uniquely expressed in the zona glomerulosa, where it catalyzes the sequential steps 11 β -hydroxylase, 18-hydroxylase, and 18-methyloxidase necessary to produce aldosterone.

2.1.2.3. STEROID SULFOTRANSFERASE AND SULFATASE

Steroid sulfates may be synthesized directly from cholesterol sulfate or by sulfating Δ^5 steroids by the main sulfotransferases, SULT2A1 and SULT2B1, and the phenolic sulfotransferase SULT1E1. The addition of a sulfate group prevents the activation of Δ^5 steroids to Δ^4 steroids by 3 β -HSD. SULT2A1, which is expressed primarily in the adrenals and to a lesser degree the liver and kidney, sulfates DHEA (45). SULT2A1 is not expressed in the ovary (45), whereas SULT2B1 and SULT1E1 are widely expressed (46). SULT2B1 and SULT1E1 sulfate the other 3 β -hydroxysteroids and estrogens, respectively. Steroid sulfates may be hydrolyzed to their native state by steroid sulfatase. Steroid sulfatase, encoded on chromosome Xp22.3, desulfates most 3 β -hydroxysteroids and is expressed in most tissues, including the ovary. The principal role of steroid sulfatase is in disposing of excess steroid sulfates. When steroid sulfatase activity is absent in X-linked ichthyosis, steroid sulfates accumulate in the stratum corneum of the skin. However, this condition has little impact on adrenal or gonadal steroidogenesis.

2.1.3. Enzymes Involved in Sex Steroid Synthesis

2.1.3.1. 17 β -HYDROXYSTEROID DEHYDROGENASES

The 17 β -hydroxysteroid dehydrogenases (17 β -HSDs) interconvert 17-ketosteroids with the corresponding 17-hydroxysteroids, thereby controlling the synthesis and metabolism of sex steroids. Although these enzymes can catalyze both oxidative and reductive reactions *in vitro*, the presence of substantial excess of a suitable cofactor *in vivo* (or in the absence of a preferred cofactor) will typically direct each enzyme in the oxidative or reductive direction (47–49). The reductive enzymes utilize NAD(P)H as the cofactor and the oxidative enzymes utilize NAD(P)⁺. Five principal human isoforms have been identified, some of which are preferential oxidases, whereas others are principally reductases. These isoforms share only 20–30% sequence identity, and yet their secondary and tertiary structures are remarkably similar.

Type I 17 β -HSD (17 β -HSD-I) is the estrogenic form expressed in the placenta, where it produces estriol, and in the ovary, where it catalyzes the last step in estradiol synthesis (50,51). 17 β -HSD-I, encoded by a gene on chromosome 17q21 near the BRCA locus, is also expressed in endometrium, breast, testis, adipose tissue, skin, liver, and prostate. 17 β -HSD-I is a homodimer that uses NADPH as its cofactor and converts estrone to estradiol. The crystallographic structure of 17 β -HSD-I shows that the active site binds steroids that have a planar A-ring and are missing the C-19 methyl group, whereas steroids with a C-19 methyl group or a nonplanar A-ring cannot be bound (52). Human ovarian granulosa cells, but not theca cells, express 17 β -HSD-I (51).

17 β -HSD-II oxidizes estradiol to estrone and testosterone to Δ^4 -androstenedione with equal efficiency using NAD⁺ as a cofactor (53,54). This enzyme can also oxidize C-20 substrates (20 α -HSD activity), converting 20 α -hydroxyprogesterone to progesterone (54). 17 β -HSD-II, encoded on chromosome 16q24, shares only 20% amino acid sequence identity with 17 β -HSD-I. 17 β -HSD-II is found in the placenta, breast, liver, small intestine, prostate, secretory endometrium, kidney, and ovary. 17 β -HSD-II is expressed in the endothelial cells of the placental intravillous vessels, consistent with its role in defending the fetal circulation from transplacental passage of maternal estradiol or testosterone (55). In the endometrium, 17 β -HSD-II is expressed in the secretory phase and maintains predominance of progestational activity by oxidizing estradiol and converting 20 α -hydroxyprogesterone back to progesterone.

17 β -HSD-III is an androgenic enzyme that uses NADPH as a cofactor and reduces androstenedione to testosterone and DHEA to androstenediol. The 17 β -HSD-III gene on chromosome 9q22 is expressed primarily in the testis and adipose tissue, but is not expressed in the ovary. This is the only form of 17 β -HSD for which a deficiency state is known, causing the classic syndrome of male pseudohermaphroditism, often termed 17-ketosteroid reductase deficiency (56).

17 β -HSD-IV, originally identified as a NAD⁺-dependent oxidase with activities similar to 17 β -HSD-II, is a widely distributed peroxisomal protein that acts primarily as a 2-enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase (47,48). The gene for 17 β -HSD-IV on chromosome 5q2 is ubiquitously expressed. Its role in sex-steroid metabolism is not established, but *in vitro* it oxidizes the C-18 and C-19 substrates using NAD⁺ as a cofactor.

17 β -HSD-V, initially cloned as a 3 β -hydroxysteroid dehydrogenase, primarily catalyzes the conversion of Δ^4 androstenedione to testosterone (57) but also has 20 β -HSD activity. 17 β -HSD-V is expressed in most tissues, including liver, kidney, blood vessels, and testis, prostate, adrenal, bone, and ovary (57). Whereas the other 17 β -HSDs are members of the short-chain dehydrogenase family, 17 β -HSD-V is a member of the aldoketoreductase family. The peripheral conversion of Δ^4 androstenedione to testosterone by 17 β -HSD-V in target tissues is apparently responsible for the “weak androgen” action of androstenedione. It is also the only form of 17 β -HSD expressed in the ovarian theca and corpus luteum, suggesting that 17 β -HSD-V is the enzyme responsible for the last step in testosterone production in the ovary.

2.1.3.2. 5 α -REDUCTASE

Testosterone can be converted to the more potent androgen dihydrotestosterone (DHT) by 5 α -reductase in target tissues, especially the skin. There are two isoforms of 5 α -reductase, which share about 50% homology. Each is a membrane-associated enzyme that utilizes NADPH as a cofactor to reduce the 4-to-5 double bond in ring A. Both enzymes are expressed in several tissues, including the urogenital tract, skin, gastrointestinal and respiratory tracts, liver, brain, and ovary. The type II enzyme is critical for the development of normal male external genitalia, but an essential role is not established in women. By contrast, type I may have a role in fecundity and parturition (58).

2.1.3.3. P450ARO: AROMATASE

Estrogens are produced from androgens by a complex series of reactions catalyzed by a single aromatase enzyme P450aro (59). This microsomal enzyme is encoded by a single gene on chromosome 15q21.1; this gene is unusual in that it uses several different alternative transcriptional start sites and first exons driven by different upstream promoter sequences, permitting the same protein to be expressed under different control in different cell types (59). P450aro in peripheral tissues, especially fat, can convert substantial portions of circulating androstenedione and testosterone in women to estrone and estradiol. The placenta expresses large amounts of aromatase, protecting the fetus from maternal androgens and permitting disposal of fetal C-19 steroids (60). Although the placenta produces huge amounts of estriol from fetally produced DHEA, estriol is not needed for normal pregnancy, as shown by the normal development, labor, and parturition of fetuses that have genetic lesions that prevent estriol production (61).

2.2. Circulating Sex Steroids

Dehydroepiandrosterone sulfate (DHEAS) is the most abundant steroid in the circulation of adults of reproductive age (62). DHEA, DHEAS, and androstenedione are produced almost exclusively by the adrenal zona reticularis. The adrenal does not express 17 β -HSD-III; the minimal adrenal production of testosterone is probably a result of 17 β -HSD-V (57). Adrenal C-19 steroids do not bind significantly to the androgen receptor; hence these steroids are primarily precursors that are converted to active androgens or estrogens by isozymes of 17 β -HSD in target tissues. The normal adrenal does not express aromatase and therefore does not synthesize estrogens, but adrenocortical carcinomas may produce estrogens.

Metabolism of steroidal precursors by skin and fat, which express aromatase and 17 β -HSD-I, -III and -V (63), produces most of the circulating testosterone. The relative expression of these enzymes determines how androstenedione will be metabolized. These tissues also express 3 β -HSD-I and steroid sulfatase, converting DHEAS to androstenedione. Most of the DHT produced in target tissues acts in an autocrine or paracrine fashion. Sebaceous glands and hair follicles express 5 α -reductase-I and -II respectively, converting testosterone to DHT at this important site of action (31).

2.3. Cell Biology of Sex Steroid Synthesis

2.3.1. In the Ovary

Ovarian theca and granulosa cells in individual follicular units synthesize both androgens and estrogens. The steroidogenic enzymes expressed in the theca or granulosa cells vary with the menstrual cycle (64). In the follicular phase, theca cells express StAR, P450scc, 3 β -HSD-II, and P450c17 to produce androstenedione, some of which is converted to testosterone by 17 β -HSD-V. Most thecal steroids diffuse to the granulosa cell, but some are secreted into the circulation. Rising thecal androstenedione production during the follicular phase plus increased expression of 17 β -HSD-I and P450aro in the granulosa cells results in abundant estradiol production by the preovulatory follicle. Theca cells continue to provide androstenedione to the granulosa cell during the luteal phase, while the granulosa cell undergoes granulosa-lutein transformation and expresses StAR, P450scc, and 3 β -HSD-II,

but not P450c17 (65). This permits the granulosa-lutein cell to produce progesterone while converting C-19 steroids from the theca to estrogens.

Numerous follicles grow simultaneously in the reproductive ovary. During the recruitment and growth phase, the granulosa cells proliferate, differentiate, and become steroidogenic. During the growth phase, most follicles undergo atresia, while some remain viable. The “antral” follicular stage is characterized by antrum formation and acquisition of differentiated steroidogenic cells (66). LH induces theca cells to synthesize androgens before FSH induces the granulosa cells to aromatize them. Only the granulosa cells surrounding the follicle express aromatase and produce estradiol. During the 2 weeks before ovulation (the follicular phase), LH increases the theca cell expression of the LH receptor, StAR, P450scc, 3 β -HSD-II, and P450c17, whereas FSH increases granulosa cell expression of aromatase and 17 β -HSD-I. The majority of ovarian C-19 steroids are produced from DHEA. While rodents and other species produce androstenedione directly from 17-OHP, the 17,20-lyase activity of human P450c17 strongly favors 17 α -hydroxypregnenolone as a substrate and utilizes 17-OHP inefficiently (24).

Intraovarian peptides, including members of the insulin-like growth factor, transforming growth factor, and epidermal growth factor families, modulate both folliculogenesis and steroidogenesis. Germ cell differentiating factor-9, expressed by the oocyte throughout folliculogenesis, appears to promote granulosa cell differentiation, stimulate theca cells, and inhibit luteal cell formation (67). Insulin-like growth factors appear to enhance responses to FSH. Both insulin-like growth factor (IGF)-1 and IGF-2 can increase granulosa cell proliferation and estradiol secretion, but only IGF-2 mRNA is expressed in the ovary, particularly in the granulosa cells (68). Women with IGF-1 deficiency can be induced to ovulate by stimulation with gonadotropins (69), consistent with the view that IGF-1 is not required for folliculogenesis.

Granulosa cells also produce inhibin, an α/β heterodimer that exists in two forms differing in the β -subunit. Serum concentrations of inhibin A and B vary with the menstrual cycle: inhibin A rises in follicular fluid with increasing follicular size, beginning just before ovulation and peaking during the luteal phase, and inhibin B increases in granulosa cells following stimulation with FSH (70). Serum concentrations of inhibin B correlate with the volume of granulosa cells, serving as an index for the size of the growing cohort of follicles (ovarian “reserve”). Inhibin B peaks in the early follicular phase and inhibits pituitary production of FSH during the later follicular phase. The antral follicle grows 1–2 mm in diameter daily during the antral phase of follicular development in response to gonadotropins (66), reaching a diameter of about 20 mm, primarily representing accumulated follicular fluid. The theca interna continues to differentiate into interstitial cells, generating more androstenedione. The granulosa cell layers differentiate into discrete zones: the membrana layer subsequently acquires LH receptors in response to FSH, whereas the cumulus layer constitutively expresses LH receptors. One dominant follicle destined for ovulation develops into a mature graafian follicle.

Following ovulation, LH induces granulosa membrana cells to differentiate into granulosa lutein cells and the remaining thecal interstitial cells to differentiate into theca lutein cells, thus forming the corpus luteum. LH also induces the granulosa lutein cells to produce vascular endothelial growth factor, which is required for neovascularization of the corpus luteum that penetrates the basement membrane to provide the LDLs necessary for steroidogenesis. After ovulation, the luteal cells accumulate more LH receptors, allowing basal LH levels to maintain the corpus luteum. Early in pregnancy, human chorionic gonadotropin stimulates these LH receptors, inducing progesterone synthesis by the corpus luteum during the first trimester. In contrast to the preovulatory follicle, the granulosa-lutein cells and the corpus luteum of pregnancy express large amounts of P450scc and 3 β -HSD, permitting the synthesis of progesterone.

2.3.2. In the Adrenal

The regulation of adrenal androgen production is poorly understood (35). The fetal adrenal produces large amounts of DHEAS, but these serve no essential role, because fetuses that cannot pro-

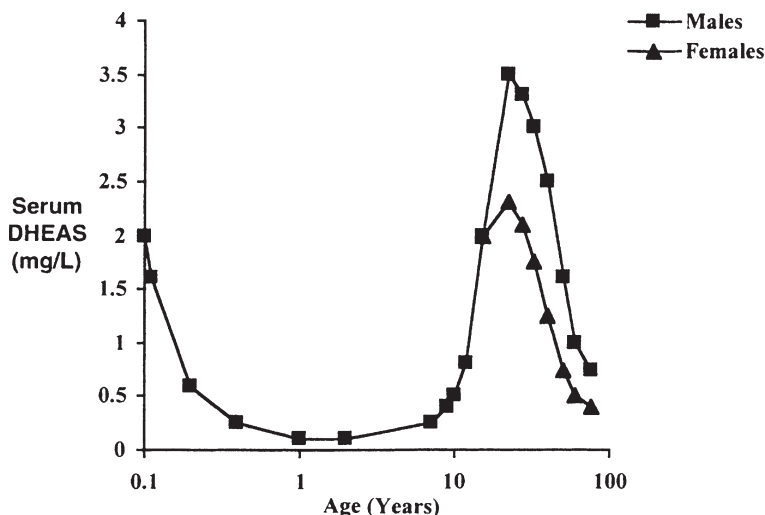


Fig. 4. Concentrations of dehydroepiandrosterone sulfate (DHEAS) as a function of age. Note that the x -axis is on a logarithmic scale.

duce these steroids (e.g., those having no P450c17) develop normally. Following birth, the fetal zone of the adrenal involutes and the production of DHEAS falls to very low levels. The adrenal zona reticularis becomes morphologically identifiable after about 3 years of age, but production of DHEAS does not begin until the onset of adrenarche at about 7–8 years of age.

Adrenarche is defined by this rise in serum DHEAS and is the basis of so-called pubarche—the onset of small amounts of pubic hair before the onset of puberty. Adrenarche typically precedes true puberty (i.e., the activation of the hypothalamic/pituitary/gonadal axis) by about 2 years and is completely independent of puberty, gonadotropins, and the gonads themselves. The stimulus to adrenarche is unknown. Adrenarche occurs only in human beings, chimpanzees, gorillas, and possibly orangutans, and hence is rather difficult to study (71). Some have hypothesized a peptide stimulator of the zona reticularis (analogous to ACTH), but no solid evidence for an adrenal androgen-stimulating hormone has appeared.

Serum concentrations of DHEAS continue to rise after puberty, reaching maximal levels at 25–30 years, and then begin to decline (“adrenopause”) (Fig. 4) (62). Despite the 100-fold change in DHEAS concentrations during adrenarche, concentrations of ACTH and cortisol do not change. Thus most contemporary studies of adrenarche focus on intra-adrenal events (35). The onset of adrenarche is accompanied by a decrease in 3 β -HSD and an increase in P450c17 and cytochrome b_5 in the zona reticularis (72), favoring the production of DHEA. Serine phosphorylation of P450c17 also favors DHEA production, but it is not known whether the phosphorylation of P450c17 changes during adrenarche.

A developmentally programmed trigger, possibly IGF-1, may induce cellular proliferation in the zona reticularis while promoting synthesis of the steroidogenic machinery (35,73). At the same time, increased synthesis of cytochrome b_5 promotes the 17,20-lyase activity of P450c17, which, together with decreased 3 β -HSD expression, favors DHEA production. Serine phosphorylation of P450c17 may also be developmentally programmed, and also facilitates 17,20-lyase activity (73). Unlike the neighboring fasciculata, the reticularis expresses sulfotransferase preferentially over steroid sulfatase, ensuring augmented DHEAS production.

Premature exaggerated adrenarche has been linked to insulin resistance, and girls with premature exaggerated adrenarche appear to be at greater risk of developing PCOS as adults (73–76). PCOS is

characterized by hirsutism, virilism, hyperandrogenism, menstrual irregularities, chronic anovulation, obesity, insulin resistance, acanthosis nigricans, high concentrations of LH, and ovarian cysts (77,78). Hyperandrogenism and insulin resistance appear to be primary lesions, and the other findings are secondary events. The hyperandrogenism in women with PCOS is of both ovarian and adrenal origin (77,78). The adrenal hyperandrogenism of PCOS resembles an exaggerated form of adrenarche, and girls with premature adrenarche are more likely to develop PCOS (74,75). A gain-of-function disorder in the pathway leading to the serine/threonine phosphorylation of P450c17 could account for such increases in both adrenal and ovarian androgen secretion and an earlier age of adrenarche (29,34), but such mutations have not yet been reported.

The hyperinsulinism and insulin resistance of PCOS is at the level of insulin receptor signal transduction (78). Serine phosphorylation of the β chain of the insulin receptor interferes with the tyrosine phosphorylation of the receptor that normally follows binding of insulin (79–81). Furthermore, some PCOS women appear to have insulin receptors in their fibroblasts that are hyperphosphorylated (78). Thus, a gain-of-function mutation in a serine-threonine kinase or its signal transduction pathway might increase the serine hyperphosphorylation of both P450c17 and the β chain of the insulin receptor, thus accounting for both the hyperandrogenism and insulin resistance of PCOS with a single molecular lesion (29,34,35,73).

3. CONCLUSION

The pathways of adrenal and ovarian steroid biosynthesis use the same enzymes for the initial steps of steroidogenesis, but express different enzymes that convert steroid precursors to the final active products. Both the adrenal and ovary produce DHEA as the key precursor of androgens and estrogens. The key enzyme in DHEA production is P450c17, which catalyzes both 17 α -hydroxylation and 17,20-lyase activities. The 17,20-lyase activity of human P450c17 strongly favors the Δ^5 pathway, so that most human androgens and estrogens derive from DHEA. Consequently, understanding the biochemistry and regulation of P450c17, and in particular its 17,20-lyase activity, is central to understanding the hyperandrogenism of PCOS.

4. FUTURE AVENUES OF INVESTIGATION

While the pathways of steroidogenesis have been described in textbooks for more than 40 years, much remains to be learned. First, the transcriptional mechanisms leading to cell-type-specific, developmentally programmed, and hormonally regulated expression of each steroidogenic enzyme and cofactor will need to be delineated in detail before the underlying genetic control of steroidogenesis can be understood. Second, the enzymology of each biosynthetic reaction, and especially the posttranslational mechanisms regulating these, require further investigation. Third, the identity, nature, and activities of steroid modifying enzymes in target tissues such as skin, uterus, breast, fat, and muscle require further investigation. Fourth, steroid synthesis in the brain and its potential role in reproductive and other behaviors are only beginning to be explored. Finally, the factors governing the hyperandrogenic states commonly grouped under the diagnosis of PCOS remain to be elucidated.

KEY POINTS

- Steroidogenic enzymes fall into two broad categories: cytochromes P450 and hydroxysteroid dehydrogenases.
- Each P450 enzyme is encoded by a single gene but has multiple activities.
- Each hydroxysteroid dehydrogenase is encoded by multiple genes, but all have similar activities.
- The adrenal and ovary share the same enzymes catalyzing early steps in steroidogenesis, but possess different enzymes leading to the different final products.
- P450c17, which catalyzes 17 α -hydroxylase and 17,20-lyase activities, is a key factor in androgen and estrogen synthesis.
- The 17,20-lyase activity of human P450c17 strongly favors the Δ^5 pathway, so that most androgen and estrogen synthesis proceeds through DHEA.

- The 17,20-lyase activity of human P450c17 is regulated posttranslationally by serine phosphorylation of P450c17 and by the allosteric action of cytochrome *b*₅.
- Serine phosphorylation may provide an important mechanistic link between the hyperandrogenism and insulin resistance of PCOS.

REFERENCES

1. Miller WL. Molecular biology of steroid hormone synthesis. *Endocr Rev* 1988;9:295–318.
2. Stocco DM, Clark BJ. Regulation of the acute production of steroids in steroidogenic cells. *Endocr Rev* 1996;17:221–244.
3. Clark BJ, Wells J, King SR, Stocco DM. The purification, cloning and expression of a novel luteinizing hormone-induced mitochondrial protein in MA-10 mouse Leydig tumor cells: Characterization of the steroidogenic acute regulatory protein (StAR). *J Biol Chem* 1994;269:28314–28322.
4. Lin D, Sugawara T, Strauss JF III, et al. Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. *Science* 1995;267:1828–1831.
5. Bose HS, Sugawara T, Strauss JF III, Miller WL. The pathophysiology and genetics of congenital lipoid adrenal hyperplasia. *N Engl J Med* 1996;335:1870–1878.
6. Arakane F, Sugawara T, Nishino H, et al. Steroidogenic acute regulatory protein (StAR) retains activity in the absence of its mitochondrial targeting sequence: Implications for the mechanism of StAR action. *Proc Natl Acad Sci USA* 1996;93:13731–13736.
7. Bose HS, Lingappa VR, Miller WL. Rapid regulation of steroidogenesis by mitochondrial protein import. *Nature* 200;417:87–91.
8. Bose HS, Whittall RM, Baldwin MA, Miller WL. The active form of the steroidogenic acute regulatory protein, StAR, appears to be a molten globule. *Proc Natl Acad Sci USA* 1999;96:7250–7253.
9. Yaworsky DC, Baker BY, Bose HS, et al. pH-dependent interaction of the carboxyl-terminal helix of steroidogenic acute regulatory protein with synthetic membranes. *J Biol Chem* 2005;280:2045–2054.
10. Baker BY, Yaworsky DC, Miller WL. A pH-dependent molten globule transition is required for activity of the steroidogenic acute regulatory protein (StAR). *J Biol Chem* 2006;280:4753–4760.
11. Watari H, Arakane F, Moog-Lutz C, et al. MLN64 contains a domain with homology to the steroidogenic acute regulatory protein (StAR) that stimulates steroidogenesis. *Proc Natl Acad Sci USA* 1997;94:8462–8467.
12. Bose HS, Whittall RM, Huang MC, Baldwin MA, Miller WL. N-218 MLN64, a protein with StAR-like steroidogenic activity is folded and cleaved similarly to StAR. *Biochemistry* 2000;39:11722–11731.
13. Papadopoulos V. Peripheral-type benzodiazepine/diazepam binding inhibitor receptor: Biological role in steroidogenic cell function. *Endocr Rev* 1993;14:222–240.
14. Chung B, Matteson KJ, Voutilainen R, Mohandas TK, Miller WL. Human cholesterol side-chain cleavage enzyme, P450scc: cDNA cloning, assignment of the gene to chromosome 15, and expression in the placenta. *Proc Natl Acad Sci USA* 1986;83:8962–8966.
15. Yang X, Iwamoto K, Wang M, Artwohl J, Mason JI, Pang S. Inherited congenital adrenal hyperplasia in the rabbit is caused by a deletion in the gene encoding cytochrome P450 cholesterol side-chain cleavage enzyme. *Endocrinology* 1993;132:1977–1982.
16. Tajima T, Fujieda K, Kouda N, Nakae J, Miller WL. Heterozygous mutation in the cholesterol side chain cleavage enzyme (P450scc) gene in a patient with 46,XY sex reversal and adrenal insufficiency. *J Clin Endocrinol Metab* 2001;86:3820–3825.
17. Katsumata N, Ohtake M, Hojo T, et al. Compound heterozygous mutations in the cholesterol side-chain cleavage enzyme gene (CYP11A) cause congenital adrenal insufficiency in humans. *J Clin Endocrinol Metab* 2002;87:3808–3813.
18. Miller WL. Regulation of steroidogenesis by electron transfer. *Endocrinology* 2005;146:2544–2550.
19. Solish SB, Picado-Leonard J, Morel Y, et al. Human adrenodoxin reductase: Two mRNAs encoded by a single gene of chromosome 17cen→q25 are expressed in steroidogenic tissues. *Proc Natl Acad Sci USA* 1988;71:7104–7108.
20. Picado-Leonard J, Voutilainen R, Kao L, Chung B, Strauss JF III, Miller WL. Human adrenodoxin: Cloning of three cDNAs and cycloheximide enhancement in JEG-3 cells. *J Biol Chem* 1988;263:3240–3244.
21. Luu-The V, Lachance Y, Labrie C, et al. Full length cDNA structure and deduced amino acid sequence of human 3 β -hydroxy-5-ene steroid dehydrogenase. *Mol Endocrinol* 1989;3:1310–1312.
22. Lorence MC, Murry BA, Trant JM, Mason JI. Human 3 β -hydroxysteroid dehydrogenase/ $\Delta^5 \rightarrow \Delta^4$ isomerase from placenta: Expression in nonsteroidogenic cells of a protein that catalyzes the dehydrogenation/isomerization of C21 and C19 steroids. *Endocrinology* 1990;126:2493–2498.
23. Morel Y, Mebarki F, Rheume E, Sanchez R, Forest MG, Simard J. Structure-function relationships of 3 β -hydroxysteroid dehydrogenase: contribution made by the molecular genetics of 3 β -hydroxysteroid dehydrogenase deficiency. *Steroids* 1979;62:176–184.
24. Auchus RJ, Lee TC, Miller WL. Cytochrome *b*₅ augments the 17,20-lyase activity of human P450c17 without direct electron transfer. *J Biol Chem* 1998;273:3158–3165.

25. Nakajin S, Hall PF. Microsomal cytochrome P-450 from neonatal pig testis. Purification and properties of A C21 steroid side-chain cleavage system (17 α -hydroxylase-C17,20-lyase). *J Biol Chem* 1981;256:3871–3876.
26. Chung B, Picado-Leonard J, Haniu M, et al. Cytochrome P450c17 (steroid 17 α -hydroxylase/17,20-lyase): cloning of human adrenal and testis cDNAs indicates the same gene is expressed in both tissues. *Proc Natl Acad Sci USA* 1987;84:407–411.
27. Picado-Leonard J, Miller WL. Cloning and sequence of the human gene encoding P450c17 (steroid 17 α -hydroxylase/17,20-lyase): Similarity to the gene for P450c21. *DNA* 1987;6:439–448.
28. Wang M, Roberts DL, Paschke R, Shea TM, Masters BSS, Kim JP. Three-dimensional structure of NADPH-cytochrome P450 reductase: Prototype for FMN- and FAD-containing enzymes. *Proc Natl Acad Sci USA* 1997;94:8411–8416.
29. Zhang L, Rodríguez H, Ohno S, Miller WL. Serine phosphorylation of human P450c17 increases 17,20-lyase activity: Implications for adrenarche and for the polycystic ovary syndrome. *Proc Natl Acad Sci USA* 1995;92:10619–10623.
30. Pandey AV, Miller WL. Regulation of 17,20-lyase activity by cytochrome b₅ and by serine phosphorylation of P450c17. *J Biol Chem* 2005;280:13265–13271.
31. Suzuki T, Sasano H, Tamura M, et al. Temporal and spatial localization of steroidogenic enzymes in premenopausal human ovaries: in situ hybridization and immunohistochemical study. *Mol Cell Endocrinol* 1993;97:135–143.
32. Geller DH, Auchus RJ, Mendonça BB, Miller WL. The genetic and functional basis of isolated 17,20-lyase deficiency. *Nature Genet* 1997;17:201–203.
33. Geller DH, Auchus RJ, Miller WL. P450c17 mutations R347H and R358Q selectively disrupt 17,20-lyase activity by disrupting interactions with P450 oxidoreductase and cytochrome b₅. *Mol Endocrinol* 1999;13:167–175.
34. Pandey AV, Mellon SH, Miller WL. Protein phosphatase 2A and phosphoprotein SET regulate androgen production by P450c17. *J Biol Chem* 2003;278:2837–2844.
35. Miller WL. The molecular basis of adrenarche: A hypothesis. *Acta Paediatr* 1999;88 (Suppl 433):60–66.
36. Flück CE, Tajima T, Pandey AV, et al. Mutant P450 oxidoreductase causes disordered steroidogenesis with and without Antley-Bixler syndrome. *Nat Genet* 2004;36:228–230.
37. Arlt W, Walker EA, Draper N, et al. Congenital adrenal hyperplasia caused by mutant P450 oxidoreductase and human androgen synthesis: analytical study. *Lancet* 2004;363:2128–2135.
38. Adachi M, Tachibana K, Asakura Y, Yamamoto T, Hanaki K, Oka A. Compound heterozygous mutations of cytochrome P450 oxidoreductase gene (POR) in two patients with Antley-Bixler syndrome. *Am J Med Genet* 2004;128A:333–339.
39. Fukami M, Horikawa R, Nagai T, et al. POR (P450 oxidoreductase) mutations and Antley-Bixler syndrome with abnormal genitalia and/or impaired steroidogenesis: molecular and clinical studies in 10 patients. *J Clin Endocrinol Metab* 2005;90:414–426.
40. Huang N, Pandey AV, Agrawal V, et al. Diversity and function of mutations in P450 oxidoreductase in patients with Antley-Bixler syndrome and disordered steroidogenesis. *Am J Hum Genet* 2005;76:729–749.
41. Morel Y, Miller WL. Clinical and molecular genetics of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Adv Hum Genet* 1991;20:1–68.
42. Mellon SH, Miller WL. Extra-adrenal steroid 21-hydroxylation is not mediated by P450c21. *J Clin Invest* 1989;84:1497–1502.
43. White PC, Curnow KM, Pascoe L. Disorders of steroid 11 β -hydroxylase isozymes. *Endocr Rev* 1994;15:421–438.
44. Fardella CE, Miller WL. Molecular biology of mineralocorticoid metabolism. *Annu Rev Nutr* 1996;16:443–470.
45. Luu-The V, Dufort I, Paquet N, Reimnitz G, Labrie F. Structural characterization and expression of the human dehydroepiandrosterone sulfotransferase gene. *DNA Cell Biol* 1995;14:511–518.
46. Miki Y, Nakata T, Suzuki T, et al. Systemic distribution of steroid sulfatase and estrogen sulfotransferase in human adult and fetal tissues. *J Clin Endocrinol Metab* 2002;87:5760–5768.
47. Peltoketo H, Luu-The V, Simard J, Adamski J. 17 β -Hydroxysteroid dehydrogenase (HSD)/17-ketosteroid reductase (KSR) family: nomenclature and main characteristics of the 17 HSD/KSR enzymes. *J Mol Endocrinol* 1999;23:1–11.
48. Mindnich R, Möller G, Adamski J. The role of 17 β -hydroxysteroid dehydrogenases. *Mol Cell Endocrinol* 2004;218:7–20.
49. Agarwal AK, Auchus RJ. Cellular redox state regulates hydroxysteroid dehydrogenase activity and intracellular hormone potency. *Endocrinology* 2005;146:2531–2538.
50. Peltoketo H, Isomaa V, Maenlavsta O, Vihko R. Complete amino acid sequence of human placental 17 β -hydroxysteroid dehydrogenase deduced from cDNA. *FEBS Lett* 1988;239:73–77.
51. Tremblay Y, Ringler GE, Morel Y, et al. Regulation of the gene for estrogenic 17-ketosteroid reductase lying on chromosome 17cen \rightarrow q25. *J Biol Chem* 1989;264:20458–20462.
52. Sawicki MW, Erman M, Puranen T, Vihko P, Ghosh D. Structure of the ternary complex of human 17 β -hydroxysteroid dehydrogenase type I with 3-hydroxyestra-1,3,5,7-tetraen-17-one (equilin) and NADP⁺. *Proc Natl Acad Sci USA* 1999;96:840–845.
53. Wu L, Einstein M, Geissler WM, Chan HK, Elliston KO, Andersson S. Expression cloning and characterization of human 17 beta-hydroxysteroid dehydrogenase type 2, a microsomal enzyme possessing 20 alpha-hydroxysteroid dehydrogenase activity. *J Biol Chem* 1993;268:12964–12969.

54. Lu ML, Huang YW, Lin SX. Purification, reconstitution, and steady-state kinetics of the trans-membrane 17 β -hydroxysteroid dehydrogenase 2. *J Biol Chem* 2002;277:22123–22130.
55. Takeyama J, Sasano H, Suzuki T, Iinuma K, Nagura H, Andersson S. 17 β -Hydroxysteroid dehydrogenase types 1 and 2 in human placenta: An immunohistochemical study with correlation to placental development. *J Clin Endocrinol Metab* 1998;83:3710–3715.
56. Geissler WM, David DL, Wu L, et al. Male pseudohermaphroditism caused by mutations of testicular 17 β -hydroxysteroid dehydrogenase 3. *Nat Genet* 1994;7:34–39.
57. Dufort I, Rheault P, Huang XF, Soucy P, Luu-The V. Characteristics of a highly labile human type 5 17 β -hydroxysteroid dehydrogenase. *Endocrinology* 1999;140:568–574.
58. Mahendroo MS, Russell DW. Male and female isoenzymes of steroid 5 α -reductase. *Rev Reprod* 1999;4:179–183.
59. Simpson ER, Mahendroo MS, Means GD, et al. Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. *Endocr Rev* 1994;15:342–355.
60. Grumbach MM, Auchus RJ. Estrogen: consequences and implications of human mutations in synthesis and action. *J Clin Endocrinol Metab* 1999;84:4677–4694.
61. Miller WL. Steroid hormone biosynthesis and actions in the materno-feto-placental unit. *Clinics Perinatol* 1998;25:799–817.
62. Orentreich N, Brind JL, Rizer RL, Vogelmann JH. Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. *J Clin Endocrinol Metab* 1984;59:551–555.
63. Corbould AM, Judd SJ, Rodgers RJ. Expression of types 1, 2, and 3 17 β -hydroxysteroid dehydrogenase in subcutaneous abdominal and intra-abdominal adipose tissue of women. *J Clin Endocrinol Metab* 1998;83:187–194.
64. Thiboutot D, Bayne E, Thorne J, et al. Immunolocalization of 5 α -reductase isozymes in acne lesions and normal skin. *Arch Dermatol* 2000;136:1125–1129.
65. Voutilainen R, Tapanainen J, Chung BC, Matteson KJ, Miller WL. Hormonal regulation of P450scc (20,22-desmolase) and P450c17 (17 α -hydroxylase/17,20-lyase) in cultured human granulosa cells. *J Clin Endocrinol Metab* 1986;63:202–207.
66. Gougeon A. Regulation of ovarian follicular development in primates: facts and hypotheses. *Endocr Rev* 1996;17:121–155.
67. McNatty KP, Moore LG, Hudson NL, et al. The oocyte and its role in regulating ovulation rate: a new paradigm in reproductive biology. *Reproduction* 2004;128:379–386.
68. Voutilainen R, Miller WL. Coordinate tropic hormone regulation of mRNAs for insulin-like growth factor II and the cholesterol side-chain cleavage enzyme, P450scc, in human steroidogenic tissues. *Proc Natl Acad Sci USA* 1987;84:1590–1594.
69. Dor J, Ben-Shlomo I, Lunenfeld B, et al. Insulin-like growth factor-I (IGF-I) may not be essential for ovarian follicular development: evidence from IGF-I deficiency. *J Clin Endocrinol Metab* 1992;74:539–542.
70. Welt CK, Smith ZA, Pauler DK, Hall JE. Differential regulation of inhibin A and inhibin B by luteinizing hormone, follicle-stimulating hormone, and stage of follicle development. *J Clin Endocrinol Metab* 2001;86:2531–2537.
71. Arlt W, Martens JWM, Song M, Wang JT, Auchus RJ, Miller WL. Molecular evolution of adrenarche: structural and functional analysis of P450c17 from four primate species. *Endocrinology* 2002;143:4665–4672.
72. Suzuki T, Sasano H, Takeyama J, et al. Developmental changes in steroidogenic enzymes in human postnatal adrenal cortex: immunohistochemical studies. *Clin Endocrinol* 2000;53:739–747.
73. Auchus RJ, Geller DH, Lee TC, Miller WL. The regulation of human P450c17 activity: relationship to premature adrenarche, insulin resistance and the polycystic ovary syndrome. *Trends Endocrinol Metab* 1998;9:47–50.
74. Ibañez L, Potau N, Virdis R, et al. Postpubertal outcome in girls diagnosed of premature pubarche during childhood: Increased frequency of functional ovarian hyperandrogenism. *J Clin Endocrinol Metab* 1993;76:1599–1603.
75. Oppenheimer E, Linder B, DiMartino-Nardi J. Decreased insulin sensitivity in prepubertal girls with premature adrenarche and acanthosis nigricans. *J Clin Endocrinol Metab* 1995;80:614–618.
76. Ibañez L, Potau N, Zampolli M, et al. Hyperinsulinemia in post-pubertal girls with a history of premature pubarche and functional ovarian hyperandrogenism. *J Clin Endocrinol Metab* 1996;81:1237–1243.
77. Ehrmann DA, Barnes RB, Rosenfield RL. Polycystic ovary syndrome as a form of functional ovarian hyperandrogenism due to dysregulation of androgen secretion. *Endocr Rev* 1995;16:322–353.
78. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanisms and implications for pathogenesis. *Endocr Rev* 1997;18:774–800.
79. Bollag G, Roth R, Beaudoin J, Mochley-Rosen D, Koshland D Jr. Protein kinase C directly phosphorylates the insulin receptor in vitro and reduces its protein-tyrosine kinase activity. *Proc Natl Acad Sci USA* 1986;83:5822–5824.
80. Stadtmayer L, Rosen OM. Increasing the cAMP content of IM-9 cells alters the phosphorylation state and protein kinase activity of the insulin receptor. *J Biol Chem* 1986;261:3402–3407.
81. Takayama S, White MF, Kahn CR. Phorbol ester-induced serine phosphorylation of the insulin receptor decreases its tyrosine kinase activity. *J Biol Chem* 1988;263:3440–3447.



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