Chapter 6
Are there other hormonal signals regulating testicular functions?
Role of adrenal steroids and estrogens

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Although the testes undeniably play a central role as the major endocrine organs of the male reproductive system, it is increasingly recognized that the adrenal glands and other organs/tissues of the male body contribute steroid products that are transported via the bloodstream. Once they reach the testis, for example, some of these compounds may be involved in either classic or alternate androgenic pathways as intermediates/precursors of sex hormones and other steroids. They can also exert further regulatory influence as end/branch points of synthesis by becoming a "sentinel molecule" in a feedback loop. Do adrenal androgens/androgen precursors or any adrenal steroid products have a relevant physiological function upon their delivery to the testis? The combination of experimental and clinical research is slowly but steadily unraveling these processes.

Adrenal gland and testis: focus on androgens and their steroid metabolites

In mammals, including humans, the testes and the entire male reproductive tract depend primarily on androgens for proper function. The classic and potent androgens - testosterone and its active metabolite DHT - are C19 carbon steroids. Testosterone, the main sex hormone in males, is predominantly produced in the testes and only in much smaller amounts by the adrenal glands. However, unlike other mammalian species, humans and primates are unique in having an adrenal cortex (zona reticularis) that produces and releases large amounts of DHEA (dehydroepiandrosterone) and its sulfated form DHEA-S (in addition to C19 carbon steroid products such as androstenedione and 11OH-androstenediol) into the bloodstream. An overview of the steroidogenesis pathways that take place in the adrenal glands for the production of androgens and other steroid hormones is outlined in Fig. 1. The major intermediates/

specific enzymes and cofactors of each androgenic pathway, as well as active metabolites of testosterone (DHT and estradiol) are also shown.

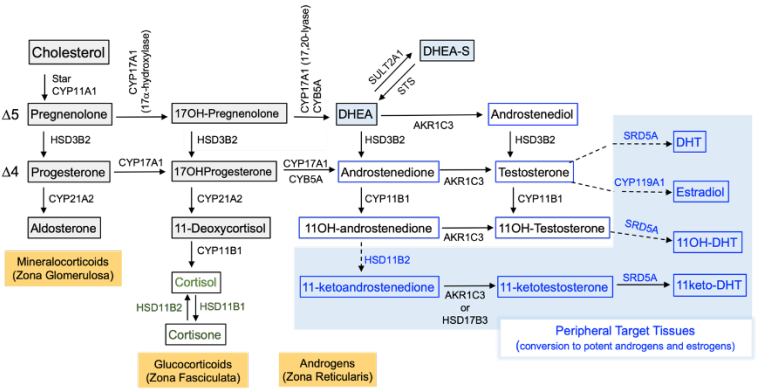

Figure 1. Overview of steroid biosynthetic pathways in human adrenal cortex. Adult cortex produces three distinct classes of steroid hormones: mineralocorticoids (ZG), glucocorticoids (ZF) and androgens (ZR). All cortical steroids are synthesized from cholesterol. The key enzymes involved in cortical steroidogenesis are either cytochrome P450 enzymes (CYPs) or hydroxysteroid dehydro-genases (HSDs). While Star and CYP11A1 are ubiquitously expressed within the adrenal cortex, the zonal segregation of other enzymes accounts for the compartmentalization of the end point steroids produced. The human adrenal ZR promotes efficient DHEA production through the Δ5 pathway; the low expression of HSB3B2 favors biosynthesis toward DHEA. CYP17A1, which competes with HSD3B2 for pregnenolone, catalyzes the conversion of pregnenolone to C19-androgens (i.e., DHEA and androstenediol). CYB5A is an allosteric enhancer of the 17,20-lyase activity of CYP17A. SULT2A1 converts DHEA in DHEA-S. STS is the primary enzyme involved in steroid desulfation. Catalytic activities of the enzymes AKR1C3 and the 11β-hydroxylase CYP11B1 produce 11-oxygenated androgens and their precursors. Conversion of adrenal steroid precursors to more potent androgens or estrogens takes place in target cells in the testes and other tissues (shown in blue background). The interconversion of active glucocorticoids (cortisol) into inactive cortisone is dependent on the tissue/cell

enzyme activity profile of HSD11B2 and HSD11B1, respectively; highlighted in green). Abbreviations: StAR, steroidogenic acute regulatory protein; CYP11A1, cytochrome P450 cholesterol side-chain cleavage; HSD3B2, 3β-hydroxysteroid dehydrogenase, Δ4/Δ5-isomerase type 2; CYP17A1, 17α-hydroxylase/17,20-lyase; CYB5A, cytochrome b5 type A; DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulfate; AKR1C3, 17β-hydroxysteroid dehydrogenase type 5; SULT2A1, steroid/DHEA sulfotransferase type 2A1; CYP11B1, 11β-hydroxylase; T, testosterone; DHT, dihydrotestosterone; 11OH-T, 11β-hydroxytestosterone; 11OH-DHT, 11β-hydroxydihydrotestosterone; SRD5A1 and SRD5A2, 5α-reductase type 1 and type 2; CYP19A1 (cytochrome P450 aromatase); HSD11B2, 11β-hydroxysteroid dehydrogenase type 2; 11OH-androstenedione, 11β-hydroxyandrostenedione; Med, adrenal medulla; STS, steroid sulfatase.

In contrast to testicular androgens, that are produced in Leydig cells under the control of the hypothalamic-pituitary-testicular axis (Chapters 3, 5), adrenocortical-derived androgens are produced in the adrenal cortex (zona reticularis; ZR) under the control of the hypothalamic-pituitary-adrenal axis, involving CRH (corticotropin-releasing hormone) from the hypothalamus and ACTH (adrenocorticotropic hormone) from the pituitary (Fig. 2).

Whereas the enzymatic machinery yields canonical pathways Δ5 (pregnenolone→DHEA→androstenedione or androstenediol), and to a lesser extend Δ4 (pregnenolone→17OH-progesterone→androstenedione) of cholesterol-derived steroids in the testis to produce testosterone (Chapter 5), in the adrenal ZR a different but similar set of enzymes produces abundant amounts of Δ5 DHEA and DHEA-S, followed by androstenedione and 11OH-androstenedione. The limited HSD3B2 expression but increased expression/activity of the 17,21-lyase function of CYP17A1 and of its allosteric regulator (cytochrome b5) favors DHEA and 11OH-androstenedione production over androstenedione/testosterone pathway synthesis in cortical ZR. In turn, high levels of sulfotransferase SULTA1 converts most of the nascent DHEA to DHEA-S for secretion. This sulfated form has a longer half-life in the blood, resulting in higher circulating concentrations compared to the non-sulfated forms. DHEA and DHEA-S not only serve as precursors for other steroids but can also serve as important biomarkers in themselves to help differentiate among different diseases of androgen excess. In lieu of the testicular enzyme

17β-HSD3, the human adrenal ZR employs AKR1C3 for the production of either androstenediol or testosterone. In addition, the ZR also produces 11-oxygenated steroids and their precursors through the catalytic activity of AKR1C3 and other adrenal specific enzyme CYP11B1. Only a small fraction of total circulating testosterone and about half of circulating androstenedione are of adrenal origin.

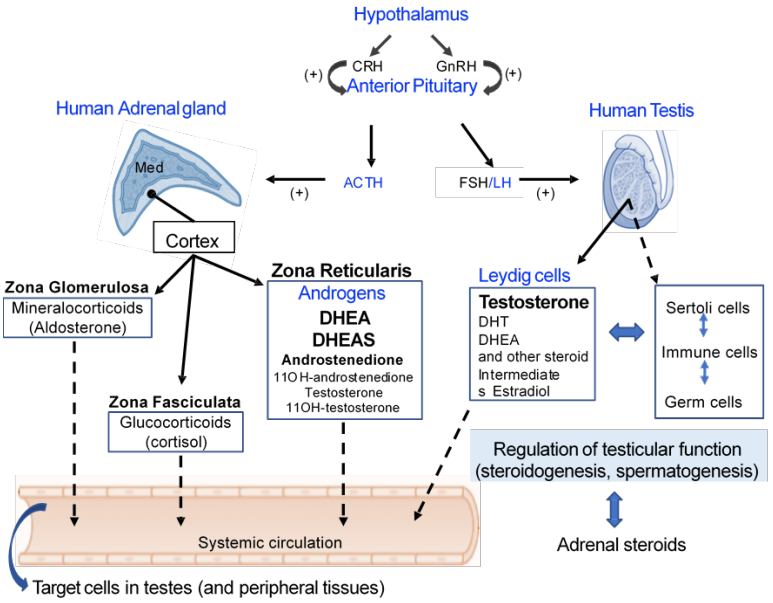

Figure 2. Schematic representation of the HPA axis components controlling steroidogenesis in the human male adult adrenal cortex and testis. Focus is given on the production of androgens and precursors in both endocrine glands. Adrenal and testicular steroidogenesis is under hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonad (HPG) axis modulation, respectively. In adult men, most of the circulating DHEA and almost all DHEA-S is of adrenal cortex origin (ZR); a portion of circulating DHEA/DHEA-S is produced by the testes. Once adrenal steroids reach the systemic circulation, they can act on target cells as they are, or they may be further converted to more potent androgens inside those cells.

Uptake of circulating DHEA-S occurs via membrane transport proteins expressed in testes and other tissues (organic anion transporting polypeptides; OATPs); its sulfate must then be removed

before it can be converted to androstenedione in target tissues. Desulfation is driven by sulfatases that are active in these tissues before further intracrine conversion into more potent androgens (testosterone/DHT) and/or estrogens (e.g., estradiol) which then exert their effect in target tissues via their cognate receptors (AR and ER, respectively). Compared to testosterone, androstenedione and DHEA have relatively little androgenic effect on their own; on the other hand, the adrenal-derived 11-oxygenated androgens, for example, are bioactive androgens, i.e., can induce androgen effects through AR activation.

In retrospect, it is easy to see that the adrenal gland is capable of making products that can be inserted into testicular steroidogenic pathways, because the testis and the adrenal gland have a common embryological origin within the urogenital ridge. They share common genetic and steroidogenic properties that contribute to steroid/androgen production in a developmentally specific manner throughout life. Adrenal androgen secretion is directly dependent on *de novo* steroid production, because there are no stored intermediate reservoirs in the adrenal cortex. The fetal adrenal gland is proportionally larger than the adult tissue, consisting mainly of the fetal zone (FZ), which resembles the ZR of the adult cortex (Fig. 3). Until birth, it secretes large amounts of DHEA and DHEA-S; one critical physiological role, after their intracrine conversion to estrogens, is to support the formation of the fetal-placental unit, thereby maintaining fetal development and pregnancy. After birth, the FZ regresses and DHEA-S synthesis decreases in the first months of life. Production of androgens by the ZR of the adrenal begins at adrenarche (~6-9 years of age in boys), just prior to puberty onset (Chapter 28). This increase in adrenal-derived androgens (primarily DHEA-S) during adrenarche serves as a precursor and possible signal for the synthesis of the more robust androgens generated through target tissue intracrine pathways. From puberty to adulthood, production of androgens by the adrenal glands becomes more closely integrated with testicular androgen steroidogenesis.

Testicular and adrenal testosterone is metabolized to DHT in the testes and other target tissues with cells expressing the enzyme 5α-reductase (SRD5A). Testosterone, but not DHT, is converted into estrogens in cells expressing the enzyme aromatase (CYP19A1). Estrogens are another group of active steroid hormones important for testicular function and male fertility. Steroidogenic precursors are also stored and secreted in smaller amounts by the testes. Once

they reach a cell with the specific enzymatic repertoire, both testicular testosterone and adrenal/testicular androgen precursors can be converted to active androgens and/or estrogens locally in target tissues (intracrine mechanisms).

Estrogens

The next steroids exerting effects in the male reproductive tract in mammals, are estrogens, chiefly estradiol. Under LH stimulus, testosterone and androstenedione are converted to estradiol and estrone, respectively, by CYP19A1 aromatase. In human testes, CYP19A1 is localized in Leydig, Sertoli and germ cells. Throughout the male lifespan, Sertoli cells are considered the earliest site of estrogen production in the testis, switching to Leydig cells during neonatal development, when gonadotropin-regulated aromatase is present. In contrast, Leydig cells are the primary site where testosterone is converted to estradiol in the adult.

In the case of estradiol, the source of estrogen can be both the cell itself, which exerts the estrogenic effect, or the traditional remote endocrine mechanism via the bloodstream. Estrogenic effects, both in reproductive and non-reproductive tissues, are exerted through at least three different estrogen receptors: ESR1 (ERα), ESR2 (ERβ), along with GPER (G protein-coupled estrogen receptor). They are abundantly expressed in testicular cells and throughout the male reproductive tract. Aromatase in human sperm allows androgens to be converted to estrogens as they pass through the reproductive tract, yielding free estrogens in seminal fluid that can act on other male reproductive organs.

A balance of intra-testicular testosterone and estrogen levels is critical for normal testicular function (steroidogenesis and spermatogenesis). In men whose spermatogenesis is impaired by Leydig cell dysfunction, there is usually increased aromatase activity resulting in elevated intra-testicular estrogen levels and a decreased testosterone/estradiol ratio. Increased aromatase activity and estrogen levels in male adipose tissue are associated with obesity and a decrease in fertility. Disturbances in aromatase or estrogen receptor activity have varying effects on fertility, ranging from minimal transitory disturbances in sperm count and function to permanent loss of fertility. In summary, testes are capable of synthesizing and responding to estrogens throughout their development.

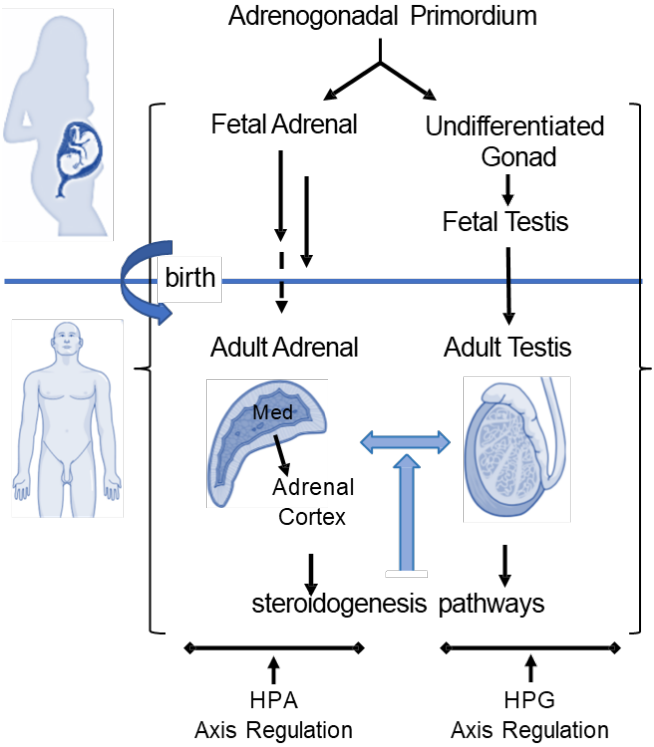

Figure 3. Human male adrenal gland cortex and testis development. The adrenal cortex and testis have a common embryonic precursor in early embryogenesis and subsequently they have common biological processes, such as the ability to produce steroid hormones. Steroid production starts in both tissues during fetal life. In the case of the adrenal gland, initially the fetal tissue consists of a large fetal zone (FZ) that secretes abundant androgen precursors DHEA/DHEA-S. Later on, neural crest-derived chromaffin cells migrate through the fetal cortex to form the future medulla. Next, the newly formed transition zone starts producing cortisol. Shortly before birth, mineralocorticoids are also secreted by the tissue. After birth, the FZ regresses, and the adrenal cortex restarts steroid production/secretion just prior to puberty. In the adult adrenal, the cortex zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR) are responsible for producing mineralocorticoids (aldosterone), glucocorticoids (cortisol) and androgens (mainly DHEA/DHEA- S), respectively.

Glucocorticoids

The adrenal cortex (zona fasciculata, ZF) also produces and secretes glucocorticoids (cortisol in human) which, like estrogens, are important regulatory steroid hormones for the development and maintenance of testicular function, male reproductive health and fertility. In adults, glucocorticoid excess has been demonstrated to adversely affect LH-Leydig cell communication through glucocorticoid receptors (GR) expressed in hypothalamic neurons, pituitary gonadotrophs, as well as in Leydig cells themselves. Taken together, these glucocorticoid-induced effects regulate the concentration and efficiency of the steroidogenic enzyme machinery of the Leydig cells. The access and biological effects of cortisol on target cells in the testis is controlled by local relative expression and activity of the isoenzymes 11β-HSD2 and 11β-HSD1 which catalyze the interconversion of active cortisol to its inactive metabolite cortisone and vice-versa, respectively. GR expression is also present in other types of human testicular cells, including Sertoli cells, immune cells (e.g., macrophages) and possibly in germ cells. There is growing evidence from animal models for the ability of testicular immune cells to synthesize their own glucocorticoids, more specifically in macrophages adjacent to Leydig cells; this testicular-immune cell source of glucocorticoids opens new avenues for analogous processes in humans.

Summary

While the testis exhibits a dominance over the male steroid synthesis pathways, other somatic sites of steroid synthesis (e.g., adrenal cortex) contribute other androgens and non-androgen steroids that modulate the diverse steroid pathways of the testis. The development of more sensitive and precise assays for characterizing adrenal and testicular steroid molecules earlier thought of as only precursors or inactive intermediates, is uncovering larger and more important roles for these compounds. Previously relegated to playing minor parts in androgenic processes, these newly recognized steroid players can now be analyzed to better assess their contributions to the biosynthetic pathways, signaling cascades and regulatory processes relating to male reproduction. These emerging androgenic participants from both within and without the male reproductive tract now offer growing opportunities for examining the molecular crosstalk in this burgeoning network of testicular processes. As we move forward, it is the increased sensitivity and

precision of the technologies used to elucidate the recently demonstrated roles of these androlological molecules that can now be harnessed to develop diagnostics to aid in the differentiation of pathologies resulting from disruptions among these steroid players and their associated pathways.

Suggested reading

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