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UPDATE ON ADRENAL STEROID HORMONE BIOSYNTHESIS AND CLINICAL IMPLICATIONS

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Abstract

Steroid biosynthesis is a complex process in which cholesterol is converted to steroid hormones with the involvement of multiple enzymes and cofactors. Inborn conditions affecting adrenal steroidogenesis are relatively common in paediatric practice and have serious implications on patient mortality and morbidity. This paper provides an overview of novel insights into human adrenal steroid biosynthesis. Inborn errors of steroidogenesis associated with congenital adrenal hyperplasia are discussed, with a particular focus on the pathophysiology and clinical features of 21-hydroxylase deficiency. The final section of the review presents more recent findings and clinical implications of adrenal-specific androgen biosynthesis.

Steroid hormone biosynthesis represents the multi-step enzymatic conversion of cholesterol via intermediate steroid precursors into biologically active steroid hormones. This biochemical process is regulated by a multitude of enzymes and co-factors. Due to their common origin from cholesterol, steroid hormones all share the same 'tetracyclic skeleton', cyclopentanoperhydrophenanthrene [1]. Despite their structural similarities, from a functional perspective, these hormones constitute a heterogeneous group contributing to a wide range of essential physiological processes.

The adrenal glands and the gonads are the principal organs involved in steroidogenesis. Other tissues with steroidogenic capacity include the placenta and the brain, which are well known sites of *de novo* steroid synthesis [1, 2]. Furthermore, the adipose tissue and the liver express a significant number of steroid-converting enzymes and therefore play important roles in the activation, metabolism and inactivation of steroid hormones [3]. The organs and tissues responsible for producing steroids express steroidogenic enzymes and co-factors differently, leading to site-specific variations and consequently pathways of steroid hormone synthesis [1]. Thus, the adrenal glands are primarily responsible for the synthesis of glucocorticoids and mineralocorticoids, as well as adrenal androgens, while the gonads represent the main site for the production of sex steroids, namely androgens, oestrogens and progesterone.

Steroidogenic cells are defined by their capacity to produce steroids *de novo* using cholesterol as substrate, a process that requires the expression of the P450 side-chain cleavage enzyme (CYP11A1, P450scc). Most of the cholesterol used for steroid hormone biosynthesis originates from the uptake of plasma low density lipoproteins through endocytosis. However, many organs, including the adrenals, are capable of producing cholesterol *de novo* from acetate [1]. Within the adrenal cortex, the steroidogenic acute regulatory protein (StAR) plays an essential part by increasing the flow of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane, where CYP11A1 initiates the first step of steroidogenesis. In contrast to polypeptide hormones, only minimal amounts of steroid hormones are stored in the steroidogenic glands and as such their release relies on rapid synthesis. This aspect has particular importance in the adrenal stress response, where steroidogenesis is a time-critical process. Steroid hormones are produced at a much slower rate in tissues that do not express StAR

(placenta and brain) through a process that is thought to involve proteins with StAR-like activity [1, 4].

Steroidogenic enzymes can be divided in two broad groups: cytochrome P450 (CYP) and hydroxysteroid dehydrogenases/ketosteroid reductase (HSD/KSR) [1]. Both play a major role in the biosynthesis of steroid hormones and a good knowledge of their structure and physiology is fundamental to understanding impaired steroidogenesis [1].

CYP enzymes contain a haeme prosthetic group that gives them the ability to activate molecular oxygen in order to catalyse oxidative reactions. They are functionally unidirectional, catalysing irreversibly the reactions they facilitate, with no feedback from downstream precursors [1]. CYP enzymes are subdivided in two types: type 1 enzymes being localised in the mitochondria and type 2 in the endoplasmic reticulum. Depending on their type CYP enzymes rely on different co-factors: ferredoxin (FDX1) or adrenodoxin and ferredoxin reductase (FDXR) for CYP type 1 and P450 oxidoreductase (POR) for CYP type 2 enzymes. Six CYP enzymes are involved in steroidogenesis of which cholesterol side-chain cleavage enzyme (CYP11A1, P450scc), 11-hydroxylase (CYP11B1, P450c11) and aldosterone synthase (CYP11B2, P450c11AS) belong to CYP type 1 enzymes, while 17-hydroxylase (CYP17A1, P450c17), 21-hydroxylase (CYP21A2, P450c21) and P450 aromatase (CYP19A1, P450aro) are CYP type 2 enzymes. Each CYP enzyme can metabolise more than one substrate and is thereby involved in multiple steps of steroidosynthesis. This impacts on the pathophysiology of inborn errors of steroidogenesis, such as 21-hydroxylase deficiency.

HSD/KSR enzymes catalyse reversible reactions. However, while *in vitro* they can drive the reactions in both directions, *in vivo* they drive the steroid flux preferentially in the oxidative or reductive mode [1]. They require NADH/NAD^+ or NADPH/$NADP^+$ as co-factors for steroid reductions or oxidations. Based on their activity, HDS enzymes are classified into dehydrogenases, which use NAD^+ to oxidate hydroxysteroids to ketosteroids, and reductases which use NADPH to reduce ketosteroids to hydroxysteroids. The HSDs involved in steroid synthesis and relevant to human pathology include the 3-hydroxysteroid dehydrogenase type 2 (HSD3B2), the 11-hydroxysteroid dehydrogenase type 1 and type 2 (HSD11B1 and HSD11B2), and a series of 17-hydroxysteroid dehydrogenases (17-HSD) [1].

Pathways of adrenal steroidogenesis

The conversion of cholesterol to pregnenolone in the mitochondria represents the first step of steroidogenesis. This enzymatic process involves three chemical reactions and is catalysed by CYP11A1 (**Figure 1**).

Mineralocorticoid synthesis

In the adrenal *zona glomerulosa,* pregnenolone is irreversibly converted into progesterone by HSD3B2. Subsequently, CYP21A2 converts progesterone to 11-deoxycorticosterone (DOC). DOC serves as substrate for CYP11B2, which has 11-hydroxylase, 18-hydroxylase and 18-oxidase activities, thus catalysing the final three reactions leading to the production of aldosterone [5]. The adrenal *zona glomerulosa* has an enzymatic profile designed for mineralocorticoid synthesis and is the only zone to express CYP11B2. By contrast CYP17A1, the enzyme directing pregnenolone towards glucocorticoid and androgen synthesis, is present in very small amounts in *zona glomerulosa* [5].

Glucocorticoid synthesis

Glucocorticoid synthesis occurs in the adrenal *zona fasciculata*, beginning with the 17-hydroxylation of pregnenolone to 17-hydroxypregnenolone, catalysed by CYP17A1, which is also responsible for converting progesterone to 17-hydroxyprogesterone (17OHP). HSD3B2 converts 17-hydroxypregnenolone to 17OHP, which is changed to 11-deoxycortisol by CYP21A2. Finally, CYP11B1 completes the process, converting 11-deoxycortisol to cortisol.

Androgen synthesis

Androgen synthesis takes place primarily in the adrenal *zona reticularis* and the gonads. Pregnenolone is converted to 17-hydroxypregnenolone, which in turn is converted to dehydroepiandrosterone (DHEA), reactions that are catalysed by CYP17A1, capable of both 17-hydroxylase and 17,20-lyase activities [1]. The 17,20-lyase activity of CYP17A1 crucially relies on the co-factor cytochrome b5, which is only expressed in the *zona reticularis.* The main role of DHEA is as a precursor for active androgens [3]. It is converted to androstenedione by HSD3B2. However, a proportion of the DHEA synthesised is converted to DHEA sulphate (DHEAS), a reaction catalysed by sulphotransferase (SULT2A), which requires the presence of 3-

phosphoadenosine-5-phosphosulfate synthase type 2 (PAPSS2) as a co-factor [1]. The sulphation of DHEA to DHEAS regulates the amount of DHEA available for androgen synthesis [6].

Two enzymes of the 17-hydroxysteroid dehydrogenase family are involved in androgen synthesis. Conversion of androstenedione to testosterone in the testes is catalysed by 17-hydroxysteroid dehydrogenase type 3 (HSD17B3), while 17-hydroxysteroid dehydrogenase type 5 or aldo-keto-reductase C3 (17HSD5 or AKR1C3) is responsible for testosterone synthesis in the adrenals [7]. Testosterone is released into the circulation and activated in peripheral tissues to dihydrotestosterone (DHT) by 5α-reductase [3].

Adrenarche represents the onset of adrenal androgen synthesis following development of the *zona reticularis*, a gradual process that begins in early childhood from three to four years of age [8], but only becomes apparent from the age of six to eight years onwards [9]. The *zona reticularis* is characterised by an increased expression of the co-factor cytochrome b5 and of SULT2A, as well as decreased expression of HSD3B2, a combination favouring the synthesis of DHEA and DHEAS [3, 9]. The widely used term of "premature adrenarche" before the age of 8 years is usually based on clinical symptoms (pubarche, acne, apocrine body odor) and lacks biochemical confirmation due to non-sensitive assays that do not detect low concentrations of adrenal androgens in earlier life.

Inborn errors of steroidogenesis

Deficiencies in the main pathways of steroid biosynthesis have now been described for all major factors and steroidogenic enzymes and the inborn errors of steroidogenesis are well-defined conditions (**Table 1**). Steroid profiling using novel analytical strategies has made clinical diagnosis more straightforward [10-13].

Enzyme/co-factor	DSD	Affected organ	Deficiency	Excess
CYP21A2	**46,XX**	**Adrenal**	**MC, GC**	**SexH**
CYP11B1	**46,XX**	**Adrenal**	**GC**	**MC, SexH**
CYP17A1	**46,XY**	**Adrenal, gonad**	**GC, SexH**	**MC**
HSD3B2	**46,XY (46,XX)**	**Adrenal, gonad**	**MC, GC, SexH**	
POR	**46,XY + 46,XX**	**Adrenal, gonad, liver**	**GC, SexH**	**(MC)**
StAR	**46,XY**	**Adrenal, gonad**	**MC, GC, SexH**	
CYP11A1	**46,XY**	**Adrenal, gonad**	**MC, GC, SexH**	
CYP11B2	**_**	**Adrenal**	**MC**	

Table 1. Clinical characteristics of inborn errors of steroidogenesis associated with congenital adrenal hyperplasia. CYP21A2: 21-hydroxylase, CYP11B1: 11-hydroxylase, CYP17A1: 17-hydroxylase, HSD3B2: 3-hydroxysteroid dehydrogenase type 2, POR: P450 oxidoreductase, StAR: steroidogenic acute regulatory protein, CYP11A1: P450 side-chain cleavage enzyme, CYP11B2: aldosterone synthase, DSD: disorder of sex development, MC: mineralocorticoid, GC: glucocorticoid, SexH: sex steroid hormone, (MC): variable MC excess.

Congenital adrenal hyperplasia (CAH) represents a group of inherited autosomal recessive diseases characterised by impaired adrenal steroid synthesis and in some cases impaired gonadal steroid production with associated genital ambiguity (**Table 1**). Seven inborn errors of steroidogenesis are commonly classified as CAH with 21-hydroxylase being the by far most common condition. The disorders included in the CAH group are defined by deficiencies of the following enzymes and co-factors: 21-hydroxylase (CYP21A2), 17-hydroxylase (CYP17A1), 11-hydroxylase (CYP11B1), 3-hydroxysteroid dehydrogenase type 2 (HSD3B2), P450 oxidoreductase (POR), StAR (lipoid CAH) and P450 side-chain cleavage enzyme (CYP11A1) [14]. The absence of electron providing co-factor P450 oxidoreductase manifests with combined CYP17A1 and CYP21A2 deficiencies, as well as variable degrees of Antley-Bixler-like bone malformations [1, 14]. Although CYP11B2 is commonly classified as a form of CAH, it does not truly belong to this group of conditions, as it only causes isolated aldosterone deficiency. This leads to salt-wasting crisis in infancy, however, patients who survive this stage often improve spontaneously and do not necessarily require replacement therapy in adulthood.

21-hydroxylase deficiency

Steroid 21-hydroxylase deficiency (21OHD) accounts for more than 95% of CAH [14, 15]. It causes impaired synthesis of glucocorticoids and mineralocorticoids, with build-up of precursors, in particular 17OHP, that is the key precursor causing androgen excess. This process is amplified by the absence of the negative feedback of cortisol on ACTH secretion under physiological conditions. The severity of disease is dictated by the residual CYP21A2 activity. The clinical presentation is commonly divided into classic and non-classic 21OHD. Classic 21OHD is often subdivided in salt wasting (SW) and simple virilising (SV) forms, with the latter characterised by an *in vitro* 21-hydroxylase activity of 1-2% that prevents adrenal crisis in neonates [14]; whereas in non-classic 21OHD up to 50% of *in vitro* enzyme activity is preserved, leading to milder androgen excess which is clinically discrete or asymptomatic [14]. The utility of this classification has been questioned as all patients with 21OHD lose salt to some degree and there appears to be a continuum rather than binary 21OHD categories [16]. Impaired cortisol production causes androgen excess leading to antenatal virilisation of genetic females, precocious puberty in boys and rapid skeletal growth in both sexes, associated with early closure of growth plates and reduced final height. Patients with classic 21OHD require life-long treatment with glucocorticoids and mineralocorticoids. Unlike other forms of adrenal insufficiency, in 21OHD glucocorticoid treatment is aimed not only at replacing deficient cortisol, but also at suppressing excessive ACTH and androgen synthesis. These therapeutic goals remain challenging in clinical practice due to the circadian pattern of ACTH secretion and supraphysiological doses of glucocorticoids are often required. Consequently, many patients with 21OHD develop comorbidities associated with poor disease control and side effects of glucocorticoids, including impaired bone health, infertility, cardiovascular and metabolic disease [17, 18].

Alternative pathways of adrenal androgen synthesis

Until recently, the adrenal contribution to circulating androgen hormones was mainly focused on DHEA and androstenedione and the potential of the adrenal cortex to produce testosterone. Both DHEA and androstenedione are weak androgens, serving as substrate for active androgen synthesis through peripheral conversion to testosterone and subsequently dihydrotestosterone (DHT) [19]. The evidence that has emerged over the last fifteen years regarding alternative

pathways to active androgens is now changing our understanding of adrenal pathology and will most likely change clinical management in the future.

The alternative pathway to dihydrotestosterone

An alternative pathway to androgens, frequently described as "the backdoor pathway", is represented by a chain of enzymatic reactions through which 17OHP is converted to DHT without testosterone as an intermediate [20]. 17OHP undergoes 5- then 3-reduction followed by 17,20-lyase cleavage, resulting in androsterone. Androsterone is converted to DHT through 17-reduction and 3-oxidation (**Figure 2**). It has been shown that this alternative pathway to androgens is active antenatally in healthy individuals and that it may play a role in male sex development during fetal life [20]. More recent evidence demonstrated its involvement in patients with 21OHD as a consequence of 17OHP excess [21]. Thus, it is believed that this alternative pathway contributes to androgen excess leading to the virilisation of the external genitalia of females with 21OHD. However, this alternative pathway of androgen synthesis was found to diminish after the first year of life in patients with 21OHD [21]. It may be that this is an explanation for the normal growth observed in patients during the first year of life [22], suggesting that a smaller dose of GC may be sufficient in young children with 21OHD [23]. The contribution of this alternative pathway to androgen excess has also been demonstrated in POR deficiency. This pathway partially explains virilisation of mothers during pregnancy in the presence of placental aromatase and the virilisation of female fetuses despite postnatal sex steroid deficiency [20].

Adrenal specific 11-oxygenated C19 steroids

Another class of active adrenal androgens extensively studied in recent years are the 11-oxygenated C19 steroids. Both androstenedione and testosterone undergo 11-hydroxylation, catalysed by CYP11B1, resulting in 11-hydroxyandrostenedione (11-OHA4) and 11-hydroxytestosterone (11-OHT) respectively, which are then reduced by HSD11B2 to 11-ketoandrostenedione (11-KA4) and 11-ketotestosterone (11-KT) respectively. 11-KT can be 5-reduced to 11-ketodihydrotestosterone (11-KDHT) (**Figure 1**).

Although 11-OHA4 was discovered in the 1950's, it received little attention as it was assumed to be part of an inactivating pathway for adrenal androgens [24]. However, more recent evidence

identified the 11-oxygentated C19 steroids as a distinct group of adrenal-derived androgens. While 11-OHA4 is a weak steroid that acts peripherally as a precursor for more active androgens [25], both 11-KT and 11-KDHT are very strong androgens transactivating the androgen receptor (AR) to a similar degree as DHT [26, 27]. Moreover, 11-KT appears to have an important physiological role during adrenarche, being the major circulating androgen in girls and women, followed by testosterone and 11-OHT [27]. There is also increasing evidence regarding the role of 11-oxygenated C19 androgens in adrenal androgen excess. High concentrations of 11-OHT, 11-KT, 11-OHA4, 11-KA4 were found in patients with 21OHD compared to healthy controls [28] and it was suggested that these 11-oxygenated C19 steroids are major contributors to clinical androgen excess in children with CAH [29]. Importantly, in adults with 21OHD 11-oxygenated C19 androgens were found to correlate with adrenal volume, an indicator of disease control [30]. Furthermore, it has been proposed that 11-oxygenated C19 steroids might serve as superior markers of adrenal androgen excess in CAH, compared to DHEA, androstenedione and testosterone, which are the commonly employed biomarkers for monitoring therapy control [28, 31]. The clinical significance of 11-oxygenated C19 steroids has also been explored in polycystic ovary syndrome (PCOS), where 11-oxygenated C19 steroids are increased and correlate with markers of metabolic risk such as the body mass index (BMI) and the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) [32]. These findings indicate the potential clinical usefulness of this group of hormones and it can be anticipated that they will be introduced as biomarkers for monitoring of androgen excess in CAH in the future.

Summary

The diagnosis for most conditions affecting adrenal steroidogenesis is straightforward and deficiencies of all adrenal enzymes and almost all co-factors have been described in humans with distinct clinical phenotypes. However, recent advances have added multiple layers of complexity by defining additional pathways to active androgens to our understanding of steroidogenesis. Clinically the management of androgen excess remains a significant challenge. Greater insights into the role of alternative pathways of androgen synthesis have led to a better understanding of adrenal pathology and novel tests may be introduced into clinical practice in the future. In particular, the “rediscovery” of 11-oxygenated C19 steroids as potential markers of adrenal androgen excess carries the prospect of developing improved biomarkers of disease control for CAH patients with 21-hydroxylase deficiency.

List of abbreviations

11-KA4: 11-ketoandrostenedione; 11-KDHT: 11-ketodihydrotestosterone; 11-KT: 11-ketotestosterone; 11-OHA4: 11-hydroxyandrostenedione; 11-OHT: 11-hydroxytestosterone; 17OHP: 17-hydroxyprogesterone; 17-HSD: 17-hydroxysteroid dehydrogenases; 21-S: 21-deoxycortisol; 21OHD: 21-hydroxylase deficiency; 3HSD: 3-hydroxysteroid dehydrogenase; ADX/Adr: adrenodoxin/adrenodoxin reductase; AKR1C3 or 17HSD5: aldo-keto-reductase C3 or 17-hydroxysteroid dehydrogenase type 5; AR: androgen receptor; CAH: congenital adrenal hyperplasia; CYP: cytochrome P450; CYP11A1 or P450scc: cholesterol side-chain cleavage enzyme; CYP11B1 or P450c11: 11-hydroxylase; CYP11B2 or P450c11AS: aldosterone synthase; CYP17A1 or P450c17: 17-hydroxylase; CYP19A1 or P450aro: P450 aromatase; CYP21A2 or P450c21: 21-hydroxylase; DHEA: dehydroepiandrosterone; DHEAS: dehydroepiandrosterone sulphate; DHT: dihydrotestosterone; DOC: 11-deoxycorticosterone; H6PDH: hexose-6-phosphate dehydrogenase; HSD/KSR: hydroxysteroid dehydrogenases/ketosteroid reductase; HSD11B1: 11-hydroxysteroid dehydrogenase type 1; HSD11B2: 11-hydroxysteroid dehydrogenase type 2; HSD17B3: 17-hydroxysteroid dehydrogenase type 3; HSD3B2: 3-hydroxysteroid dehydrogenase type 2; NAD: nicotinamide adenine dinucleotide; NADPH: nicotinamide adenine dinucleotide phosphate (reduced form); PAPSS2: 3-phosphoadenosine-5-phosphosulfate synthase type 2; PCOS: polycystic ovary syndrome; POR: P450 oxidoreductase; StAR: steroidogenic acute regulatory protein; SULT2A: sulphotransferase.

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Figure legends

Figure 1. Diagram of adrenal steroid biosynthesis.

The first and second columns represent mineralocorticoid and glucocorticoid synthesis. The third and fourth columns correspond to androgen synthesis, including synthesis of 11-oxygenated C19 steroids. The large arrows indicate chemical reactions. The small arrows indicate the metabolites derived from the steroid hormones. Adjacent to each large arrow are the enzymes and co-factors involved in catalysing the respective reaction. Grey boxes are used to indicate steroidogenic enzymes, green boxes correspond to adrenodoxin/adrenodoxin reductase, orange box to 3-phosphoadenosine-5-phosphosulfate synthase type 2, yellow ovals to P450 oxidoreductase, orange balls to cytochrome b5 and blue ovals to the coenzyme hexose-6-phosphate dehydrogenase. (abbreviations: THDOC: tetrahydro-11-deoxycorticosterone; THA: tetrahydro-11-dehydrocorticosterone; THB: tetrahydrocorticosterone; THALDO: tetrahydroaldosterone; THS: tetrahydrodeoxycortisol; THF: tetrahydrocortisol).

Figure 2. Diagram of the alternative pathway to dihydrotestosterone.

The “alternative” pathway is indicated by the pale-blue background while the “classic” pathway is marked by the bright-blue background, both leading to dihydrotestosterone (grey-blue background). The pink background marks the oestrogens pathway. The large arrows indicate chemical reactions. Adjacent to each arrow are the enzymes and co-factors involved in catalysing the respective reaction. Grey boxes are used to indicate steroidogenic enzymes and yellow ovals correspond to P450 oxidoreductase.