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A guide to understanding the steroid pathway: New insights and diagnostic implications

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ABSTRACT

Steroid analysis has always been complicated requiring a clear understanding of both the clinical and analytical aspects in order to accurately interpret results. The literature relating to this specialised area spans many decades and the intricacies of the steroid pathway have evolved with time. A number of key changes, including discovery of the alternative androgen pathway, have occurred in the last decade, potentially changing our understanding and approach to investigating disorders of sexual development. Such investigation usually occurs in specialised paediatric centres and although preterm infants represent only a small percentage of the patient population, consideration of the persistence of the foetal adrenal zone is an additional important consideration when undertaking steroid hormone investigations. The recent expanded role of mass spectrometry and molecular diagnostic methods provides significant improvements for accurate steroid quantification and identification of enzyme deficiencies. However analysis of steroids and interpretation of results remain complicated. This review aims to provide an insight into the complexities of steroid measurement in children and offers an updated guide to interpretation, of serum and urine steroids through the presentation of a refined steroid pathway.

Introduction

Since the development of the early radioimmunoassays (RIA) for serum steroids [1] and gas chromatography (GC) metabolomic methods for urine steroids [2–5] in the 1960's, there have been significant advances in our understanding and quantification of these remarkable hormones. Recent refinements in mass spectrometry and molecular techniques have resulted in a paradigm shift in steroid analysis. Appreciation of the advantages and limitations of the analytical methods aids clinical interpretation. Even so, due to the number and similarity of many steroids the interpretation of results remains complicated; particularly in neonates. A clear understanding of steroid structure, the genetic basis of steroid biosynthesis and the relationship between steroids in the pathway is essential for accurate interpretation. With the recognition of the newly discovered alternative androgen pathway and the advent of mass spectrometry in the clinical diagnostic laboratory, it is timely to examine the current advances in steroid biosynthesis and analysis.

This review aims to provide an insight into the complexities of steroid measurement in children and offers an updated guide to the interpretation of serum and urine steroids through the presentation of a refined steroid pathway.

Structure and naming of steroids

Historically many of the current trivial steroid hormone names owe their origin to scientific endeavours of the early 20th century[6,7]. The isolation and naming of the first steroids related to their broad function, such as the names coined for “estrone” (estr(us)=fertile female; and one = ketone), “androsterone” (andro = male; ster = sterol; and one = ketone) and “testosterone” (testo = testes; ster = sterol; and one = ketone)[8]. As the repertoire of steroids of clinical interest expanded individual research groups began to name steroids either alphabetically or numerically in order of isolation[9–12]. The names assigned inevitably varied between these groups[13]. Some examples which still persist colloquially today include Kendall's compound “A” (11 dehydrocortisone), “B” (corticosterone), “E” (cortisone), and “F” (cortisol) and Reichtein's compound “S” (11 deoxy cortisol). Even when groups took steps to harmonise the names[13], the approach proved inadequate as new steroids were purified. In addition, these names did not recognise the steroids' important structural features. Hence it became evident that the alphabetical naming system was clumsy and a solution was sought.

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The 1989 recommendations by the Joint Commission on Biochemical Nomenclature (JCBN) for The Nomenclature of Steroids serve as the foundation for our current formal naming rules[14–16]. Understanding the basis for names (trivial and expanded chemical names) of individual steroids provides insight into the steroid pathway; and avoids confusion with similar sounding names. However, with continued routine use of trivial names some confusion persists.

A common example where mistakes arise, due to similar spelling, is with the three androgens androstenediol, androstanediol and androstenedione. In this example each compound is a 19 carbon (C19) steroid, but each differs in double bonds, hydroxyl and/or keto groups. Androstenediol (C19 with a double bond and two hydroxyl groups) is the substrate of 3β-hydroxy steroid dehydrogenase (HSD) enzyme whilst androstenedione (C19 with a double bond and two keto groups) is the product of this enzyme activity from a slightly different but distinct section of the steroid pathway. Moreover, androstanediol (C19 with no double bonds and two hydroxyl groups) is not directly related to activity of the 3β-HSD enzyme and is a metabolite of the "alternative" steroid pathway. Whilst the trivial names are routinely used, this example highlights the benefits of appreciating the basis of the steroid structure (Fig. 1), understanding the steroid naming rules (Table 1), and the steroid pathway (Fig. 2), along with the action and location of the enzymes (Table 2). Together these provide clarity to aid interpretation of complex patient results.

Steroid synthesis

All steroids are synthesised from cholesterol. Tissues that can convert cholesterol with the cytochrome P450 side chain cleavage (P450scc) enzyme have the ability to produce steroids. In humans several organs are capable of steroidogenesis: adrenal cortex (zona glomerulosa, zona fasciculata and zona reticularis); Leydig cells of the testes; granulosa and theca cells of the ovary; and syncytiotrophoblasts of the placenta. It is also possible that some steroidogenesis takes place in the human brain[17]. Hence, steroid expression can be ubiquitous or tissue specific.

The local production of steroids is governed by the expression of enzymes and cofactors. For example the absence of 17α-hydroxylase activity diverts pregnenolone to aldosterone in the zona glomerulosa. Similarly, the presence of cytochrome b5 is required for 17,20-lyase activity in the zona reticularis, leading to production of dehydroepiandrosterone (DHEA). Many tissues, such as fat and skin, also have enzymes that convert one steroid to another but this is typically not classified as steroidogenesis as they lack the P450scc enzyme. Instead these intracrine tissues utilise steroid products such as DHEA which are present in the circulation[18]. Tissue specific locations of steroid enzymes are detailed in Table 2.

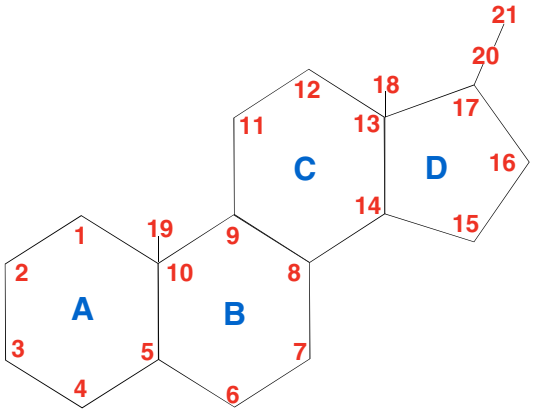

Fig. 1. Basic steroid structure showing a fully saturated 21 carbon steroid with the alphabetical naming of the individual rings and the numbering sequence of the carbon atoms. All steroids share the same basic 17 carbon structure with the presence of four linked rings (three six sided and one five sided) known as the cyclopentanophenanthrene (or cyclopentanoperhydrophenanthrene) ring. The rings are alphabetically labelled with the carbon atoms are numbered sequentially. Cholesterol is recognised as the parent steroid and contains 27 carbon atoms, whereas the three main groups of steroids of interest in clinical endocrinology consist of 18, 19 or 21 carbon atoms, representing the estrane, androstane and pregnane skeleton.

Table 1
Steroid naming rules, illustrating the steps to naming of the common steroids measured for the investigation of DSD in childhood.

Basic steroid naming rules
1. Choose a core name that most closely matches the steroid molecule. This will be either "andro" for carbon 19 steroids, "estra" for carbon 18 steroids and "preg" for carbon 21 steroids.
2. Add a suffix to this name to the number of unsaturated double bonds and specify the location of the unsaturated double bonds as a prefix number. The addition of an "an" or "ane" to the name indicates no double bonds whilst the addition of an "ene", "diene" or "triene" indicates one, two or three double bonds respectively. Note that these additions are commonly written both with and without the "e" at the end.
3. Indicate the number of hydroxyl groups prefixing with the position number of each group and also the orientation of the group to the plain of paper i.e. alpha or beta. The addition of "ol", "diol", "triol" or "tetrol" indicates one, two, three or four hydroxyl groups respectively. The relative location is given immediately before the naming addition.
4. Indicate the number (if any) of keto groups, prefixing with the position of each group. No addition to the name is given if there are no keto groups. The addition of "one", "dione", "trione" or "tetrone" indicates one, two, three or four keto groups respectively. The relative location is given immediately before the naming addition.
5. Prefixing important section of the steroid to highlight its relevance. Steroids may be prefixed with compounds for any structurally important component elements e.g. delta 4 androstenedione or 17 β oestradiol.
6. Suffixing standard derivatives i.e. steroids coupled to another compound. In general these standard derivatives are suffixed to the steroid name with the best known example being dehydro-epiandrosterone sulphate (DHEAS).

Note A — Position of hydrogen bonds: When the hydrogen bonds are designate as alpha (α) the orientation is below the plane of the paper, whilst the beta (β) orientation is above the plane of the paper. The configuration of hydrogen at position 5 (i.e. the ring-junction between A and B) should always be designated as either alpha or beta by placing this numeral and letter being placed immediately before the stem name. The trivial steroid name often includes "allo" for steroids with the alpha configuration at position 5 e.g. allo-tetrahydro-cortisol.
Note B — Position of double bonds: To specify where the double bond is always use the lower number of the ring structure. If double bond along the ring junction e.g. between positions 5 and 6, label as 5. If it is between positions 5 and 10, label as 5 (10).
Note C — Isomerisation. An isomer is a chemical with the same molecular formula but with a different structure and an epimer is also an isomer but with only one change to the structure e.g. epi-testosterone which is the result of a change in configuration of the OH group at position 17 of the steroid molecule. Whereas the terms cis (on the same side) and trans (on the opposite side) relate to a change in the position of a functional group.
Note D — Use of the term delta (Δ). Whilst many trivial names for steroids now relate to the steroid structure, the full chemical name or at least additional information about an important structural characteristic/component is often used for identification and analysis. As such with the example of androstenedione, it is often referred to as delta 4 androstenedione to recognise the important change in the position of the double bond from position five with the conversion from DHEA which has the double bond at position four.
Note E — Highlighting specific chemical components. Frequently specific sections of the steroid compound are named to highlight a function property. Examples include 17β for estradiol, 17-keto for a number of androgens and 16-hydroxy for steroids of the foetal adrenal pathway are often added as descriptors of the important functional components.

Steroidogenic enzymes groups

The various steps of steroidogenesis are catalysed by several enzymes. These enzymes are essentially classified into two groups, the cytochrome P450 (CYP) enzymes and the hydroxysteroid dehydrogenase (HSD) enzymes.

The cytochrome P450 enzymes are heme containing oxidative enzymes. Their name is derived from their spectrophotometric characteristic of strong absorbance at 450 nm. Based on their intracellular location, CYPs are further classified into type 1 (mitochondrial) and type 2 (endoplasmic reticulum). Type 1 CYPs receive electrons from