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Review

Gas chromatography/mass spectrometry (GC/MS) remains a pre-eminent discovery tool in clinical steroid investigations even in the era of fast liquid chromatography tandem mass spectrometry (LC/MS/MS)☆

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ABSTRACT

Liquid chromatography tandem mass spectrometry (LC/MS/MS) is replacing classical methods for steroid hormone analysis. It requires small sample volumes and has given rise to improved specificity and short analysis times. Its growth has been fueled by criticism of the validity of steroid analysis by older techniques, testosterone measurements being a prime example. While this approach is the gold-standard for measurement of individual steroids, and panels of such compounds, LC/MS/MS is of limited use in defining novel metabolomes. GC/MS, in contrast, is unsuited to rapid high-sensitivity analysis of specific compounds, but remains the most powerful discovery tool for defining steroid disorder metabolomes. Since the 1930s almost all inborn errors in steroidogenesis have been first defined through their urinary steroid excretion. In the last 30 years, this has been exclusively carried out by GC/MS and has defined conditions such as AME syndrome, glucocorticoid remediable aldosteronism (GRA) and Smith–Lemli–Opitz syndrome. Our recent foci have been on P450 oxidoreductase deficiency (ORD) and apparent cortisone reductase deficiency (ACRD).

In contrast to LC/MS/MS methodology, a particular benefit of GC/MS is its non-selective nature; a scanned run will contain every steroid excreted, providing an integrated picture of an individual's metabolome. The "Achilles heel" of clinical GC/MS profiling may be data presentation. There is lack of familiarity with the multiple hormone metabolites excreted and diagnostic data are difficult for endocrinologists to comprehend. While several conditions are defined by the absolute concentration of steroid metabolites, many are readily diagnosed by ratios between steroid metabolites (precursor metabolite/product metabolite). Our work has led us to develop a simplified graphical representation of quantitative urinary steroid hormone profiles and diagnostic ratios.

Contents

☆ Article from special issue on "Steroid profiling and analytics: going towards Sterome".

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1. Introduction

Urinary steroid profiling has been a critical part of the diagnosis of disorders of steroid hormone synthesis and metabolism since the 1960s, first by thin layer chromatography, followed by gas chromatography and gas chromatography–mass spectrometry (GC/MS). However, immunoassays and related techniques have been widely used for quantification of plasma steroid hormones in routine diagnostic processes for established conditions. While such techniques have been central to our ability to diagnose clinical disorders, data are frequently compromised since the problem of cross-reactivity has never been completely solved for several analytes, thus impacting specificity. Another problem commonly occurs when low concentrations of hormones are quantified, as in pediatric patients or postmenopausal women. Furthermore, large interassay variability exists even for common measurements such as testosterone, estradiol, and progesterone [1]. These difference have been significantly lowered between laboratories using liquid chromatography tandem mass spectrometry (LC/MS/MS) [1]. The latest generation of GC/MS and LC/MS/MS techniques are superior to immunoassays regarding specificity and limits of quantification, providing a good linearity even down to low concentrations. Since LC/MS/MS high-sensitivity and high-specificity measurement can be allied with high throughput, many diagnostic laboratories are adopting this new technology. While LC/MS/MS will be the mainstream technology in steroid hormone analysis in the near future, it is not without its limitations, with one drawback being that analyte measurements are always targeted, even when panels of steroids are measured in single runs.

The targeted analytes for radioimmunoassays (RIAs) or tandem MS analysis are selected steroid hormones and their precursors illustrated in the steroid biosynthetic pathway (Fig. 1, abbreviations listed in Table 1). Historically the analytes requested for patient studies have been kept to a minimum because of cost and lack

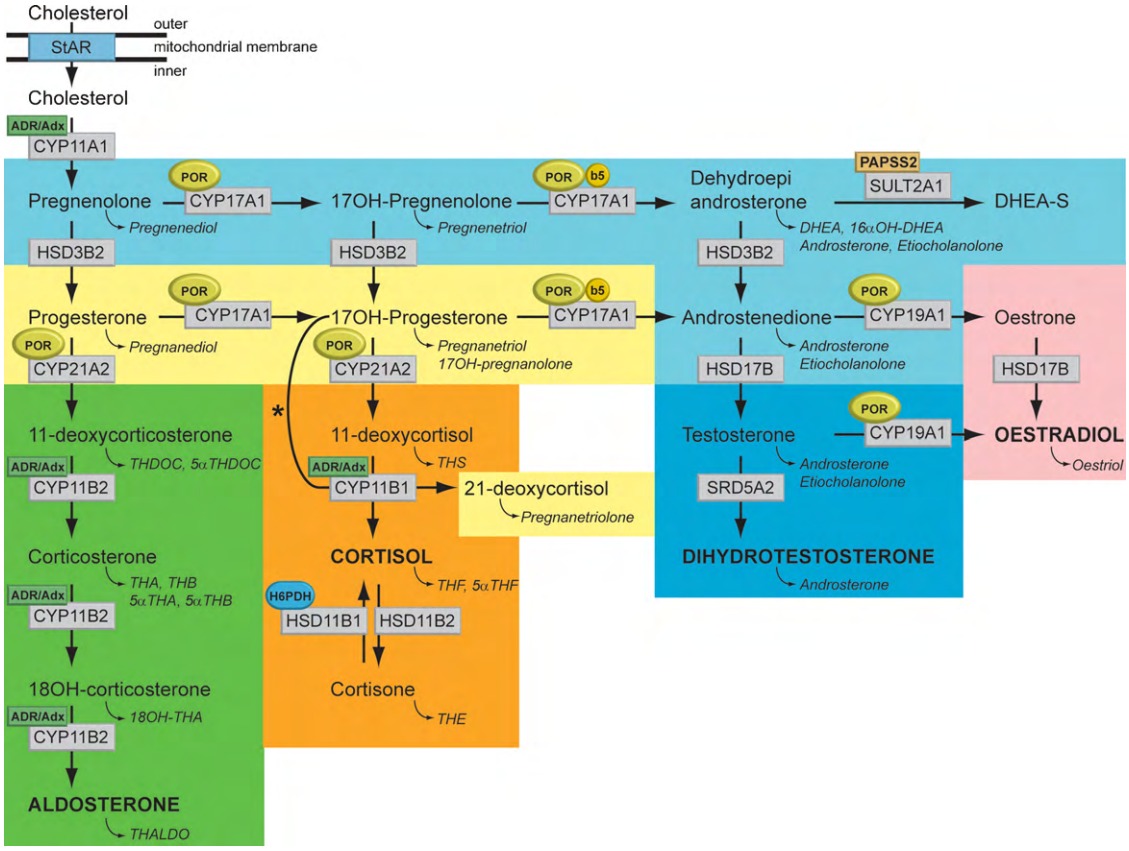

Fig. 1. Synthesis and metabolism of hormonal steroids. This figure illustrates the formation of the major hormone classes from cholesterol. Steroid names in conventional script are steroid hormones and precursors; those in italics are urinary metabolites of the aforementioned. The major transformative enzymes are in rectangular boxes, the cofactor ("facilitator") enzymes in ovals. The pale blue area contains common intermediate steps; the yellow preliminary steps in glucocorticoid synthesis; the green mineralocorticoids; the orange, glucocorticoids; dark blue, androgens and pink, estrogens. Mitochondrial CYP type I enzymes requiring electron transfer via adrenodoxin reductase (ADR) and adrenodoxin (Adx) CYP11A1, CYP11B1, CYP11B2, are marked with a labelled box ADR/Adx. Microsomal CYP type II enzymes receive electrons from P450 oxidoreductase (POR), CYP17A1, CYP21A2, CYP19A1, are marked by circled POR. The 17,20-lyase reaction catalyzed by CYP17A1 requires in addition to POR also cytochrome b5 indicated by a circled b5. Similarly, hexose-6-phosphate dehydrogenase (H6PDH) is the cofactor-generating enzyme for 11β-HSD1. The asterisk (*) indicates the 11-hydroxylation of 17OHP to 21-deoxycortisol in 21-hydroxylase deficiency. The conversion of androstenedione to testosterone is catalyzed by HSD17B3 in the gonad and AKR1C3 (HSD17B5) in the adrenal. StAR, steroidogenic acute regulatory protein; CYP11A1, P450 side-chain cleavage enzyme; HSD3B2, 3β-hydroxysteroid dehydrogenase type 2; CYP17A1, 17α-hydroxylase; CYP21A2, 21-hydroxylase; CYP11B1, 11β-hydroxylase; CYP11B2, aldosterone synthase; HSD17B, 17β-hydroxysteroid dehydrogenase; CYP19A1, P450 aromatase; SRD5A2, 5α-reductase type 2; SULT2A1, sulfotransferase 2A1; PAPPS2, 3′-phosphoadenosine 5′-phosphosulfate synthase 2.