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The art of measuring steroids
Principles and practice of current hormonal steroid analysis

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ABSTRACT

Steroids are small and highly important structural or signalling molecules in living organisms and their metabolism is complex. Due to the multiplicity of enzymes involved there are many different steroid related disorders. E.g., an individual enzyme defect is rather rare but can share various clinical symptoms and can thus be hardly diagnosed clinically. Therefore, reliable hormonal determination still presents the most reasonable initial diagnostic approach and helps to avoid uncritical and expensive attempts at molecular diagnostic testing. It also presents a backbone of monitoring these complex patients. In science, reliable hormone measurement is indispensable for the elucidation of new mechanisms of steroid hormone actions.

Steroid analytics is highly challenging and should never be considered trivial. Most common methods for steroid determination comprise traditionally immunoassay, or more recently, mass spectrometry based methods. It is absolutely necessary that clinicians and scientists know the methods they are applying by heart. With the introduction of automated direct assays, a loss of quality could be observed over the last two decades in the field of steroid immunoassays.

This review wants to meet the need for profound information and orientation in the field of steroid analysis. The pros and cons of the most important methods, such as immunoassays and mass spectrometry based methods will be discussed. The focus of the latter will lie on gas chromatography-mass spectrometry (GC–MS) as well as liquid chromatography-mass spectrometry (LC–MS). Selected analytical applications from our Deutsche Forschungsgemeinschaft Research Group FOR 1369 “Sulfated Steroids in Reproduction” will illustrate the contents.

In brief, immunoassays have for long presented the traditional technique for steroid analysis. They are easy to set up. Only one analyte can be measured per immunoassay. Specificity problems can arise and caution has to be exerted especially regarding direct assays lacking purification steps. Mass spectrometry based methods provide structural information on the analyte and thus higher specificity. In combination with chromatographic techniques, they permit the simultaneous determination of a multitude of analytes. Highest specificity can be obtained using GC–MS, a sophisticated but most powerful tool for characterizing steroid metabolomes. LC–MS is a true high throughput technique and highly suited for detecting complex steroids. GC–MS and LC–MS are not competing but complementary techniques.

Since reliable steroid determination requires extremely high expertise in the field of analytics as well as steroid biochemistry, it is recommended that collaborations and networking with highly specialized centers of expertise are developed.

1. Introduction

1.1. Measuring steroids, an art?

It is not easy to reliably measure steroids. When one looks up the meaning of art, e.g. in Merriam-Webster’s Dictionary [1] one of the definitions of art is *“an occupation requiring knowledge or skill”*. These two elements, in depth knowledge as well as skill are indispensable prerequisites for successful steroid determination. Both, clinicians and scientists, ordering tests for steroid hormones, have to know the

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methods applied by heart. Conversely, laboratory scientists involved in steroid measurements have not only to be skilled analysts; they also need to have in depth knowledge of steroid metabolism and its dysfunctions. Furthermore, clients and analysts need to communicate and cooperate as closely as possible to select the right assay and to interpret the results correctly [2].

1.2. Measuring steroids in times of political deregulation

As with all societal events, political circumstances also influence medical and scientific actions. The determination of steroid hormones, their precursors and their metabolites has once been a mainstay of endocrinology, the science devoted to the action of hormones. It is inconceivable but true that during the last two to three decades, primarily austerity measures have led to a dissociation of hormone laboratories from endocrinology and finally to their absorption by often remote huge centralized, supposedly more economic laboratory platforms, where steroids represent just one class of parameters among a multitude of others. Little wonder that this development went hand in hand with a loss of expertise in assay development, selection, interpretation, and inadequate economic valuation, finally leading to a crisis in the quality of steroid analysis in the clinic and research.

This development is more worrisome than one tends to think and it is typical against the background of the current political situation. It is embedded in a world of politically intended increased deregulation, leading to augmented and fierce competition and finally to the survival of the economically strongest. Furthermore, current reasoning tends to equalize frequent occurrence with importance. These changes are fatal if transferred to medicine and basic science. Disorders of steroid metabolism are rare and belong to the most complex disorders. It goes without saying that these latter qualities will neither put patients with steroid disorders nor scientists trying to elucidate the underlying mechanisms in a position to present a democratically or an economically strong community. It is therefore high time for politicians to eventually realize that the current socio-economic system highly discriminates against and threatens both patients with rare and complex disorders as well as the activity of scientists devoted to investigate these diseases.

1.3. Why measuring steroids in times of molecular genetics?

Truly, we have seen many impressive advances in the field of molecular genetics, but admittedly not all phenomena in a patient can be explained by characterizing his genome. We have learnt that a fingerprint of his metabolic profile is often much more informative and concordant with the clinical phenotype. In addition, comprehensive metabolic assessment is not only a powerful diagnostic tool but also allows for monitoring of the disease.

This article is devoted to all of those who wish to be informed on the principles and practice of current analysis of natural steroid hormones, their precursors and metabolites. The article addresses clinicians, as well as hormone analysts, and researchers. To keep its scope at a reasonable limit, steroids such as bile acids or vitamin D and its metabolites have not been enclosed in this review.

While immunoassay has for long been the predominant assay technique for measuring steroids, the introduction of mass spectrometry (MS) based techniques has increased the variety of analytical methods. Especially one of the recent developments, liquid chromatography-mass spectrometry (LC–MS) is about to “flood” the market. The need for profound information and orientation in the field of steroid analysis is further reflected in an increasing number of conflicting publications showing that obviously competition and confusion has arisen in the field of steroid analysis particularly between immunoassay and MS based techniques [3]. But also in the field of MS based steroid analytics there seems uncertainty about the roles of gas chromatography-mass spectrometry (GC–MS), the older, and LC–MS, the younger technique. Thus it seems to be an opportune time to provide a differentiated view and perspective reconsidering the pros and cons of each analytical approach.

1.4. Role of DFG research group 1369 “Sulfated Steroids in Reproduction”

To exemplify the contents of this article, the inclined reader will find examples of typical applications of the analytical techniques mentioned and discussed. These examples were taken from collaborations of the authors within Research Group 1369 “Sulfated Steroids in Reproduction”, a worldwide unique research consortium investigating hitherto unknown biological functions of sulfated steroids, which has been funded by the Deutsche Forschungsgemeinschaft (DFG). To ensure most reliable steroid analysis within this research project, one subproject was devoted to MS based steroid analytics (“LC–MS and GC–MS based Steroidomics”, Stefan A. Wudy, principal investigator). As this special edition of the Journal of Steroid Biochemistry and Molecular Biology will entirely be devoted to essential outcomes of this research group, the reader will find more applications in further publications of this volume.

1.5. Steroids, structure and nomenclature

Steroids are small molecules. However, they are essential for practically all forms of life. On the one hand, they are indispensable for the formation of cell structures. On the other hand, they can act as systemic or local signaling molecules (hormones, paracrine or intracrine regulatory factors), constituting an elaborate and highly important information transfer system. Already tiny structural changes can result in dramatic functional changes.

The structural common feature of steroids is the 4-membered hydrocarbon ring system (sterane, cyclopentanoperhydrophenanthrene) consisting of three six-membered carbon rings A, B, C and one 5-membered carbon ring D (Fig. 1). All thousands of natural and synthetic steroids are derivatives of that core. Most steroid compounds are derived from the following six basic hydrocarbons: the C_{18} steroids estranes, their name rings in trivial names like estradiol, estrone; the C_{19} steroids androstanes, their name reminds us of androgens; the C_{21} steroids resulting from pregnane, its echo – pregnancy – points to gestagens; the C_{24} steroids cholanes, to be found in cholic alcohols and acids; the C_{27} steroids cholestanes, who form the foundation of sterols;

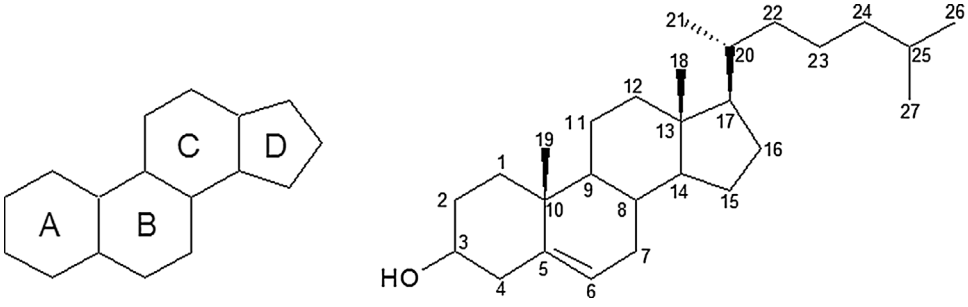

Fig. 1. Sterane (cyclopentanoperhydrophenanthrene) and cholesterol.
The structural common feature of steroids is the 4-membered hydrocarbon ring system consisting of three six-membered carbon rings A, B, C and one 5-membered carbon ring D. The numbering of the carbon sceleton is shown for cholesterol.