Journal of Steroid Biochemistry & Molecular Biology 167 (2017) 182–191

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

The steroid metabolite 16(β)-OH-androstenedione generated by CYP21A2 serves as a substrate for CYP19A1

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ARTICLE INFO

Article history:
Received 30 September 2016
Received in revised form 2 January 2017
Accepted 3 January 2017
Available online 5 January 2017

Keywords:
Steroidogenesis
CYP21A2
CYP19A1
androstenedione
16(beta)- OH-androstenedione
LC–MS/MS

ABSTRACT

The 21-hydroxylase (CYP21A2) is a steroidogenic enzyme crucial for the synthesis of mineralo- and glucocorticoids. It is described to convert progesterone as well as 17-OH-progesterone, through a hydroxylation at position C21, into 11-deoxycorticosterone (DOC) and 11-deoxycortisol (RSS), respectively. In this study we unraveled CYP21A2 to have a broader steroid substrate spectrum than assumed. Utilizing a reconstituted *in vitro* system, consisting of purified human CYP21A2 and human cytochrome P450 reductase (CPR) we demonstrated that CYP21A2 is capable to metabolize DOC, RSS, androstenedione (A4) and testosterone (T). In addition, the conversion of A4 rendered a product whose structure was elucidated through NMR spectroscopy, showing a hydroxylation at position C16-beta. The androgenic properties of this steroid metabolite, 16(β)-OH-androstenedione (16bOHA4), were investigated and compared with A4. Both steroid metabolites were shown to be weak agonists for the human androgen receptor. Moreover, the interaction of 16bOHA4 with the aromatase (CYP19A1) was compared to that of A4, indicating that the C16 hydroxyl group does not influence the binding with CYP19A1. In contrast, the elucidation of the kinetic parameters showed an increased K_m and decreased k_{cat} value resulting in a 2-fold decreased catalytic efficiency compared to A4. These findings were in accordance with our docking studies, revealing a similar binding conformation and distance to the heme iron of both steroids. Furthermore, the product of 16bOHA4, presumably 16-hydroxy-estrone (16bOHE1), was investigated with regard to its estrogenic activity, which was negligible compared to estradiol and estrone. Finally, 16bOHA4 was found to be present in a patient with 11-hydroxylase deficiency and in a patient with an endocrine tumor. Taken together, this study provides novel information on the steroid hormone biosynthesis and presents a new method to detect further potential relevant novel steroid metabolites.

1. Introduction

Steroid hormones are synthesized from cholesterol in a series of enzymatic reactions, involving six cytochromes P450 (CYPs), as well as three hydroxysteroid-dehydrogenases (HSDs). The six cytochromes P450 belong to two different CYP classes: the mitochondrial CYPs (CYP11A1, CYP11B1 and CYP11B2) and the microsomal CYPs (CYP17A1, CYP21A2 and CYP19A1). The mitochondrial CYPs are arranged at the inner mitochondrial membrane. The electrons needed to catalyze their hydroxylation reactions are transferred from NADPH via a FAD containing adrenodoxin reductase (AdR) and an iron-sulphur containing adrenodoxin (Adx). In case of the microsomal CYPs, which are attached to the endoplasmatic reticulum (ER) the electrons are delivered through a FAD and FMN containing NADPH-dependent oxidoreductase (CPR) [1,2].

Abbreviations: 16bOHA4, 16beta hydroxy-androstenedione; DOC, deoxycorticosterone; A4, androstenedione; E1, estrone; AdR, adrenodoxin reductase; E2, estradiol; Adx, adrenodoxin; ER, estrogen receptor; AR, androgen receptor; FAD, flavin adenine dinucleotide; CPR, cytochrome P450 reductase; FMN, flavin mononucleotide; CYP, cytochrome P450; p16bOHE1, putative 16beta hydroxy-estrone; DHEA, dehydroepiandrosterone; RSS, 11-deoxycortisol; DLPC, dilauroyl phosphatidylcholin; T, testosterone.

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http://dx.doi.org/10.1016/j.jsbmb.2017.01.002
0960-0760/

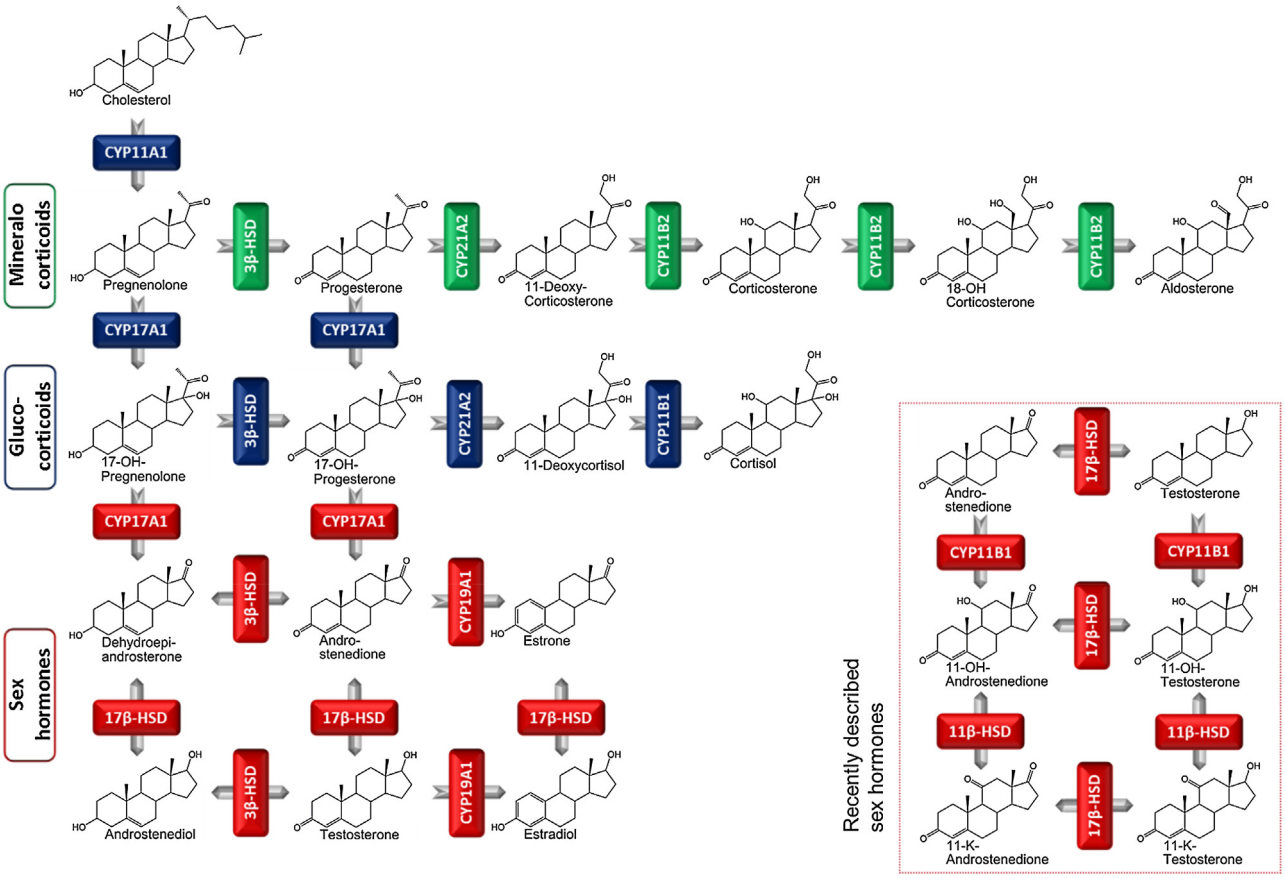

Fig. 1. Scheme of classical steroidogenesis including recently described sex hormones (inset) [6,7].

In mammals, steroid hormones are crucial for life and reproduction. They are synthesized in steroidogenic tissues and organs such as the adrenal gland (Fig. 1), gonads, brain or the testis. Depending on their mode of action, steroid hormones are classified into mineralo- and glucocorticoids, sex hormones and neurosteroids. Through activation of the respective nuclear receptors, they participate in many different vital processes, such as the regulation of the salt and water homeostasis through the action of aldosterone, the main mineralocorticoid or the development of secondary sexual characteristics through the action of estrogens and androgens. Also, several steroid intermediates have been shown to possess biological relevance, such as DHEA or progesterone, which are involved in a variety of biological processes [3–5].

To date, the six CYPs participating in steroid hormone biosynthesis were reported to be successfully expressed in bacteria and subsequently purified, allowing their characterization and the investigation of the impact of diverse compounds on their activities on a molecular level [6–11]. Thereby researchers gained a deeper insight into the mechanistic mode of action of steroidogenic CYPs. In 2012, Hobler and colleagues [9] using recombinantly expressed and purified human CYP11B2 demonstrated its ability to hydroxylate the substrates corticosterone and deoxycorticosterone at position C19. Albeit the synthesis of 19OH-corticosterone has not yet been described so far in humans, the 19-hydroxylase activity towards deoxycorticosterone (DOC) was previously characterized by Kawamoto et al. [12] using COS-7 cells transfected with a plasmid encoding for human CYP11B2. These 19-hydroxylated products were proposed to be precursors of 19-normineralocorticoids such as 19-noraldosterone showing hypertensinogenic activities and, therefore, a relation to primary aldosteronism. Moreover, CYP11B2 was shown to be able to hydroxylate deoxycorticosterone at position C18 yielding 18OH-deoxycorticosterone. It was concluded that aldosterone synthesis can alternatively be conducted via 18-hydroxylation of DOC, followed by 11β-hydroxylation and 18-oxidation of 18OH-DOC [9].

Very recently, a systematic analysis of the effect of steroid hormone intermediates on the action of CYP11A1 was performed in our laboratory. It was demonstrated that beside its side-chain cleavage activity, CYP11A1 possesses a 2β-, a 6β- and a 16β-hydroxylase activity towards different steroid hormone intermediates [13]. These novel enzymatic activities of CYP11A1 result in the formation of 2β- and 6βOH-deoxycorticosterone, 2β- and 6βOH-androstenedione, 6βOH-testosterone and 16βOH-DHEA. The hydroxylations at positions 6β and 16β of these steroids are already described, but mostly attributed to liver CYPs [14,15]. There are, however no reports available about the production 2βOH-deoxycorticosterone and 2βOH-androstenedione. Referring to the physiological role of 2βOH-deoxycorticosterone and 2βOH-androstenedione no data is available and thus, their function still has to be unravelled.

All of the above information indicates that the enzymatic properties of steroidogenic CYPs still have not been fully elucidated. The observation that CYP11A1, besides performing the side-chain cleavage of cholesterol, is also able to perform highly selective hydroxylations on steroid scaffolds opens up new paths for the understanding of the steroid hormone biosynthesis.

In this study we describe a novel activity of CYP21A2. This steroidogenic CYP hydroxylates progesterone and 17OH-progesterone at position C21, yielding DOC and deoxycortisol (RSS),