Journal of Steroid Biochemistry & Molecular Biology 114 (2009) 57–63

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

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

Epimerase activity of the human 11β-hydroxysteroid dehydrogenase type 1 on 7-hydroxylated C_{19}-steroids☆

Olivier Hennebert[a], Matthieu Montes[b], Alain Favre-Reguillon[c], Henry Chermette[d], Clotilde Ferroud[c], Robert Morfin[a,*]

[a] Chaire de Génie Biologique, EA-3199, Biotechnologie, Conservatoire National des Arts et Métiers, 2 rue Conté, 75003 Paris, France
[b] Chaire de Bioinformatique, EA-3199, Conservatoire National des Arts et Métiers, 292 rue Saint Martin, 75141 Paris Cedex 03, France
[c] Chaire de Transformations chimiques et pharmaceutiques, UMR-7084, Conservatoire National des Arts et Métiers, 2 rue Conté, 75003 Paris, France
[d] CNRS, UMR-5180 Sciences Analytiques, Chimie Physique Théorique, Université de Lyon 1, Bât 210, 43 Boulevard du 11 novembre 1918, 69622 Villeurbanne Cedex, France

ARTICLE INFO

Article history:
Received 27 November 2008
Accepted 31 December 2008

Keywords:
11β-HSD1
DHEA
Epiandrosterone
NADPH
Oxidoreduction
Cortisol
Cortisone

ABSTRACT

Cytochrome P4507B1 7α-hydroxylates dehydroepiandrosterone (DHEA), epiandrosterone (EpiA) and 5α-androstane-3β,17β-diol (Adiol). 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) interconverts 7α- and 7β-forms. Whether the interconversion proceeds through oxido-reductive steps or epimerase activity was investigated. Experiments using [^{3}H]-labelled 7β-hydroxy-DHEA, 7β-hydroxy-EpiA and 7β-hydroxy-Adiol showed the ^{3}H-label to accumulate in the 7-oxo-DHEA trap but not in 7-oxo-EpiA or 7-oxo-Adiol traps. Computed models of 7-oxygenated steroids docked in the active site of 11β-HSD1 either in a flipped or turned form relative to cortisone and cortisol. 7-Oxo-steroid reduction in 7α- or 7β-hydroxylated derivatives resulted from either turned or flipped forms. 11β-HSD1 incubation in $H_2{}^{18}O$ medium with each 7-hydroxysteroid did not incorporate ^{18}O in 7-hydroxylated derivatives of EpiA and Adiol independently of the cofactor used. Thus oxido-reductive steps apply for the interconversion of 7α- and 7β-hydroxy-DHEA through 7-oxo-DHEA. Epimerization may proceed on the 7-hydroxylated derivatives of EpiA and Adiol through a mechanism involving the cofactor and Ser_{170}. The physiopathological importance of this epimerization process is related to 7β-hydroxy-EpiA production and its effects in triggering the resolution of inflammation.

1. Introduction

Testosterone, the male hormone, derives from dehydroepiandrosterone (DHEA) and is a precursor for epiandrosterone (EpiA) and 5α-androstane-3β,17β-diol (Adiol). Testosterone is mainly produced in the testis and ovaries, but also in other tissues through an intracrine process [1]. Peripheral testosterone metabolism proceeds first through reduction of the $\Delta^{4,5}$ double bond to 5α-dihydrotestosterone (5α-DHT) by microsomal 5α-reductase. 5α-DHT is the sole native ligand for the androgen receptor. Several enzymatic steps are involved in the control of resulting 5α-DHT levels in tissues. Most of these enzymes are specific hydroxysteroid oxidoreductases (HSOR), namely 17β-HSOR, 3α-HSOR and 3β-HSOR (Fig. 1). Thus, EpiA formation results both from 17β-oxidation of 5α-androstane-3β,17β-diol and from 3β-reduction of 5α-androstane-3,17-dione. Cytochrome P450-7B1 (CYP7B1) is the enzyme responsible for 7α-hydroxylation of oxysterols [2], including all 3β-hydroxysteroids bearing oxygen in the side chain or at the 17-position, such as pregnenolone, DHEA, EpiA and Adiol [3,4]. 7α-Hydroxy-DHEA, 7α-hydroxy-EpiA and 7α-hydroxy-Adiol are produced from their respective precursors via CYP7B1 in tissues such as brain, liver, skin and joints where the hydroxylase is expressed [5–8] (Fig. 1). These tissues also contain NADP(H)-dependent 11β-HSD1 [7] which converts 7α-hydroxysteroids to the corresponding 7β-hydroxysteroids [9–11]. This conversion has been observed in humans by characterizing 7α- and 7β-hydroxy-EpiA metabolites in urine after injection of radio-labelled 5α-androstane-3β,17β-diol [12].

Human 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) catalyzes the interconversion of cortisone into active cortisol. 11β-HSD1 inhibition is a tempting target for the treatment of a host of human disorders that might benefit from blockade of glucocorticoid action, such as obesity, metabolic syndrome, and type 2 diabetes. Numerous studies have been devoted to finding potent 11β-HSD1 inhibitors among natural and chemical molecules [13–17]. It is also possible that steroid molecules naturally produced in the organism

☆ Lecture presented at the '18th International Symposium of the Journal of Steroid Biochemistry and Molecular Biology', 18–21 September 2008, Seefeld, Tyrol, Austria.

* Corresponding author. Tel.: +33 140272572; fax: +33 140272380.
E-mail address: morfin@cnam.fr (R. Morfin).

0960-0760/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2008.12.015

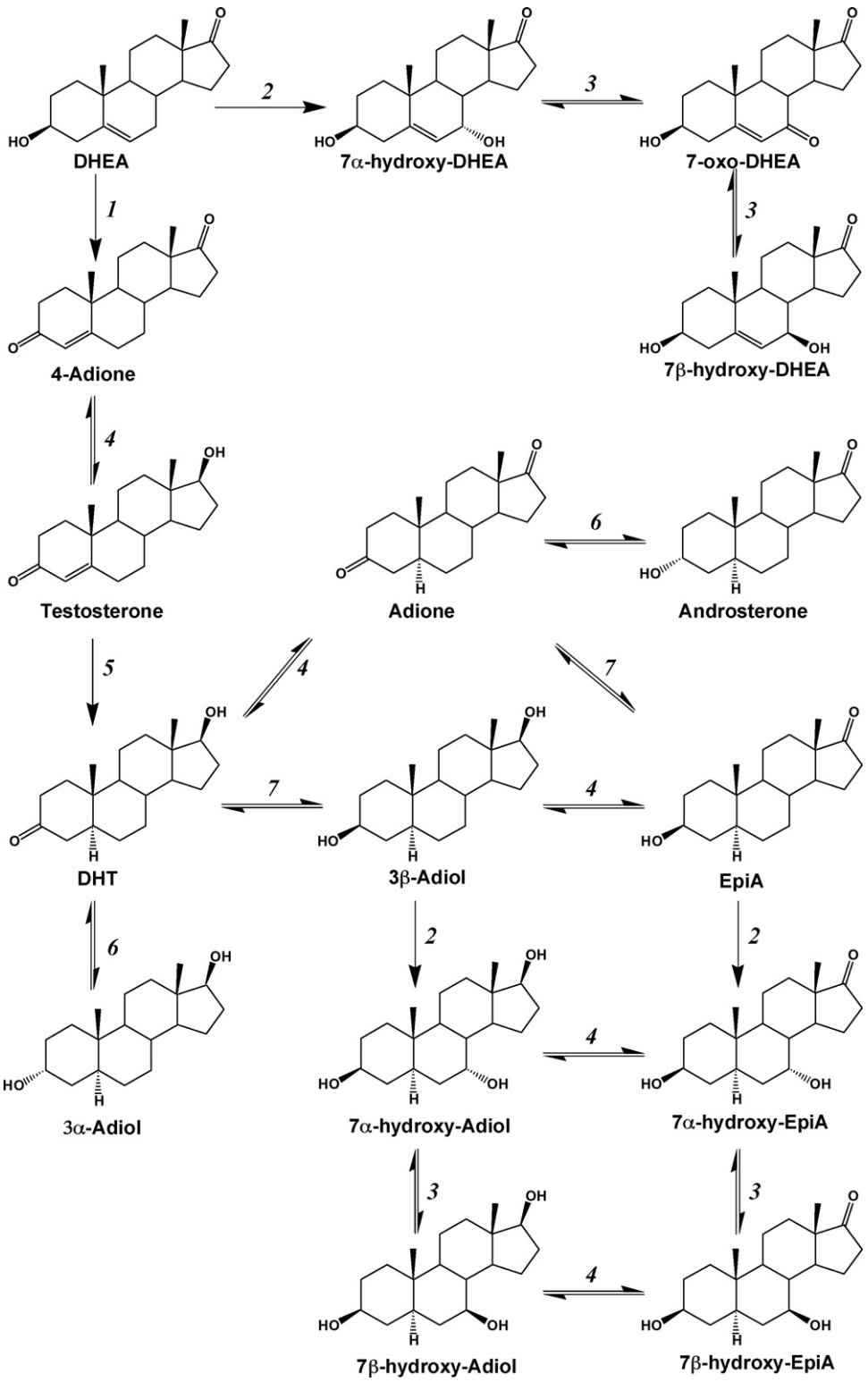

Fig. 1. Epiandrosterone (EpiA) derives from testosterone and is a substrate for CYP-7B1 (2) producing 7α-hydroxy-EpiA which is then interconverted into 7β-hydroxy-EpiA by 11β-hydroxysteroid dehydrogenase type 1 (3). Other enzymes are 3-hydroxysteroid dehydrogenase-isomerase (1), 5α-reductase (5), 3α-hydroxysteroid dehydrogenase (6), 3β-hydroxysteroid dehydrogenase (7), 17β-hydroxysteroid dehydrogenase (4). Steroids are 5α-dihydrotestosterone (DHT), 5α-androstane-3,17-dione (Adione), 5α-androstane-3α,17β-diol (3α-Adiol), 5α-androstane-3β,17β-diol (3β-Adiol), 5α-androstane-3β,7α,17β-triol (7α-hydroxy-Adiol), 5α-androstane-3β,7β,17β-diol (7β-hydroxy-Adiol).

control 11β-HSD1 activity. Our investigations helped identify several native C_{19}-steroids that were substrate for the 11β-HSD1 and inhibited the interconversion of cortisone and cortisol. Therefore, our objective is to review these findings and to examine both the mechanism of action at stake and its physiological importance.

2. 11β-HSD1-mediated oxidoreduction of 7-oxo-steroids

In the presence of NADPH, the recombinant human 11β-HSD1 reduced 7-oxo-DHEA into 7β-hydroxy-DHEA in preference to 7α-hydroxy-DHEA as indicated by a higher V_{max}/K_M ratio (Table 1) [9].

Table 1
11β-HSD1-mediated oxidoreduction of cortisone and 7-oxo-steroids and reduced products. The kinetic parameters were previously reported.

Substrate	Product	K_M (μM)	V_{max}/K_M
NADPH-dependent 11β-HSD1-mediated reduction or epimerization			
Cortisone	Cortisol	2.8	0.4
7-Oxo-DHEA	7α-Hydroxy-DHEA	1.15	0.5
7-Oxo-DHEA	7β-Hydroxy-DHEA	1.13	7.4
7-Oxo-EpiA	7α-Hydroxy-EpiA	0.57	23.7
7-Oxo-EpiA	7β-Hydroxy-EpiA	0.52	5.8
7-Oxo-Adiol	7α-Hydroxy-Adiol	5.1	3.43
7-Oxo-Adiol	7β-Hydroxy-Adiol	6.8	0.22
7α-Hydroxy-EpiA	7β-Hydroxy-EpiA	8.2	2.2
7β-Hydroxy-EpiA	7α-Hydroxy-EpiA	»21[a]	<0.1[a]
7α-Hydroxy-Adiol	7β-Hydroxy-Adiol	1.1	4.5
7β-Hydroxy-Adiol	7α-Hydroxy-Adiol	22	1.5
NADP⁺-dependent 11β-HSD1-mediated oxidation or epimerization			
Cortisol	Cortisone	17	0.4
7α-Hydroxy-DHEA	7-Oxo-DHEA	70	0.2
7β-Hydroxy-DHEA	7-Oxo-DHEA	9.5	1.9
7α-Hydroxy-EpiA	7β-Hydroxy-EpiA	8.1	0.9
7β-Hydroxy-EpiA	7α-Hydroxy-EpiA	»21[a]	<0.1[a]
7α-Hydroxy-Adiol	7β-Hydroxy-Adiol	1.2	2
7β-Hydroxy-Adiol	7α-Hydroxy-Adiol	21	0.5

[a] Conversion yields were too low for accurate measurements.

This preference for the 7β-epimer was maintained in the NADP⁺-dependent oxidation of 7β-hydroxy-DHEA and 7α-hydroxy-DHEA. Other tests with 7α- and 7β-hydroxylated derivatives of EpiA [10] and Adiol [11] gave results conflicting with those obtained through DHEA derivatives. Thus, the NADPH-dependent 7α-reduction of 7-oxo-EpiA and 7-oxo-Adiol was preferred over the 7β-reduction by the enzyme through V_{max}/K_M values shown in Table 1. In contrast to DHEA, the NADP⁺-dependent oxidation of 7α- and 7β-hydroxy-EpiA and 7α- and 7β-hydroxy-Adiol did not result in 7-oxo derivative productions. Instead, interconversion of the 7α- and 7β-hydroxylated forms was observed, with a preference for the production of the 7β-hydroxylated epimers [10,11] through V_{max}/K_M values shown in Table 1.

From these findings in 5α-reduced steroids, the mechanism driven by the recombinant human 11β-HSD1 remained open. Did the interconversion proceed through oxido-reductive steps or through a direct epimeric transformation? Oxidoreduction should proceed through ketone formation. Our previous kinetic studies indicated through apparent K_M and V_{max} determinations that the conversion of 7α- into 7β-derivatives was preferred, as well as the reduction of 7-oxo into 7α-derivatives. On this basis, and because a very rapid oxidoreduction process could deplete the medium of a putative 7-oxo intermediate, we chose to use the 7β-hydroxysteroids as substrates for the recombinant human 11β-HSD1 with and without NADP⁺ or NADPH supplementations.

Search for a putative 7-oxo intermediate was assessed with use of the [^{3}H]-labelled 7β-hydroxysteroid together with the relevant non-radioactive 7-oxo-steroid for radioactivity trapping. In order to validate this model we used the known NADP⁺-dependent 11β-HSD1-catalyzed oxidation of 7β-hydroxy-DHEA into 7-oxo-DHEA. Our evidence obtained for [^{3}H]-label in the 7-oxo-DHEA originating from [^{3}H]-7β-hydroxy-DHEA indicated that the model was functional. No label occurred in the expected 7α-hydroxy-DHEA fraction with use of NADP⁺ supplementation. Use of NADPH supplementation did not yield any [^{3}H]-7β-hydroxy-DHEA transformation product, thus indicating that the NADP⁺-dependent oxidation step was a prerequisite for further transformation. The validated model, used with either [^{3}H]-7β-hydroxy-EpiA or [^{3}H]-7β-hydroxy-Adiol, should yield [^{3}H]-label accumulation in the respective 7-oxo-EpiA and 7-oxo-Adiol traps. No label was found in the two 7-oxo-steroid traps, but small amounts of radioactivity occurred at the level of 7α-hydroxylated derivatives. These formations did not occur in the absence of the cofactor and were more dependent on NADPH than NADP⁺. Two conclusions may be drawn from these findings. Firstly, the recombinant human 11β-HSD1 does not trigger the NADP⁺-dependent oxidation of 7β-hydroxy-EpiA and 7β-hydroxy-Adiol into 7-oxo derivatives. Secondly, the enzyme causes

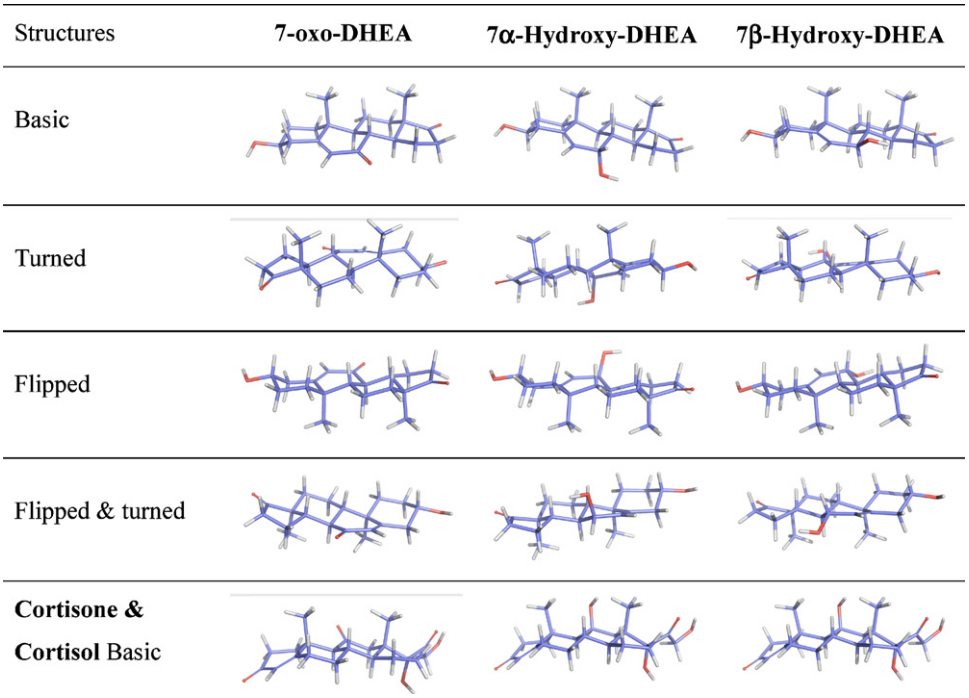

Fig. 2. Possible positioning relative to cortisone or cortisol for 7-oxygenated DHEA derivatives in the 11β-HSD1 active site. Structural models were generated from computation using the Khon-Sham methodology (mol. 2 data). The 12 7-oxygenated DHEA derivative positions were selected for this tabular figure. Steroid backbone, oxygen and hydrogen are depicted in blue, red and white, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 2
11β-HSD1-mediated reduction of cortisone and 7-oxo-steroids. Kinetic parameters were previously reported. Distances between oxygen borne at the steroid 7-position and Tyr_{183} phenolic group and active hydrogen-bearing C_4 of nicotinamide (NA) in NADPH are given. The distances in brackets are those measured in each product deriving from the reduction process. Each steroid model was positioned and docked at minimum energy settings into the computerized crystal structure of the human 11β-HSD1. Distances in Å were obtained through use of PyMol software.

Substrate (position)	Product (position)	K_M (μM)	V_{max}/K_M	Tyr_{183} (Å)	NA (Å)
Cortisone	Cortisol	2.8	0.4	2.85 [1.81]	1.90 [2.30]
7-Oxo-DHEA (turned)	7α-Hydroxy-DHEA (turned)	1.15	0.5	5.00 [3.16]	2.45 [2.34]
7-Oxo-DHEA (flipped)	7β-Hydroxy-DHEA (flipped)	1.13	7.4	2.31 [3.81]	2.29 [4.97]
7-Oxo-EpiA (turned)	7α-Hydroxy-EpiA (turned)	0.57	23.7	4.40 [3.91]	1.91 [1.93]
7-Oxo-EpiA (flipped)	7β-Hydroxy-EpiA (flipped)	0.52	5.8	2.34 [2.06]	2.29 [2.57]
7-Oxo-Adiol (turned)	7α-Hydroxy-Adiol (turned)	5.1	3.4	5.60 [2.94]	1.84 [1.96]
7-Oxo-Adiol (flipped)	7β-Hydroxy-Adiol (flipped)	6.8	0.22	3.11 [1.81]	2.11 [2.42]

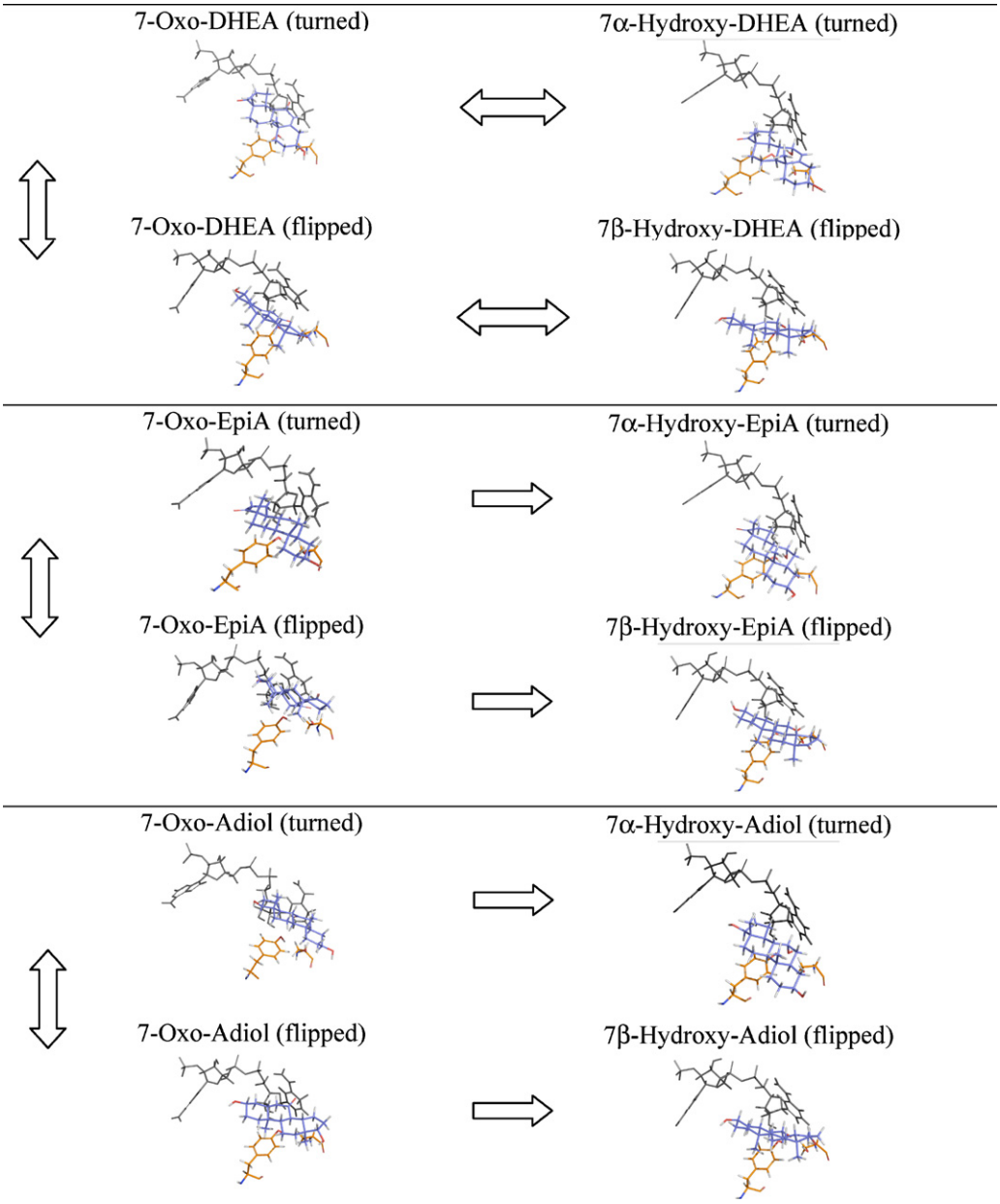

Fig. 3. Mechanism generating both 7α- and 7β-hydroxysteroids after 11β-HSD1-catalyzed NADPH-dependent reduction of 7-oxo-steroid precursors. The crystal structure of the human 11β-HSD1 retrieved from the Protein Data Bank (PDB code 1ILT) was used in conjunction with QuacPac program from Openeye Scientific Software. Docking of the different steroids was performed using the flexible docking program Surflex. The steroid substrates (blue) and products were docked in the site relative to Tyr_{183}, Ser_{170} (orange) and nicotinamide from NADP(H) (black) at minimum energy settings for each flipped or turned structure. Enzymatic reduction of turned structures results in 7α-hydroxylated product while flipped structures result in 7β-hydroxylated products. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 3
11β-HSD1-mediated epimerization of 5α-reduced 7-hydroxysteroids. Kinetic parameters were previously reported [9–11]. Distances between the hydroxyl borne at the steroid 7-position and Tyr_{183}, Ser_{170} hydroxyl groups and C-4 of nicotinamide (NA) in $NADP^+$ are given. The distances in brackets are those measured in each product deriving from the epimerization process. Each steroid model was positioned and docked at minimum energy settings into the computerized crystal structure of the human 11β-HSD1. Distances were obtained through use of PyMol software.

Substrate (position)	Product (position)	K_M (μM)	V_{max}/K_M	Tyr_{183} (Å)	Ser_{170} (Å)	NA (Å)
7α-Hydroxy-EpiA (turned)	7β-Hydroxy-EpiA (turned)	8.1	0.9	3.91 [4.65]	1.77 [2.27]	1.93 [4.03]
7β-Hydroxy-EpiA (flipped)	7α-Hydroxy-EpiA (flipped)	>21[a]	<0.1[a]	2.06 [5.31]	1.98 [3.41]	2.57 [3.35]
7α-Hydroxy-Adiol (turned)	7β-Hydroxy-Adiol (turned)	1.2	2	2.94 [2.50]	2.04 [3.34]	1.96 [4.02]
7β-Hydroxy-Adiol (flipped)	7α-Hydroxy-Adiol (flipped)	21.0	0.5	1.81 [5.37]	1.96 [3.44]	2.42 [3.35]

[a] Transformation yields were too low for a precise measurement.

epimerization of the 5α-reduced 7β-hydroxysteroids independently of the cofactor oxidation state and prefers NADPH over $NADP^+$ for epimer production. Nevertheless, presence of a cofactor is necessary for such epimerization. It should be noted that the NADPH-dependent reduction of 7-oxo-EpiA and 7-oxo-Adiol was described with a preferred production of the 7α-hydroxylated derivatives [10,11], while such derivatives are preferably epimerized into 7β-hydroxylated compounds. It should also be noted that these findings obtained with the recombinant human 11β-HSD1 correlated well with other studies using the native enzyme in human liver and intestinal preparations [5,6].

The present findings were also assessed through close examination and comparison of the steroid structures relative to enzyme activity. Cortisone and all 7-oxo steroids are substrates for NADPH-dependent reduction using the recombinant 11β-HSD1. For the 11-oxo and 7-oxo groups, an equivalent positioning in close proximity to NADPH within the active site of the enzyme could be expected.

3. Examination of 7-oxidized steroid structures

The modelling of cortisol, cortisone and the 7-oxygenated steroids was computed after use of the Khon-Sham density functional theory [18]. Free access to these models, namely cortisol, cortisone, 7-oxo-DHEA, 7-oxo-EpiA, 7-oxo-Adiol, 7α-hydroxy-DHEA, 7α-hydroxy-EpiA, 7α-hydroxy-Adiol, 7β-hydroxy-DHEA, 7β-hydroxy-EpiA and 7β-hydroxy-Adiol, is provided [http://bioinfo.cnam.fr/bioinfo/structuresteroidsinmol2/]. The structure of each 7-oxidized steroid may be positioned as cortisol or either flipped or turned (Fig. 2). The 7-oxo groups of all flipped or turned 7-oxosteroids could match correctly with the 11-oxo group of cortisone. In 7α-hydroxysteroids, the axial 7α-position is only able to match with the axial 11β-hydroxyl of cortisol after a flip of the molecule. This was not the case with the equatorial 7β-position which stood opposite to the axial 11β-hydroxyl when either flipped or flipped and turned. The turned molecule would be the only structure bringing the 7β-hydroxyl into the vicinity of the 11β-hydroxyl. It was also noticed that the structures of Δ_5-steroids did not superpose exactly with those of 5α-reduced steroid structures. These comparisons led us to examine the fit of each steroid relative to Tyr_{183} and Ser_{170} in the active site of the cofactor-supplemented 11β-HSD1.

4. Docking of 7-oxo-steroid derivatives in the 11β-HSD1 active site

Cortisone docked in the NADPH-fortified 11β-HSD1 active site in proximity to the cofactor and to Tyr_{183} with minimum energy required (Table 2). No other cortisone position would dock properly within the site. This positioning results in cortisol production from cortisone reduction. Once either turned or flipped, all 7-oxo-steroids were positioned in the site with the 7-oxo in proximity to NADPH and to Tyr_{183}. Distances between the steroid-borne ketone and both Tyr_{183} and the active hydrogen-bearing C_4 of nicotinamide in NADPH correspond well with kinetic parameters (Table 2). Thus, the highest V_{max}/K_M values were obtained with the shortest distances from nicotinamide and were modulated by those from Tyr_{183}. The NADPH-dependent reduction occurred on either turned or flipped 7-oxo-steroids, and led to the production of 7β- and 7α-reduced derivatives, respectively (Table 2 and Fig. 3). This structural positioning approach permits assessment of the events taking place in the active site. It indicates that production of 7α- and 7β-reduced forms result from the two different docking positions of the 7-oxo-steroid substrates in the active site. Thus, flipped formulae of 7-oxo and 7β-hydroxysteroids were docked in the site, while turned formulae of 7-oxo-steroids and 7α-hydroxysteroids were also docked in the site (Fig. 3). When applied to 7-oxo-steroids, these two docking forms lead to the mechanism depicted in Fig. 3, where 7-oxosteroids are reduced in the α- or β-position when docked in a turned or flipped position. This proposal is attractive as it may explain the stereospecific reduction of 7-oxo-cholesterol to 7β-hydroxy-cholesterol, exclusively [19–21]. Our model shows that due to its large side chain, 7-oxo-cholesterol docking into the 11β-HSD1 active site may occur in the flipped position only, thereby leading to its reduction to 7β-hydroxy-cholesterol only.

5. Epimerization and docking of 5α-reduced 7-hydroxysteroids in the 11β-HSD1 active site

The epimerization mechanism derived from our examination of 7-oxosteroid oxidoreduction by the 11β-HSD1. Nevertheless, this did not apply satisfactorily in a direct epimerization of 7α- and 7β-hydroxylated 5α-reduced steroids and further examination of steroid docking showed that turned 5α-reduced 7α-hydroxysteroid structures met with minimum energy requirements as the 7α-hydroxyl was positioned closer to Ser_{170} than Tyr_{183} (Table 3). With 5α-reduced 7β-hydroxysteroids, the flipped formulae met with minimum energy requirements and the 7β-hydroxyl positioned was in close vicinity to Ser_{170} and nicotinamide C_4 while Tyr_{183} became more distal (Table 3). Differences in positioning of either 7α-hydroxy-EpiA and 7α-hydroxy-Adiol or 7β-hydroxy-Adiol and 7β-hydroxy-Adiol led us to question the absence of 7-oxo intermediate production and the interconversion process taking place in the presence of both NADPH and $NADP^+$. The possibility of an epimerization process taking place led to several hypotheses: (i) once docked and oxidized, the turned 7α-hydroxy-EpiA carbanion could react with H_2O and form an unstable ketone-hydrate leading to 7-oxo-EpiA immediately reduced to 7β-hydroxy-EpiA; (ii) the turned 7α-hydroxy-EpiA carbanion could react with the proximal Ser_{170}, to form a stable hemi-ketal reduced then by NADPH in 7β-hydroxy-EpiA (Fig. 4); (iii) the formed 7-oxo intermediate is kept locked into the enzyme active site where it flips or rotates prior to reduction into the epimeric form. These mechanisms could apply to 7β-hydroxy-EpiA substrate after docking in the flipped position, and to 7α- and 7β-hydroxy-Adiol. Testing the first hypothesis required the use of $H_2{}^{18}O$. We lyophilized 1 mg portions of the recombinant 11β-HSD1 in the presence of either $NADP^+$ or NADPH, and tested their activity before carrying out incubations in the presence of $H_2{}^{18}O$. As expected, no ^{18}O enrichment was detected in 7α-hydroxy-DHEA

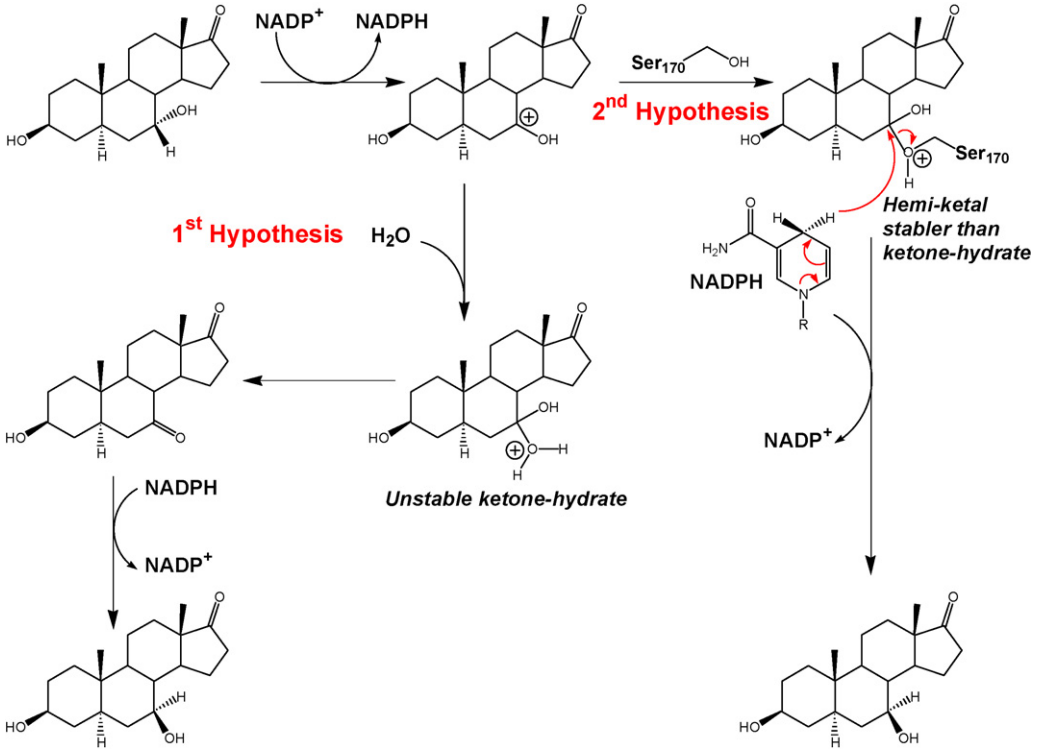

Fig. 4. Hypotheses for 11β-HSD1-catalyzed epimerization of 5α-reduced 7-hydroxysteroids. The steroid depicted is 7α-hydroxy-EpiA docked in the enzyme site with the 7α-hydroxyl in proximity to Ser_{170} and distant from Tyr_{183}. The first hypothesis involves one molecule of H_2O which provides the –OH necessary for epimerization to proceed through ketone-hydrate formation and a 7-ketone intermediate. Absence of ^{18}O enrichment after use of $H_2{}^{18}O$ eliminated this hypothesis. The second hypothesis implies reaction of Ser_{170} for hemi-ketal production prior to reduction. This model applies as well to 7α-hydroxy-Adiol and 7β-hydroxy-Adiol under turned and flipped structures, but requires $NADP^+$ to proceed and does not explain the preferred NADPH-mediated epimerization.

or 7β-hydroxy-DHEA after incubation of 7-oxo-DHEA in the presence of NADPH. Both 7α-hydroxy-DHEA and 7β-hydroxy-DHEA were oxidized into 7-oxo-DHEA in $NADP^+$-fortified incubations without ^{18}O incorporation. As expected from the mechanism proposed above, the reduction of 7-oxo-EpiA by NADPH produced both 7α-hydroxy-EpiA and 7β-hydroxy-EpiA containing no ^{18}O. In the presence of $NADP^+$ as well as NADPH, 7α-hydroxy-EpiA and 7α-hydroxy-Adiol were converted to 7β-hydroxy-EpiA and 7β-hydroxy-Adiol containing no ^{18}O. Thus, the 11β-HSD1 interconverts both 7α- and 7β-epimers through a NADPH-dependent process which does not involve H_2O in the medium. These results imply that the second hypothesis identifying Ser_{170} as possibly responsible for the epimerization process may be valid. This proposal relates well to the 7-oxygen distances from Ser_{170} and the cofactor as well as to the measured K_M and V_{max}/K_M ratios (Table 3) and shows that once produced, epimerized products become distant from Ser_{170} and the cofactor, thus releasing them and excluding a back reaction to proceed. Ser_{170} replacement by other amino acids through site-directed mutagenesis could provide proof for Ser_{170} involvement in the process. Nevertheless, at present we cannot provide any proof validating this hypothesis. The last hypothesis implies that once produced from their 7-hydroxylated precursor, both 7-oxo 5α-reduced derivatives are docked into the active site of the enzyme and allowed to flip or turn without leaving the site. This would first require $NADP^+$ for the oxidation to proceed and then NADPH for reduction and epimer production. The fact that NADPH is preferred for the epimerization process does not support this hypothesis. In addition, computational examination of the steroid positions in the active site of the enzyme did not provide evidence for possible flip or turn of the docked steroids. In conclusion, we were able to demonstrate the 11β-HSD1-mediated epimerization of 5α-reduced 7-hydroxysteroids, but unable to assess its exact mechanism.

6. Rationale for the epimerization process to proceed

The immediate question raised by these findings concerns the utility of 11β-HSD1-containing cells generating epimers of 5α-reduced-7-hydroxysteroids. The present knowledge of steroid metabolism and effects of 7β-hydroxysteroids may provide answers to that question. Firstly, most tissues (liver, brain, skin...) express the 11β-HSD1 in order to convert the 11β-HSD2-generated cortisone into active cortisol [7,22]. Secondly, these tissues contain CYP7B1 which is responsible for the 7α-hydroxylation of DHEA, EpiA and Adiol [7,23]. Thirdly, 11β-HSD1 produces 7β-hydroxy-EpiA from the 7α-hydroxy-EpiA precursor [5,6,10]. Fourthly, 7β-hydroxy-EpiA specifically triggers the production of 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2) in animals [24] and humans [25]. Since 15d-PGJ_2 is directly implied in the resolution of inflammation [26,27] and is the native ligand for PPAR-γ [28–30], the role of 7β-hydroxy-EpiA is key in these processes.

Through measured K_M and V_{max}/K_M values, our studies indicate that 7β-hydroxy-EpiA production from the 7α-hydroxy-EpiA precursor is greatly favoured. Therefore, native production of 7β-hydroxy-EpiA through the 11β-HSD1-mediated interconversion process could be important in controlling inflammation and related consequences for the organism.

Acknowledgement

This work was sponsored by Hunter-Fleming Ltd. (UK).

References

[1] F. Labrie, V. Luuthe, C. Labrie, J. Simard, DHEA and its transformation into androgens and estrogens in peripheral target tissues: intracrinology, Front. Neuroendocrinol. 22 (2001) 185–212.

[2] M. Schwarz, E.G. Lund, R. Lathe, I. Bjorkhem, D.W. Russell, Identification and characterization of a mouse oxysterol 7 alpha- hydroxylase cDNA, J. Biol. Chem. 272 (1997) 23995–24001.
[3] K.A. Rose, G. Stapleton, K. Dott, M.P. Kieny, R. Best, M. Schwarz, D.W. Russell, I. Bjorkhem, J. Seckl, R. Lathe, Cyp7b, a novel brain cytochrome p450, catalyzes the synthesis of neurosteroids 7alpha-hydroxy dehydroepiandrosterone and 7alpha-hydroxy pregnenolone, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 4925–4930.
[4] S.B. Kim, S. Chalbot, D. Pompon, D.H. Jo, R. Morfin, The human cytochrome P4507B1: catalytic activity studies, J. Steroid Biochem. Mol. Biol. 92 (2004) 383–389.
[5] S. Chalbot, R. Morfin, Human liver S9 fractions: metabolism of dehydroepiandrosterone, epiandrosterone, and related 7-hydroxylated derivatives, Drug Metab. Dispos. 33 (2005) 563–569.
[6] S. Chalbot, R. Morfin, Neurosteroids: metabolism in human intestine microsomes, Steroids 70 (2005) 319–326.
[7] O. Hennebert, S. Chalbot, S. Alran, R. Morfin, Dehydroepiandrosterone 7alpha-hydroxylation in human tissues: possible interference with type 1 11beta-hydroxysteroid dehydrogenase-mediated processes, J. Steroid Biochem. Mol. Biol. 104 (2007) 326–333.
[8] C. Trap, F. Nato, S. Chalbot, S.B. Kim, P. Lafaye, R. Morfin, Immunohistochemical detection of the human cytochrome P4507B1: production of a monoclonal antibody after cDNA immunization, J. Neuroimmunol. 159 (2005) 41–47.
[9] C. Muller, D. Pompon, P. Urban, R. Morfin, Inter-conversion of 7alpha- and 7beta-hydroxy-dehydroepiandrosterone by the human 11beta-hydroxysteroid dehydrogenase type 1, J. Steroid Biochem. Mol. Biol. 99 (2006) 215–222.
[10] O. Hennebert, C. Pernelle, C. Ferroud, R. Morfin, 7Alpha- and 7beta-hydroxy-epiandrosterone as substrates and inhibitors for the human 11beta-hydroxysteroid dehydrogenase type 1, J. Steroid Biochem. Mol. Biol. 105 (2007) 159–165.
[11] O. Hennebert, S. Le Mee, C. Pernelle, R. Morfin, 5Alpha-androstane-3beta,7alpha,17beta-triol and 5alpha-androstane-3beta,7beta,17beta-triol as substrates for the human 11beta-hydroxysteroid dehydrogenase type 1, Steroids 72 (2007) 855–864.
[12] F. Jacolot, F. Berthou, Y. Dreano, J.P. Bercovici, H.H. Floch, In vivo metabolism of ^{14}C-labelled 5α-androstane-3β,17β-diol, J. Steroid Biochem. 14 (1981) 663–669.
[13] J.R. Seckl, B.R. Walker, 11Beta-hydroxysteroid dehydrogenase type 1 as a modulator of glucocorticoid action: from metabolism to memory, Trends Endocrinol. Metab. 15 (2004) 418–424.
[14] M. Wang, Inhibitors of 11beta-hydroxysteroid dehydrogenase type 1 for the treatment of metabolic syndrome, Curr. Opin. Investig. Drugs 7 (2006) 319–323.
[15] D.J. Wake, B.R. Walker, Inhibition of 11beta-hydroxysteroid dehydrogenase type 1 in obesity, Endocrine 29 (2006) 101–108.
[16] U. Oppermann, Type 1 11beta-hydroxysteroid dehydrogenase as universal drug target in metabolic diseases? Endocr. Metab. Immune Disord. Drug Targets 6 (2006) 259–269.
[17] L. Miguet, Z. Zhang, M. Barbier, M.G. Grigorov, Comparison of a homology model and the crystallographic structure of human 11beta-hydroxysteroid dehydrogenase type 1 (11betaHSD1) in a structure-based identification of inhibitors, J. Comput. Aided Mol. Des. 20 (2006) 67–81.
[18] W. Kohn, L.J. Sham, Self-consistent equations including exchange and correlation effects, Phys. Rev. 140 (1965) A1133–A1138.
[19] R.A. Schweizer, M. Zurcher, Z. Balazs, B. Dick, A. Odermatt, Rapid hepatic metabolism of 7-ketocholesterol by 11beta-hydroxysteroid dehydrogenase type 1: species-specific differences between the rat, human and hamster enzyme, J. Biol. Chem. 279 (2004) 18415–18424.
[20] M. Hult, B. Elleby, N. Shafqat, S. Svensson, A. Rane, H. Jornvall, L. Abrahmsen, U. Oppermann, Human and rodent type 1 11beta-hydroxysteroid dehydrogenases are 7beta-hydroxycholesterol dehydrogenases involved in oxysterol metabolism, Cell. Mol. Life Sci. 61 (2004) 992–999.
[21] S. Arampatzis, B. Kadereit, D. Schuster, Z. Balazs, R.A. Schweizer, F.J. Frey, T. Langer, A. Odermatt, Comparative enzymology of 11beta-hydroxysteroid dehydrogenase type 1 from six species, J. Mol. Endocrinol. 35 (2005) 89–101.
[22] J.R. Seckl, B.R. Walker, Minireview: 11beta-hydroxysteroid dehydrogenase type 1—a tissue-specific amplifier of glucocorticoid action, Endocrinology 142 (2001) 1371–1376.
[23] M.W. Khalil, B. Strutt, D. Vachon, D.W. Killinger, Metabolism of dehydroepiandrosterone by cultured human adipose stromal cells: identification of 7alpha-hydroxydehydroepiandrosterone as a major metabolite using high performance liquid chromatography and mass spectrometry, J. Steroid Biochem. Mol. Biol. 46 (1993) 585–595.
[24] O. Hennebert, M.A. Pelissier, S. Le Mée, E. Wülfert, R. Morfin, Anti-inflamatory effects and changes in prostaglandin patterns induced by 7β-hydroxy-epiandrosterone in rats with colitis, J. Steroid Biochem. Mol. Biol. 110 (2008) 255–262.
[25] S. Le Mee, O. Hennebert, C. Ferec, E. Wülfert, R. Morfin, 7β-Hydroxy-epiandrosterone-mediated regulation of the prostaglandin synthesis pathway in human peripheral blood monocytes, Steroids 73 (2008) 1148–1159.
[26] O. Haworth, C.D. Buckley, Resolving the problem of persistence in the switch from acute to chronic inflammation, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 20647–20648.
[27] R. Rajakariar, M. Hilliard, T. Lawrence, S. Trivedi, P. Colville-Nash, G. Bellingan, D. Fitzgerald, M.M. Yaqoob, D.W. Gilroy, Hematopoietic prostaglandin D2 synthase controls the onset and resolution of acute inflammation through PGD2 and 15-deoxyDelta12 14 PGJ2, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 20979–20984.
[28] Z. Ou, X. Zhao, L.A. Labiche, R. Strong, J.C. Grotta, O. Herrmann, J. Aronowski, Neuronal expression of peroxisome proliferator-activated receptor-gamma (PPARgamma) and 15d-prostaglandin J2-mediated protection of brain after experimental cerebral ischemia in rat, Brain Res. 1096 (2006) 196–203.
[29] N.S. Wayman, Y. Hattori, M.C. McDonald, H. Mota-Filipe, S. Cuzzocrea, B. Pisano, P.K. Chatterjee, C. Thiemermann, Ligands of the peroxisome proliferator-activated receptors (PPAR-gamma and PPAR-alpha) reduce myocardial infarct size, FASEB J. 16 (2002) 1027–1040.
[30] M. Abdelrahman, M. Collin, C. Thiemermann, The peroxisome proliferator-activated receptor-gamma ligand 15-deoxyDelta12,14 prostaglandin J2 reduces the organ injury in hemorrhagic shock, Shock 22 (2004) 555–561.