Journal of Steroid Biochemistry & Molecular Biology 119 (2010) 1–13

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

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

Review

# The glucocorticoid-activating enzyme 11β-hydroxysteroid dehydrogenase type 1 has broad substrate specificity: Physiological and toxicological considerations

Alex Odermatt*, Lyubomir G. Nashev

*Swiss Center for Applied Human Toxicology and Division of Molecular and Systems Toxicology, Department of Pharmaceutical Sciences, University of Basel, Klingelbergstrasse 50, CH-4056 Basel, Switzerland*

ARTICLE INFO

*Article history:*
Received 25 October 2009
Received in revised form 12 January 2010
Accepted 15 January 2010

*Keywords:*
11β-Hydroxysteroid dehydrogenase
Glucocorticoid
Steroid metabolism
Hexose-6-phosphate dehydrogenase
Detoxification
7-Ketocholesterol
Carbonyl reductase

ABSTRACT

The primary function of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) is to catalyze the conversion of inactive to active glucocorticoid hormones and to modulate local glucocorticoid-dependent gene expression. Thereby 11β-HSD1 plays a key role in the regulation of metabolic functions and in the adaptation of the organism to energy requiring situations. Importantly, elevated 11β-HSD1 activity has been associated with metabolic disorders, and recent investigations with rodent models of obesity and type 2 diabetes provided evidence for beneficial effects of 11β-HSD1 inhibitors, making this enzyme a promising therapeutic target. Several earlier and recent studies, mainly performed *in vitro*, revealed a relatively broad substrate spectrum of 11β-HSD1 and suggested that this enzyme has additional functions in the metabolism of some neurosteroids (7-oxy- and 11-oxyandrogens and -progestins) and 7-oxysterols, as well as in the detoxification of various xenobiotics that contain reactive carbonyl groups. While there are many studies on the effect of inhibitors on cortisone reduction and circulating glucocorticoid levels and on the transcriptional regulation of 11β-HSD1 in obesity and diabetes, only few address the so-called alternative functions of this enzyme. We review recent progress on the biochemical characterization of 11β-HSD1, with a focus on cofactor and substrate specificity and on possible alternative functions of this enzyme.



**Contents**



*Abbreviations:* 11β-HSD, 11β-hydroxysteroid dehydrogenase; AKR, aldo-keto reductase; CYP450, cytochrome P450; ER, endoplasmic reticulum; G6P, glucose-6-phosphate; G6PT, endoplasmic reticulum glucose-6-phophate transporter; GR, glucocorticoid receptor; H6PDH, hexose-6-phosphate dehydrogenase; MR, mineralocorticoid receptor; SDR, short-chain dehydrogenase/reductase.

* Corresponding author. Tel.: +41 61 267 1530; fax: +41 61 267 1515.
*E-mail address:* alex.odermatt@unibas.ch (A. Odermatt).

0960-0760/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2010.01.007

## 1. Introduction

Glucocorticoids are essential hormones involved in the regulation of nearly every physiological process. They modulate lipid synthesis, carbohydrate metabolism and protein turnover, and they are key regulators of stress responses, growth and differentiation, neuronal functions, blood pressure and immune functions [1]. The importance of appropriate glucocorticoid regulation is best exemplified by glucocorticoid receptor (GR) knock-out mice, which die shortly after birth [2] and by the lethality of adrenalectomy. Moreover, disturbed glucocorticoid action, as a result of impaired endogenous regulation or due to environmental influences including infection, high-fat food and exposure to xenobiotics, contributes to various diseases [3]. Thus, therapeutic applications aim at enhancing glucocorticoid effects in cases of insufficient endogenous responses, *e.g.* inducing immune suppressive effects during inflammation, or inhibiting excessive glucocorticoid action to reduce adverse metabolic complications. Especially for systemic applications, the multiple roles of glucocorticoids and their actions have to be considered in order to avoid adverse effects during therapy.

The expression of up to 10% of all genes in a higher organism is modulated by glucocorticoids, and a tightly controlled regulatory network is required for a highly time- and tissue-specific regulation and to allow a fine-tuned control of metabolic processes [4]. Glucocorticoids exert most of their effects through binding to corticosteroid receptors of the nuclear hormone receptor family. Two distinct types of receptors exist, the type I or high affinity corticosteroid receptor, also known as mineralocorticoid receptor (MR), and the type II lower affinity or classical glucocorticoid receptor (GR) [5–7]. Of both receptors several transcriptional and translational variants exist [8–10]. Ligand binding to MR and GR regulates gene transcription either directly by binding of the receptor to the promoter of the corresponding gene or indirectly by interaction of the receptor with other transcription factors, as exemplified by the interaction of GR with AP-1 and NF-κB [11]. The active GR (and MR) complex consists of several tissue-specifically regulated proteins, and the receptor and its associated proteins are modulated by multiple post-translational modifications, making glucocorticoid responses highly dynamic.

It was long thought that the magnitude of glucocorticoid action is determined by the circulating concentration of active steroid and the intracellular concentration of GR in the target tissues. However, in the past two decades, the intracellular interconversion of inactive and active glucocorticoids by 11β-hydroxysteroid dehydrogenases (11β-HSD) emerged as a key mechanism of tissue-specific regulation of glucocorticoid action. Glucocorticoids are produced in the adrenal glands, and humans secrete approximately 10–20 mg of cortisol every day. Glucocorticoids exist as active 11β-hydroxysteroids (cortisol in human, corticosterone in rodents), which are regulated by the circadian rhythm and show highest concentrations of about 800 nM in the morning and about 200 nM at midnight in humans, and as inactive 11-ketosteroids (cortisone in human, 11-dehydrocorticosterone in rodents) that remain relatively constant throughout the day at about 100 nM [12]. Whereas cortisol is bound at over 90% to α-2 cortisol-binding globulin (CBG) in blood and at 5–6% to albumin, cortisone exists mainly in the free form [13]. Two distinct 11β-HSD enzymes have been characterized. 11β-HSD2 (SDR9C3, according to a recently introduced nomenclature [14]) catalyzes the conversion of active to inactive glucocorticoids (Fig. 1) [15,16]. It is expressed in cortical collecting ducts and distal tubules of the kidney and in distal colon, where it protects MR from high circulating 11β-hydroxyglucocorticoids. It is also expressed in placenta, where its role is to protect the fetus from high maternal glucocorticoids. In contrast, 11β-HSD1 (SDR26C1) catalyzes the reverse reaction and plays an important role in the activation of GR in metabolically relevant tissues such as liver, adipose and skeletal muscle [17,18]. Interestingly, 11β-HSD1 is also expressed in the hippocampus, in mature adipocytes and in macrophage, tissues expressing MR and GR. It is thought that the MR is occupied by glucocorticoids in these tissues; however, the mechanism of MR activation and function in these tissues remains unclear [19].

Thus, in the absence of 11β-HSDs, a given target cell depends on the circulating level of active 11β-hydroxyglucocorticoids. In contrast, cells expressing 11β-HSD1 are able to generate active steroid from the relatively constant pool of unbound circulating 11-ketoglucocorticoid, and glucocorticoid responses are mainly dependent on the activity of this enzyme. Cells expressing 11β-HSD2 are relatively insensitive to glucocorticoids and are expected to respond to high circulating 11β-hydroxyglucocorticoids only, for example in situations of severe stress. The expression of the respective 11β-HSD enzyme allows a highly tissue- and cell-specific modulation of glucocorticoid sensitivity.

Numerous studies in animals and humans provided evidence for an association between the development of metabolic diseases and the prolonged exposure to elevated levels of active glucocorticoids, either due to administration of synthetic glucocorticoids or as a result of enhanced 11β-HSD1 activity (reviewed in [18,20]). Moreover, treatment of transgenic and diet-induced rodent models of obesity, type 2 diabetes and atherosclerosis with 11β-HSD1 inhibitors demonstrated the improvement of various metabolic parameters, including reduced food intake, decreased glucose levels, enhanced insulin sensitivity, improved lipid profiles and reduced atherosclerotic plaque progression (reviewed in [21–25]). Based on these observations, 11β-HSD1 is currently considered a promising target for therapeutic interventions, with several clinical trials ongoing. Because 11β-HSD1, in addition to its function in the interconversion of glucocorticoids, metabolizes various carbonyl containing chemicals, including non-steroidal compounds, it will be important to investigate the physiological relevance of these alternative functions and to assess the consequences of inhibition.

## 2. Amino acid sequence and structural features of 11β-HSD1

The 11β-HSD enzymes belong to the superfamily of short-chain dehydrogenases/reductases (SDR). According to a recent *in silico* analysis, over 46,000 members have been identified in a wide range of organisms, with at least 73 genes in the human genome [14,26]. SDRs exert a variety of functions and have a broad substrate spectrum, including steroids, retinoids, prostaglandins, lipids, bile acids and xenobiotics [27]. They usually display relatively low sequence identities (typically as low as 10–30%) but share common sequence motifs and structural properties. The common motifs include a structurally conserved binding site for the cofactor NAD(P)(H) in the N-terminal region (Thr-Gly-$(Xaa)_3$-Gly-Xaa-Gly) and the catalytic tetrad (consisting of Asn, Ser, Tyr, and Lys), which is essential for the proton transfer between substrate and cofactor [28]. The 3D-structures of SDRs deposited in the protein database PDB show a nearly superimposable α/β-folding pattern with a central β-sheet typical of a Rossmann-fold (for binding of the nicotinamide ring and its ribose), which is flanked by helices on either side, and a unique substrate binding cavity responsible for the large substrate specificities found in this protein superfamily.

Initially, 11β-HSD1 was identified in a search for an 11β-hydroxyglucocorticoid inactivating dehydrogenase. 11β-HSD1 was purified from rat liver [29] and the cDNA cloned [17]. Subsequently, the human enzyme was cloned and the recombinant enzyme characterized [30]. Activity measurements using cell

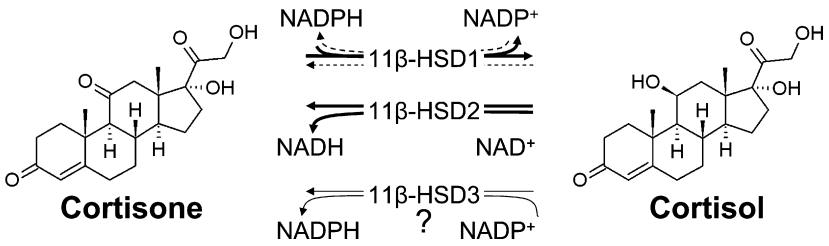


**Fig. 1.** Conversion of cortisone and cortisol by 11β-HSD enzymes.

lysates and microsomal preparations revealed that 11β-HSD1 is a reversible enzyme with 11-ketoglucocorticoid reductase and 11β-hydroxyglucocorticoid dehydrogenase activity, respectively, whereby the dehydrogenase activity was found to be more stable [31,32]. Later, in an attempt to achieve higher protein yields, the full-length human and rat enzymes were expressed in the yeast *P. pastoris* and purified with preserved enzymatic activity [33]. N-terminally truncated 11β-HSD1 proteins were also expressed and purified for structural and functional analyses [34–36]. Despite deleting the single N-terminal transmembrane helix, the truncated proteins are still hydrophobic and remain associated with the ER membrane.

Important novel information was provided by the recently solved 3D-structures of 11β-HSD1 with cofactor, substrate and/or inhibitor, including those from different species [37–39]. The various 11β-HSD1 3D-structures reveal an overall folding pattern similar to those of other SDRs. A central seven-stranded parallel β-sheet is flanked by six parallel α-helices, with four short α-helix insertions relative to the consensus fold. Although an early 3D-structure of human 11β-HSD1 suggested a tetrameric overall fold of the subunits (PDB entries 1xu7, 1xu9) [37], other studies on 3D-structures of 11β-HSD1 from human, guinea-pig and mouse consistently demonstrated that the functional unit of 11β-HSD1 exists as a homodimer formed by two monomers (PDB entry 2bel) [38–40]. This is also in line with an earlier functional analysis using purified protein [41]. The two subunits superimpose well on each other and they are related by a pseudo-2-fold axis, with a relatively large total buried surface area of the dimer interface [38,39]. The amino-termini are on the same face of the dimer and the amino acids for each chain point in the same direction toward the membrane, which is important for the interaction of the hydrophobic C-terminal part of the enzyme with the membrane. Furthermore, the C-terminal helical segment interacts with two of the substrate binding loops in the other subunit, suggesting a tripartite role of the C-terminal region in active site architecture, subunit interactions and interactions with the lipid bilayer. While the two N-terminal single membrane helices from the dimer anchor the enzyme in the membrane, the C-terminal helical segments form a non-polar plateau that is thought to be located within the membrane. The 3D-structures suggest that lipophilic substrates such as steroid hormones and oxysterols, which are enriched in the membrane, enter the catalytic site of the enzyme from the hydrophobic lipid bilayer. This model may explain why low concentrations of cortisone are efficiently converted to cortisol in preparations containing membranes despite the rather high $K_m$ values reported for the enzyme. It may also explain why some lipophilic inhibitors show lower $IC_{50}$ values in intact cells compared with isolated enzyme preparations. Highly lipophilic compounds are expected to reach high local concentrations in membranes and may inhibit the enzyme specifically by occupying the hydrophobic ligand binding site or rather unspecifically by disturbing proper folding and/or insertion of the enzyme in the membrane.

Although 11β-HSD1 and 11β-HSD2 both interconvert glucocorticoids (Fig. 1), a sequence comparison with other SDRs indicates a different origin of these two enzymes that share only 18% identical amino acid sequence, suggesting convergence of substrate specificity during evolution. While 11β-HSD2 is most closely related to 17β-HSD2 (SDR9C2) that converts estradiol to estrone, 11β-HSD1 shares highest sequence similarity with the putative 11β-HSD3 (HSD11B1L, SDR26C2), which has recently been suggested to catalyze the $NADP^+$-dependent dehydrogenation of cortisol [42], and the orphan SDR DHRS7C (SDR32C2) [14,26] whose function remains unknown. Moreover, 11β-HSD2 contains three N-terminal membrane spanning helices with the catalytic domain facing the cytoplasm, whereas 11β-HSD1 has a single N-terminal transmembrane helix with two positively charged lysines at positions 5 and 6 and two negatively charged glutamates at positions 25 and 26 that determine the orientation of the catalytic moiety into the endoplasmic reticulum lumen [43,44]. Furthermore, 11β-HSD2 uses cofactor $NAD^+$ while 11β-HSD1 preferentially accepts NADPH (see below). Despite the orientation into different compartments and the low sequence similarity of 11β-HSD1 and 11β-HSD2, the selectivity of 11β-HSD1 inhibitors is usually assessed using 11β-HSD2 as an initial counter-screen [21–23]. Thus, it remains to be investigated whether some of the many "selective" inhibitors described in the scientific and patent literature may act on other, functionally more related SDRs.

11β-HSD1 is a glycoprotein; however, the role of glycosylation remains unclear [31,45,46]. There are species-specific differences in the number of glycosylation sites. The human and canine enzyme contain three, the mouse, hamster and rat enzymes two and the guinea-pig enzyme one predicted glycosylation site, with evidence from experiments using cells transfected with recombinant 11β-HSD1 that these sites are indeed glycosylated [47]. The sites in human 11β-HSD1 correspond to $Asn^{123}$, $Asn^{162}$ and $Asn^{207}$, whereby the latter is conserved throughout all species. While mutation of $Asn^{203}$ on rat 11β-HSD1 led to complete loss of activity in intact Chinese hamster ovary cells [48], complete deglycosylation of human 11β-HSD1 as well as expression in yeast and bacteria, where the enzyme is not glycosylated, did not affect enzyme activity [34,46]. It remains to be determined whether glycosylation affects 11β-HSD1 protein stability or its interaction with H6PDH or whether it might be responsible for some of the species-specific effects of 11β-HSD1 inhibitors.

## 3. Preference for co-substrate NADP(H)

Inspection of the amino acid sequence of 11β-HSD1 shows the presence of a lysine ($Lys^{44}$) in the conserved Gly-$(Xaa)_3$-Gly-Xaa-Gly motif and an arginine residue at position 66 that is thought to stabilize the 2′-phosphate group of NADP(H). Analogous to $Arg^{66}$, $NAD^+$-selective SDR enzymes contain a glutamate or aspartate residue at the end of β-strand B [49–51]. Structural analysis of 11β-HSD1 revealed that the 2′-phosphate of NADPH is bound to $Arg^{66}$ and $Ser^{67}$, but not to $Lys^{44}$ as in other NADPH-dependent SDRs [38].

Based on kinetic analyses and protein–ligand binding experiments Monder et al. proposed an ordered sequential mechanism with NADPH binding first, followed by substrate bind-

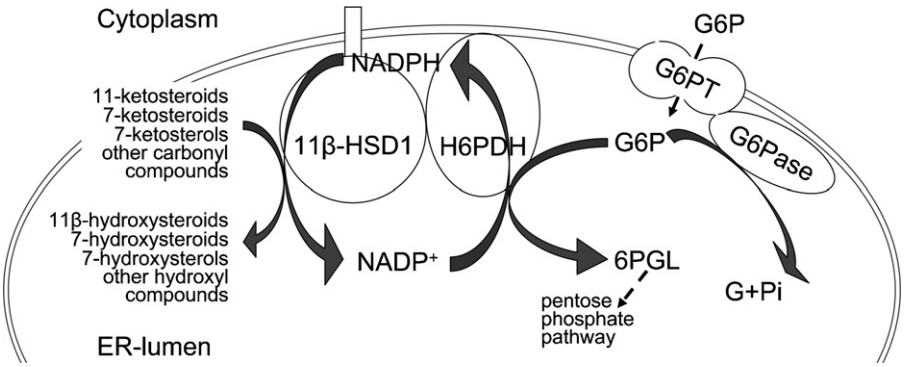


**Fig. 2.** Schematic representation of 11β-HSD1 as a multi-functional ER-luminal enzyme. Hexose-6-phosphate dehydrogenase, H6PDH; glucose-6-phosphate, G6P; ER-luminal glucose-6-phosphate transporter, G6PT; glucose, G; phosphate, Pi; 6-phosphogluconolactone, 6PGL.

ing [32]. A much higher association constant was obtained for NADPH compared with 11-dehydrocorticosterone, and 11-dehydrocorticosterone binding was barely detectable in the absence of NADPH. Similarly, a recent study on the impact of co-substrate concentrations on the effect of 11β-HSD1 inhibitors reported no specific binding of cortisone in the absence of NADPH and provided further evidence that NADPH binds prior to the substrate [52].

Early functional analyses of recombinant rat 11β-HSD1 in intact cells and in cell lysates revealed bi-directional activity with efficient catalysis of both the reduction of 11-dehydrocorticosterone and the oxidation of corticosterone [17,30]. Similar observations were made with 11β-HSD1 from other species, including the human enzyme [33,36,43,44,53]. Using purified protein, microsomal preparations and cell lysates, it was observed that 11β-HSD1 accepts both NAD(H) and NADP(H) as co-substrate, with a preference to utilize NADPH for reduction and about equal efficiencies with $NAD^+$ and $NADP^+$ for oxidation [36,44,53,54]. Thus, the cellular availability of the respective co-substrate in the ER lumen is an important factor regarding glucocorticoid balance.

In the cytoplasm, and probably in other cellular compartments including the ER, NADPH and $NAD^+$ concentrations exceed those of $NADP^+$ and NADH, respectively, by an order of magnitude or more [55–57]. Using microsomal preparations, we recently demonstrated that an approximately 10-fold excess of NADPH over $NADP^+$ is required for an efficient reductase activity of 11β-HSD1 [58]. Furthermore, in intact cells a depletion of glucose shifted the glucocorticoid ratio at steady-state conditions to the oxidized metabolite. Because the cellular nicotinamide nucleotide concentrations exceed those of steroid hormones by far, the co-substrate levels can be expected to determine the reaction direction of steroid metabolizing enzymes, suggesting that steroid reductases primarily function with NADPH and dehydrogenases with $NAD^+$ [59]. It might be speculated that the reversible enzyme 11β-HSD1 utilizes NADPH for reduction and $NAD^+$ for oxidation of substrates in cells not expressing H6PDH. Changes in cellular conditions such as cell fuel abundance and oxidative stress are expected to influence steroid hormone metabolism through altered ratios of oxidized to reduced nicotinamide co-substrates.

## 4. Interaction with H6PDH

The recent discovery of a role for H6PDH in determining the reaction direction of 11β-HSD1 (Fig. 2) revealed another level of tissue-specific glucocorticoid regulation [60–63]. 11β-HSD1 was found to act mainly as a dehydrogenase in undifferentiated human omental adipose stromal cells, in contrast to differentiated mature adipocytes where it acts predominantly as a reductase [64]. It was then proposed that the switch of 11β-HSD1 dehydrogenase to reductase activity correlates with the expression of H6PDH [65]. Other investigators found that 11β-HSD1 functions predominantly as a reductase in pre-adipocytes [66,67]. A switch from dehydrogenase to reductase activity has also been observed during bone differentiation [68], and it may be relevant in other cell types during the transition from proliferation to the differentiated state. Recently, Gomez-Sanchez et al. provided an extensive overview of the H6PDH/11β-HSD1 mRNA and protein expression ratios in various rat tissues [69]. The highest ratio of H6PDH/11β-HSD1 was found in liver, macrophage, spleen, thymus, skeletal muscle and adipose tissue. Lower H6PDH protein expression was found in the stomach and in testes and kidneys where in addition to the 90 kDa band immunoreactive bands at approximately 80 and 65 kDa, respectively, were detected. In the brain the 90 kDa H6PDH band was absent and a 55 kDa protein was observed. The functions of these lower molecular weight H6PDH variants remain to be uncovered.

The full-length 90 kDa H6PDH catalyzes the first two steps of the pentose-phosphate pathway in the ER and converts glucose-6-phosphate to 6-phosphogluconolactone to generate NADPH (Fig. 2) [70,71]. Because the ER membrane is not freely permeable for NADPH, H6PDH plays a key role in the maintenance of the NADPH/$NADP^+$ redox couple in the ER [72]. Piccirella et al. provided evidence that changes in the glutathione redox couple do not influence the redox state of the NAD(P)(H) system [73]. Using microsomal preparations we recently showed that 11β-HSD1 requires an NADPH/$NADP^+$ ratio of 10 or greater to efficiently function as a reductase, suggesting that this intraluminal ratio is even higher in hepatocytes, adipocytes, macrophage and myocytes where 11β-HSD1 predominantly acts as a reductase [58]. The generation of NADPH by H6PDH is dependent on glucose-6-phosphate availability in the ER and on the cellular metabolic state. In transfected HEK-293 cells and in Leydig cells, glucose depletion from the culture medium results in a dramatic shift from reductase to dehydrogenase activity of 11β-HSD1 [58,74,75]. Interestingly, supplementation of the DMEM culture medium with $NAD^+$, NADH or adenosine led to an increase in ATP levels and a stimulation of the reductase and inhibition of the dehydrogenase activity, whereas $NADP^+$ stimulated dehydrogenase activity and NADPH had no effect [74]. These observations suggest that a fall of energy level with a subsequent intraluminal change in the NADPH/$NADP^+$ redox couple causes a shift from 11β-HSD1 reductase to dehydrogenase activity.

Several groups recently demonstrated the impact of H6PDH on 11β-HSD1 function using microsomal preparations [61], coexpression of recombinant proteins [60,62], gene silencing [62,76], and transgenic mouse models [63,77,78]. Coexpression of 11β-HSD1 and H6PDH in HEK-293 or CHO cells led to a 6-fold increase of the reductase activity with a concomitant decrease of dehydrogenase

activity [60]. An opposite effect of similar magnitude was observed in H6PDH-deficient mice [63]. These transgenic mice suffer from myopathy and show an impaired muscle fibre type differentiation. In skeletal muscle, an activation of the unfolded protein response was observed, suggesting that a decreased NADPH/$NADP^+$ ratio in the sarcoplasmic reticulum may lead to impaired protein folding. Furthermore, the lack of H6PDH resulted in an impaired storage and mobilization of lipids from adipose tissue in mice, with a decreased expression of key lipogenic enzymes such as acetyl CoA carboxylase, adiponutrin and stearoyl-coenzyme A desaturase-2 [79].

The enzymatic cooperativity between 11β-HSD1 and H6PDH depends on an intact microsomal complex and is lost upon solubilisation of the membrane by detergents [60,61]. Recently, two independent studies demonstrated a direct protein–protein interaction between 11β-HSD1 and H6PDH [76,80]. Using recombinant proteins and performing coimmunoprecipitation, Far-Western blotting and fluorescence resonance energy transfer assays, Atanasov et al. demonstrated an interaction between 11β-HSD1 and H6PDH with purified proteins as well as in intact cells [80]. Moreover, using chimeric proteins containing N- or C-termini that were exchanged between 11β-HSD1 and 11β-HSD2 they provided evidence that the N-terminal region upstream of the co-substrate binding site of 11β-HSD1 is involved in the interaction with H6PDH. Zhang et al. confirmed the interaction between the two enzymes using purified proteins [76]. Furthermore, they showed that the N-terminal domain of H6PDH is sufficient for interaction with 11β-HSD1. Together, these findings suggest that H6PDH stimulates 11β-HSD1 reductase activity by direct delivery of co-substrate NADPH rather than by an overall increase of the luminal NADPH/$NADP^+$ ratio. Therapeutics specifically disrupting the 11β-HSD1–H6PDH interaction might offer an alternative approach to decrease local glucocorticoid production without affecting other NADPH-dependent functions.

## 5. Other 11-oxysteroids as substrates and inhibitors of 11β-HSD1

Several 11-oxygenated derivatives of progesterone and pregnenolone were found to act as potent inhibitors of 11β-HSD1 and 11β-HSD2 [81,82] and to confer mineralocorticoid properties upon corticosterone in adrenalectomized rats [82]. Infusion of the 11β-HSD inhibitor 11β-hydroxy-3α,5α-tetrahydro-progesterone into normotensive Sprague–Dawley rats resulted in increased blood pressure [83]. Furthermore, 11β-hydroxyprogesterone and 11-ketoprogesterone potently inhibited 11β-HSD1 dehydrogenase and reductase activity, respectively, in primary rat vascular smooth muscle cells [84]. In rat aortic rings, 11β-hydroxyprogesterone enhanced the phenylephrine-induced contractile response and showed an additive effect with corticosterone, while incubation with 11-ketoprogesterone attenuated the contractile response. Galigniana et al. using rat liver and kidney homogenates, provided qualitative evidence that 11β-HSD1 and 11β-HSD2 can catalyze the reduction and dehydrogenation of 11-ketoprogesterone and 11β-hydroxyprogesterone, respectively [85]. Furthermore, their results indicated that 11-ketoprogesterone and 11β-hydroxyprogesterone both exhibit sodium retaining properties by their own, whereby a higher affinity to bind MR was observed for 11-ketoprogesterone [85,86]. In contrast, 11β-hydroxyprogesterone displayed much higher affinity than 11-ketoprogesterone to activate GR. These observations indicate complex interactions between corticosteroids and 11-oxyprogesterones at the level of metabolism and receptor activation.

According to earlier studies, a significant proportion of corticosterone and its 5α-ring A-reduced derivatives is excreted via the bile in humans and rodents [87,88] that can then be metabolized by intestinal microorganisms to 11-oxygenated derivatives of progesterone and 5α-tetrahydro derivatives. The secretion rate of 11β-hydroxyprogesterone was found to be approximately 50-fold lower than that of glucocorticoids in pigs and dogs [89]. Regarding the low circulating concentrations, it seems unlikely that 11-oxygenated progesterone metabolites contribute to sodium retention. However, these metabolites may reach higher concentrations in specific tissues such as the placenta during pregnancy, where disturbed regulation of 11-oxyprogesterone metabolism might interfere with fetal development [90].

In addition to 11-oxyprogesterone metabolites, 11β-HSD1 has the ability to catalyze the interconversion of several 11-oxyandrogen metabolites (own unpublished observations). In humans, a substantial amount of 11β-hydroxyandrostenedione is generated from cortisol in the adrenal gland and in vascular and renal tissue [91,92]. 11β-Hydroxyandrostenedione can be further metabolized by 17β-HSDs to 11β-hydroxytestosterone and by 5α-reductase to their 5α-tetrahydro derivatives. Although significant amounts of 11β-hydroxy and 11-keto metabolites of androstenedione, androsterone and etiocholanolone are excreted in human urine, the biological function of these metabolites remains unclear. Morris and co-workers analyzed a large number of steroid metabolites for their inhibition of 11β-HSD1 dehydrogenase and reductase activity, respectively [93,94]. Whereas 5α-dihydro- and 3α,5α-tetrahydrocorticosterone and 11β-hydroxy derivatives of progesterone, allopregnanolone, testosterone and androstanediol were potent inhibitors of the dehydrogenase activity, the 5β-reduced steroids showed weak or no inhibitory activity. The 11-keto derivatives 3α,5α-tetrahydro-11-dehydrocorticosterone, 11-ketoallopregnanolone and 11-keto-3β,5α-tetrahydrotestosterone inhibited the reductase activity at submicromolar concentrations, whereas 11-ketoprogesterone, 11-ketotestosterone and 11-ketoandrostenedione were an order of magnitude less potent. The 5β-reduced derivatives 11-ketopregnanolone and 11-ketoetiocholanolone were inactive. Thus, on one hand 11β-HSDs play an important role in the local regulation of the ratio of these 11β-hydroxy to 11-keto steroids and in determining their activities towards their cognate receptors, and, on the other hand, the availability of these steroids modulates glucocorticoid metabolism.

11-Oxygenated androgen metabolites may play an important role in the regulation of the balance between catabolic glucocorticoids and anabolic androgens. Several of the metabolites mentioned above that modulate 11β-HSD1 activity can be synthesized by 11β-hydroxylase, which is expressed not only in the adrenals but also in testicular Leydig cells [95]. Whether 11β-HSD1 functions as a dehydrogenase or reductase in Leydig cells is still controversial. While Leckie et al. observed primarily reductase activity in rat Leydig cells [96], Gao et al. reported that 11β-HSD1 functions predominantly as a dehydrogenase, thereby protecting the testis from glucocorticoids [97,98]. As discussed above, the reaction direction of 11β-HSD1 in Leydig cells may depend on glucose availability and on the redox state in the ER [74]. Also, some of the dehydrogenase activity may be explained by the expression of 11β-HSD2 that was observed in recent studies with Leydig cell preparations, blood capillaries and sertoli cells [99–101].

Nevertheless, glucocorticoids suppress steroidogenesis and induce apoptosis in Leydig cells [102,103]. Testosterone synthesis by Leydig cells was found to be inhibited by 50% in the presence of 1.5 nM dexamethasone or 400 nM corticosterone, respectively, but not by 11-dehydrocorticosterone [104]. GR antagonist RU-486 blocked both dexamethasone and corticosterone-mediated inhibition of testosterone synthesis, whereas the 11β-HSD1 inhibitor glycyrrhetinic acid affected exclusively the effect by corticosterone. Dexamethasone, in contrast to corticosterone, cannot be inactivated by 11β-HSDs [105]. Thus, 11β-HSD1 seems to have an important role in Leydig cells in the control of local glucocorticoid

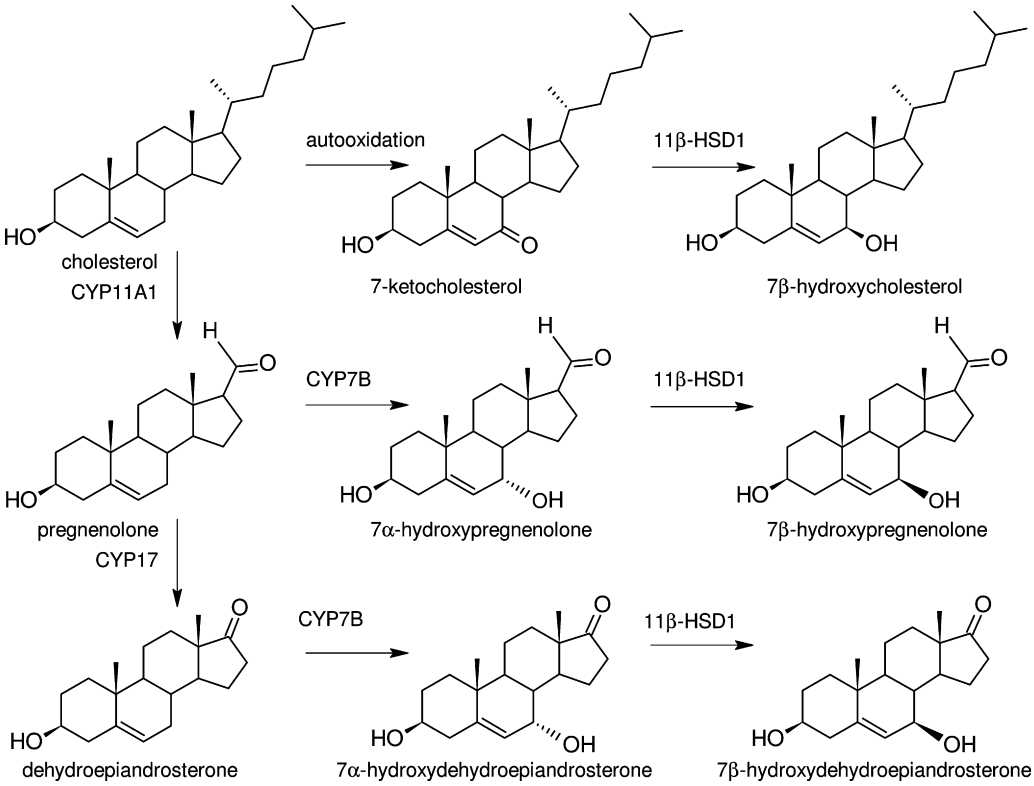


**Fig. 3.** Role of 11β-HSD1 in the generation of 7β-hydroxylated sterols and steroids.

availability and in the interconversion of 11-oxygenated androgen metabolites. The consequences of long-term treatment of patients with metabolic disease with 11β-HSD1 inhibitors on androgen production should be investigated by assessing the total amounts of androgens and the androgen metabolite profile in blood and urine.

## 6. 7-Oxygenated steroids as substrates of 11β-HSD1

7-Oxygenated steroids and sterols are found in almost every living system including mammals, birds, fish and plants; however, their physiological roles and mechanisms of action still remain unclear. The plasma concentrations of these metabolites are in the nanomolar range, thus clearly lower than the concentrations of the parental steroids such as DHEA and pregnenolone, but they may reach higher concentrations in specific cells where they are generated. In the liver, 7α-hydroxylation of cholesterol by CYP7A1 represents the rate-limiting step of bile acid synthesis and metabolic elimination. DHEA, pregnenolone and other related sex steroid precursors are 7α-hydroxylated by CYP7B1 in brain and several other peripheral tissues (Fig. 3) [106–109].

It was proposed that 7-oxygenated-DHEA metabolites may be responsible for some of the neuroprotective, anti-glucocorticoid and immune-modulatory functions of DHEA [106]. In young and aged mice treated with a neuron-damaging agent, 7-keto-DHEA at pharmacological doses showed memory-enhancing and neuroprotective effects [110]. Pringle et al. observed a significant reduction of hypoxia-induced neurotoxicity in hippocampal slices upon incubation with nanomolar concentrations of 7α- and 7β-hydroxyepiandrosterone [111]. Moreover, they reported neuroprotective effects of 7β-hydroxyepiandrosterone in rat models of cerebral ischemia. Interestingly, decreased CYP7B1 mRNA expression was found in patients with Alzheimer's disease [107]. Alzheimer's patients displayed decreased production of 7-hydroxy-DHEA metabolites with significantly higher ratios of DHEA/(7α-hydroxy-DHEA + 7β-hydroxy-DHEA) in cerebrospinal fluid [112,113]. Several studies provide evidence for immune-modulatory effects of 7-oxygenated steroids. In mice a more pronounced immune-stimulatory effect was observed for 7α-hydroxy-DHEA and 7α-hydroxypregnenolone than for the parental steroids [114]. Recently, Dulos and Boots observed increased CYP7B1 expression in synovial tissue and increased 7α-hydroxy-DHEA concentrations in the serum of mice with collagen-induced arthritis [115]. Interestingly, the severity of arthritis correlated with elevated CYP7B1 activity. There is increasing evidence for an impact of 7-keto-DHEA on the regulation of carbohydrate and lipid metabolism. When given orally to rats, 7-keto-DHEA was more potent than DHEA in activating the key "thermogenic" enzymes glycerol-3-phosphate dehydrogenase and malic enzyme in the liver [116]. Ihler and Chami-Stemmann proposed the use of 7-keto-DHEA in the treatment of patients with Raynaud's phenomenon to increase basal metabolic rate and inhibit vasospasms [117]. In another clinical study, 7-keto-DHEA, applied as a gel externally on healthy individuals, led to significantly increased serum high-density lipoprotein and lipoprotein A-I levels [118]. Thus, 7-oxygenated steroids are involved in the regulation of important physiological functions.

It was demonstrated that liver microsomal preparations can produce equal amounts of 7-keto- and 7β-hydroxy-DHEA from 7α-hydroxy-DHEA [119]. However, experiments with different CYP enzymes expressed in baculoviral systems showed no formation of 7-keto-DHEA and only traces of 7β-hydroxy-DHEA when 7α-hydroxy-DHEA was supplied as substrate [120]. Several studies then suggested the formation of 7β-hydroxy-DHEA by hydroxysteroid dehydrogenases [121–123].

Recently, 11β-HSD1 was reported to catalyze the interconversion of several 7-oxygenated sterols and steroids (Fig. 4 and Table 1) [124–128]. Muller et al. applied microsomes from yeast expressing recombinant human 11β-HSD1 and demonstrated the interconversion of 7-hydroxy- and 7-keto-DHEA [127]. The $K_m$ values obtained for the reduction of 7-keto-DHEA and the oxida-

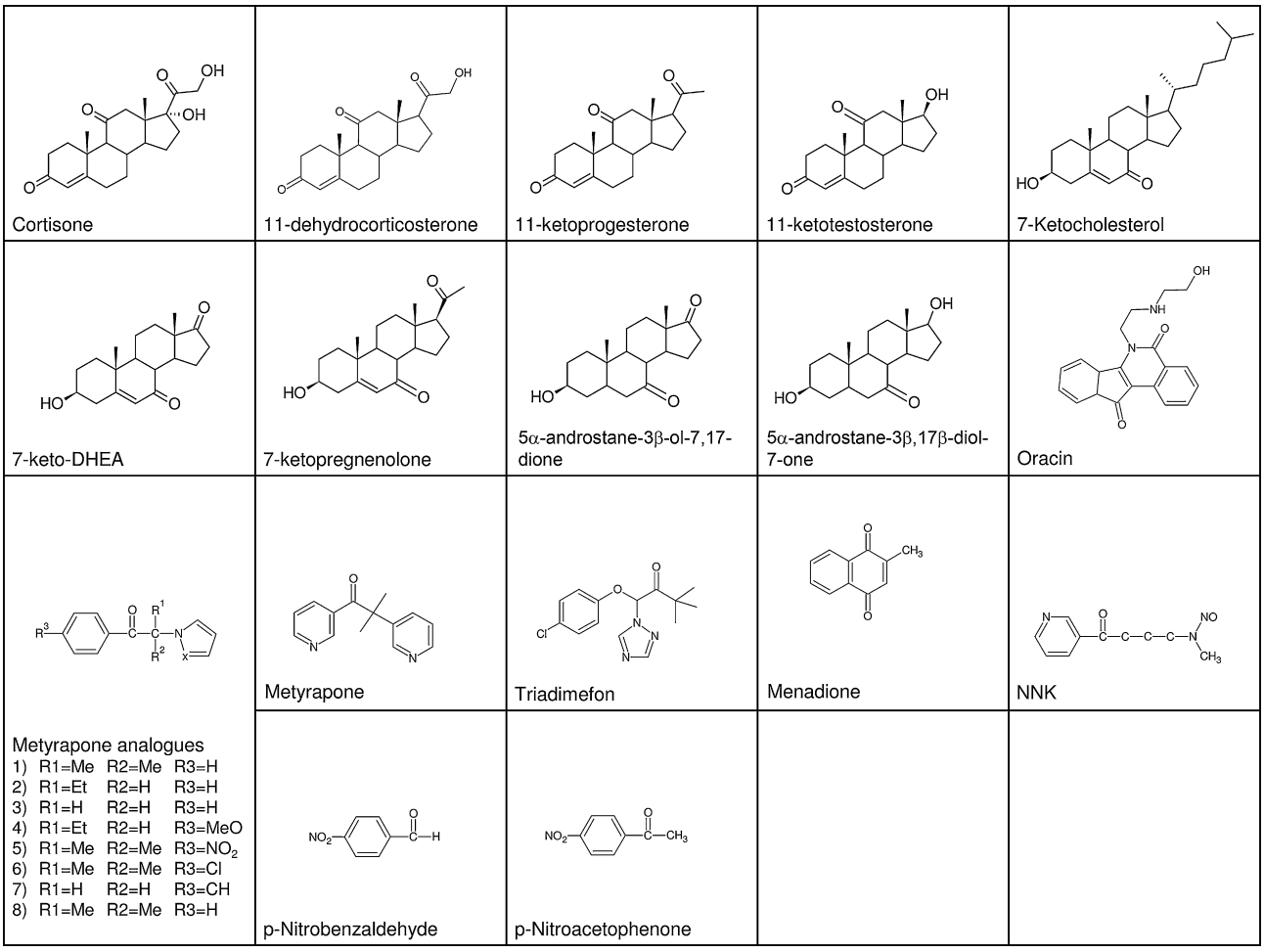


**Fig. 4.** Structures of 11β-HSD1 substrates.

tion of 7β-hydroxy-DHEA were about half the values for cortisone and cortisol, respectively, indicating a higher affinity of 11β-HSD1 for the 7-oxy-DHEA metabolites than for glucocorticoids. Moreover, these authors provided evidence that 11β-HSD1 can function as an epimerase catalyzing the interconversion of 7α- and 7β-hydroxyepiandrosterone and 5α-androstane-3β,7α,17β-triol and 5α-androstane-3β,7β,17β-triol, respectively, with a preferential formation of the 7β-hydroxy metabolite [128,129]. In a recent study, we applied intact HEK-293 cells transfected with human 11β-HSD1 in the presence or absence of H6PDH [126] and observed preferential formation of 7β-hydroxy-DHEA from 7-keto-DHEA as well as some conversion of 7α-hydroxy-DHEA to 7β-hydroxy-DHEA. These results are in agreement with the data of Muller et al. who observed the highest catalytic $V_{max}/K_m$ value for the reduction of 7-keto- to 7β-hydroxy-DHEA using purified enzyme [127]. When cells expressing only 11β-HSD1 were incubated for 24 h (under steady-state conditions), equal amounts of 7-keto-DHEA and 7β-hydroxy-DHEA with minor amounts of 7α-hydroxy-DHEA were observed, independent of the metabolite supplied initially. Upon incubation of cells coexpressing 11β-HSD1 and H6PDH for 24 h, 7β-hydroxy-DHEA was the predominant metabolite with minor amounts of the two other metabolites. Similar observations were made with 7-oxygenated pregnenolone, indicating that 11β-HSD1/H6PDH is responsible for the generation of the 7β-hydroxylated steroids and that the ratio of 11β-HSD1/H6PDH determines the availability of each metabolite. These experiments emphasize the importance of using intact cell systems to assess enzyme reaction direction.

The 7-keto steroids 7-keto-DHEA, 7-keto-pregnenolone and 5α-androstane-3β-ol-7,17dione were found to potently compete with cortisone for binding to 11β-HSD1, with $IC_{50}$ values in the nanomolar range both in cell lysates and intact cells [130]. Under physiological conditions, these 7-ketosteroids are unlikely to affect 11β-HSD1-dependent cortisone reduction because they are present in the circulation at one to two orders of magnitude lower than those of the glucocorticoids. However, upon administration of pharmacological doses they are likely to competitively inhibit glucocorticoid activation.

These recent findings identified 11β-HSD1 as the enzyme responsible for the generation of various 7β-hydroxysteroid metabolites. Further research needs to assess the consequences of altered 11β-HSD1 activity on the role of 7-oxygenated steroids in the regulation of brain function and the immune system.

## 7. Role of 11β-HSD1 in the metabolism of 7-ketocholesterol

In addition to 7-oxygenated steroids, human 11β-HSD1 efficiently metabolizes 7-ketocholesterol, with $K_m$ values comparable with those for glucocorticoids but 2–3-fold lower $V_{max}$ [124,125]. There are significant species-specific differences in the metabolism of 7-ketocholesterol by 11β-HSD1. Whereas the human, rat and murine enzymes efficiently convert 7-ketocholesterol to 7β-hydroxycholesterol, the dog and guinea-pig enzymes show very low activity and the hamster enzyme is not stereospecific and generates 7α- and 7β-hydroxycholesterol [47,125,131]. Importantly, an accumulation of 7-ketocholesterol was observed in the liver

**Table 1**
Reported $K_m$ values of 11β-HSD1 for various carbonyl substrates.

| Substrate | $K_m$ [μM] | $V_{max}$ [nmol h$^{-1}$ mg$^{-1}$] | Protein source | Comment | Ref. |
|---|---|---|---|---|---|
| 7-Ketocholesterol | 0.49 | 0.64 | Cell lysate | Human 11β-HSD1, transfected HEK | [125] |
| 7-Ketocholesterol | 0.42 | 0.61 | Cell lysate | Rat 11β-HSD1, transfected HEK | [125] |
| 7-Ketocholesterol | 0.38 | 0.63 | Cell lysate | Hamster 11β-HSD1, transfected HEK | [125] |
| 11-Dehydrocorticosterone | 0.31 | 1.4 | Cell lysate | Human 11β-HSD1, transfected HEK | [125] |
| 11-Dehydrocorticosterone | 0.31 | 1.4 | Cell lysate | Rat 11β-HSD1, transfected HEK | [125] |
| 11-Dehydrocorticosterone | 0.40 | 1.5 | Cell lysate | Hamster 11β-HSD1, transfected HEK | [125] |
| 7-Ketocholesterol | 51 | – | Purified protein | Human 11β-HSD1 expressed in *P. pastoris* | [124] |
| 7-Ketocholesterol | 7.7 | – | Purified protein | mouse 11β-HSD1 expressed in *P. pastoris* | [124] |
| 7-Ketocholesterol | 85 | – | Purified protein | Rat 11β-HSD1 expressed in *P. pastoris* | [124] |
| 7-Keto-DHEA | 1.13 | 504 | Microsomes | Human 11β-HSD1 expressed in *S. cerevisiae* | [127] |
| Cortisone | 2.8 | 74 | Microsomes | Human 11β-HSD1 expressed in *S. cerevisiae* | [127] |
| 7-Ketoepiandrosterone to 7α-hydroxyepiandrosterone | 0.57 | 738 | Microsomes | Human 11β-HSD1 expressed in *S. cerevisiae* | [129] |
| 7-Ketoepiandrosterone to 7β-hydroxyepiandrosterone | 0.52 | 186 | Microsomes | Human 11β-HSD1 expressed in *S. cerevisiae* | [129] |
| Cortisone | 4.1 | 69 | Microsomes | Human 11β-HSD1 expressed in *S. cerevisiae* | [129] |
| 7-Keto-5α-androstane-3β,17β-diol to 7α-hydroxyl | 5.1 | 870 | Microsomes | Human 11β-HSD1 expressed in *S. cerevisiae* | [128] |
| 7-Keto-5α-androstane-3β,17β-diol to 7β-hydroxyl | 6.8 | 90 | Microsomes | Human 11β-HSD1 expressed in *S. cerevisiae* | [128] |
| Cortisone | 4.4 | 90 | Microsomes | Human 11β-HSD1 expressed in *S. cerevisiae* | [128] |
| Oracin to (+)-dihydrooracin | 790 | 262 | Purified protein | Mouse liver 11β-HSD1 | [155] |
| Oracin to (−)-dihydrooracin | 230 | 18.8 | Purified protein | Mouse liver 11β-HSD1 | [155] |
| NNK | 1750 | 8100 | Purified protein | Mouse liver 11β-HSD1 | [170] |
| Cortisone | 220 | 320 | Purified protein | Mouse liver 11β-HSD1 | [170] |
| Oracin to (+)-dihydrooracin | 190 | 50 | Purified protein | Mouse liver 11β-HSD1 | [171,172] |
| Oracin to (−)-dihydrooracin | 150 | 150 | Purified protein | Mouse liver 11β-HSD1 | [171,172] |
| Cortisone | 14 | 318 | Purified protein | Mouse liver 11β-HSD1 | [160,171] |
| 11-Dehydrocorticosterone | 20 | 240 | Purified protein | Mouse liver 11β-HSD1 | [160,171] |
| NNK | 12,030 | 14,580 | Purified protein | Mouse liver 11β-HSD1 | [160,171] |
| 11-Dehydrocorticosterone | 35 | 2.9 | Microsomes | Mouse lung 11β-HSD1 | [173] |
| NNK | 629 | 39.2 | Microsomes | Mouse lung 11β-HSD1 | [173] |
| Metyrapone | 500 | 660 | Purified protein | Mouse liver 11β-HSD1 | [150] |
| ρ-Nitrobenzaldehyde | 1190 | 1580 | Purified protein | Mouse liver 11β-HSD1 | [150] |
| ρ-Nitroacetophenone | 1670 | nd | Purified protein | Mouse liver 11β-HSD1 | [150] |
| 11-Dehydrocorticosterone | 220 | 320 | Purified protein | Mouse liver 11β-HSD1 | [150] |
| Cortisone | 184 | 298 | Purified protein | Mouse liver 11β-HSD1 | [150] |
| Metyrapone analogue 1 | 130 | 340 | Purified protein | Mouse liver 11β-HSD1 | [152] |
| Metyrapone analogue 2 | 200 | 400 | Purified protein | Mouse liver 11β-HSD1 | [152] |
| Metyrapone analogue 3 | 630 | 460 | Purified protein | Mouse liver 11β-HSD1 | [152] |
| Metyrapone analogue 4 | 490 | 1060 | Purified protein | Mouse liver 11β-HSD1 | [152] |
| Metyrapone analogue 5 | 260 | 660 | Purified protein | Mouse liver 11β-HSD1 | [152] |
| Metyrapone analogue 6 | 100 | 360 | Purified protein | Mouse liver 11β-HSD1 | [152] |
| Metyrapone analogue 7 | 260 | 520 | Purified protein | Mouse liver 11β-HSD1 | [152] |
| Metyrapone analogue 8 | 95 | 520 | Purified protein | Mouse liver 11β-HSD1 | [152] |
| Ketoprofen | 20 | 2.64 | Microsomes | Human 11β-HSD1 expressed in *P. pastoris* | [154] |
| Metyrapone | 370 | 336 | Microsomes | Human 11β-HSD1 expressed in *P. pastoris* | [154] |
| Cortisone | 3.8 | 12 | Microsomes | Human 11β-HSD1 expressed in *P. pastoris* | [154] |
| Triadimefon | 77 | 115 | Microsomes | Rat liver 11β-HSD1 | [169] |

For metyrapone analogues see Fig. 4.

of rats receiving 7-ketocholesterol by gavage and coadministered with the 11β-HSD1 inhibitor carbenoxolone [125], demonstrating an important role of this enzyme in the metabolism of food-derived 7-ketocholesterol.

The human organism is exposed to 7-ketocholesterol by two distinct routes. On one hand, 7-ketocholesterol is generated at high quantities upon heating cholesterol-rich food [132]. In industrialized countries, the common diet includes a frequent consumption of fried or highly processed cholesterol-rich products, containing relatively high amounts of oxidized lipids, including 7-ketocholesterol that is absorbed in the intestine and metabolized in the liver. Following ingestion 7-ketocholesterol is rapidly metabolized in the liver. It can be hydroxylated at position 27 by CYP27A1 (27-hydroxylase) and reduced at position 11 by 11β-HSD1, followed by further metabolism to bile acids and excretion. On the other hand, 7-ketocholesterol can be generated locally in the plasma membrane of cells by autooxidation of cholesterol during oxidative stress [132]. Concentrations of 7-ketocholesterol up to 10 μM are found in macrophage-derived foam cells of atherosclerotic plaques and in cataract lenses, probably as a result of excessive autooxidation [133–136]. Importantly, in patients with coronary artery disease, significantly elevated serum 7-ketocholesterol levels compared with control subjects (19 ng/ml *versus* 32 ng/ml (48 nM *versus* 80 nM)) were observed [137]. Serum 7-ketocholesterol levels strongly correlated with the presence of acute myocardial infarction, the number of affected blood vessels and high sensitive C-reactive protein concentrations, suggesting a close association between elevated serum 7-ketocholesterol and the progression of coronary atherosclerosis and inflammation.

Despite the high dietary 7-ketocholesterol content, there is no evidence for a direct correlation between oral 7-ketocholesterol uptake and the formation of atherosclerotic plaques or cataract [132,133,138], suggesting that the dietary 7-ketocholesterol is efficiently metabolized in the liver and that local ROS production and generation of oxidized lipids are responsible for the high 7-ketocholesterol levels observed in atherosclerotic plaques and cataract lenses. Intuitively, one might assume that pharmacological inhibition of 11β-HSD1 leads to an accumulation of

7-ketocholesterol in macrophage-derived lipid-rich foam cells, which may contribute to the progression of atherosclerotic plaques. However, administration of a selective 11β-HSD1 inhibitor for only 11 days lowered serum cholesterol, free fatty acids and triglycerides, and reduced atherosclerotic lesion progression by over 80% in apolipoprotein E knock-out mice on a high-fat diet [139]. Moreover, inhibition of 11β-HSD1 by the non-selective carbenoxolone reduced atherosclerosis progression in mice [140], and 11β-HSD1 knock-out animals display an atheroprotective phenotype, including lower triglyceride and cholesterol concentrations, and increased insulin sensitivity and glucose tolerance [141]. It is not clear at present whether the beneficial effects of 11β-HSD1 inhibition are solely due to reduced adverse metabolic effects as a result of the decreased intracellular concentration of active glucocorticoids or whether the prevention of the formation of high levels of 7β-hydroxycholesterol may be relevant as well. Recent studies in differentiated human THP-1 macrophage and in mouse 3T3-L1 and 3T3-F442 adipocytes demonstrated that high concentrations of 7-ketocholesterol, as might occur in foam cells and adipocytes, compete with the reduction of cortisone by 11β-HSD1 and lead to decreased GR activation [130,142]. The pathophysiological relevance of these observations is unclear and further research, including studies with 11β-HSD1-deficient mice and clinical studies applying selective inhibitors, is required to understand the role of 11β-HSD1 in regulating the interactions between 7-oxysterols and glucocorticoids. Although the molecular mechanisms are currently unclear, the beneficial effects of 11β-HSD1 inhibition on the cardiovascular system seem to be independent of blood pressure and may involve direct protective effects on the vasculature (for a comprehensive review see [143]).

## 8. Non-steroidal carbonyl compounds as substrates of 11β-HSD1

Carbonyl compounds comprise a large number of aldehydes, ketones and quinones, containing some reactive intermediate metabolites of physiological reactions and of the metabolism of xenobiotics [144]. The carbonyl group of these chemicals can be metabolized to a hydroxyl in phase I biotransformation reactions, followed by phase II conjugation reactions and excretion of the soluble product. The reduction of reactive carbonyl compounds to their alcohol represents an essential detoxification function. Many enzymes belonging to different families, including cytochrome P450, AKR and SDR, are involved in carbonyl reduction. Often several enzymes can catalyze the reduction of a given chemical, and there are significant species-specific differences in the expression and substrate spectrum of these enzymes. Covering the complexity of carbonyl reduction is out of the scope of this article and we refer to recent comprehensive reviews [145,146]. Here, we will focus on 11β-HSD1 and its substrates.

Most of the carbonyl reductases described so far localize to the ER membrane and face the cytoplasm or they are soluble cytoplasmic enzymes, with exception of 11β-HSD1 that protrudes into the ER lumen. The relevance of the luminal orientation of 11β-HSD1 for xenobiotics metabolism remains to be uncovered. The formation of hydroxyls in the ER lumen may favor the conjugation and subsequent secretion of certain chemicals. The role of 11β-HSD1 in the metabolism of xenobiotics was first described by Maser and Netter [147]. Cloning and determination of the primary structure of mouse 11β-HSD1 revealed its identity with a previously described ER membrane bound carbonyl reductase that catalyzed the reductive metabolism of metyrapone, ρ-nitrobenzaldehyde and ρ-nitroacetophenone, and that preferentially utilized NADPH as cofactor and was inhibited by 5α-dihydrotestosterone but not by the classic carbonyl reductase inhibitors quercitrin or barbitone [147–149]. Purified mouse 11β-HSD1 catalyzed the reduction of metyrapone, ρ-nitrobenzaldehyde and ρ-nitroacetophenone with $K_m$ values that were approximately 3-, 7- and 9-fold higher than that for cortisone, suggesting that these xenobiotics compete with glucocorticoid substrates for the same binding site (Fig. 4 and Table 1) [150]. Later, metyrapone, a drug used to inhibit 11β-hydroxylase (CYP11B1)-dependent adrenal glucocorticoid biosynthesis, was shown to competitively inhibit 11β-HSD1 reductase activity, although with a relatively high apparent $K_i$ of 30 μM [151]. Thus, in addition to 11β-hydroxylase, inhibition of 11β-HSD1 may contribute to the anti-glucocorticoid effects of metyrapone. In insects, metyrapone inhibits the biosynthesis of 20-hydroxyecdysone, a hormone responsible for moulting, metamorphosis and reproduction of insects. Metyrapone and some novel analogues used as insecticides are metabolized in mice by hepatic 11β-HSD1 to the less toxic alcohol derivatives that can then be eliminated from the body following conjugation by glucuronic acid at the hydroxyl [152]. In order to determine the role of 11β-HSD1 in the total metabolism of metyrapone and to distinguish its contribution from that of other microsomal and cytoplasmic enzymes [145,153], studies in knock-out mice may be performed.

Furthermore, using microsomal preparations it was shown that 11β-HSD1 catalyzes the reduction of the non-steroidal anti-inflammatory drug ketoprofen [154]. $K_m$ values for ketoprofen and cortisone of 20 and 3.8 μM, respectively, were obtained in this study. In addition, purified 11β-HSD1 was found to catalyze the conversion of the potential anti-cancer drug oracin (6-[2-(2-hydroxyethyl)aminoethyl]-5,11-dioxo-5,6-dihydro-11H-indeno[1,2-c] isoquinoline) to dihydrooracin [155,156]. The relevance of 11β-HSD1 in the metabolism of oracin remains unclear, because its $K_m$ for oracin was at least 10-times higher than that for cortisone, and other enzymes mainly of the aldo-ketoreductase family have been identified to catalyze the carbonyl reduction of oracin [157,158]. Using microsomal preparations of yeast overexpressing human 11β-HSD1, oxidation of DFU-lactol, the prodrug for the selective cyclooxygenase type 2 (COX-2) inhibitor DFU, was reported, although with low affinity and velocity [154].

Moreover, purified 11β-HSD1 was found to convert the tobacco carcinogen nicotine-derived nitrosamine ketone (NNK) to the less toxic nitrosamine alcohol (NNAL) [159,160]. NNK can either be activated to a carcinogenic hydroxylated form that has the potential to cause DNA methylation and DNA pyridyloxobutylation, or it is reduced to NNAL and conjugated with glucuronic acid [161]. A study with microsomes of rat liver and lung proposed that the contribution of 11β-HSD1 to the production of NNAL is approximately 12% and 32%, respectively [162]. Other enzymes involved in NNK metabolism include the aldo-reductases AKR1C1, AKR1C2, AKR1C4 and AKR1B10, and the cytosolic carbonyl reductase CBR1 [158,163]. The elucidation of the relevance of 11β-HSD1 in the detoxification of NNK is important with respect to therapeutic applications of inhibitors of this enzyme in patients with metabolic diseases who are smoking. Soldan et al. observed up to 20-fold differences in 11β-HSD1 expression levels among different individuals in the lung and suggested that lower activity might represent a risk factor for the development of lung cancer [164]. Inhibitors of the carbonyl metabolizing enzymes are considered risk factors for the development of lung cancer. Excessive alcohol consumption has been associated with an increased frequency of p53 mutations in non-small cell lung cancer in cigarette smokers [165], and ethanol was shown to inhibit 11β-HSD1 and AKR1C1, AKR1C2 and AKR1C4 [166]. Despite these earlier studies on a potential role of 11β-HSD1 in the detoxification of tobacco carcinogens, animal experiments addressing tobacco carcinogen metabolism in the presence of selective 11β-HSD1 inhibitors or large clinical

studies testing a possible association of 11β-HSD1 activity and cancer risk in smokers are still missing. Thus, patients treated with 11β-HSD1 inhibitors should be carefully monitored for potential adverse effects due to the interference with detoxification functions.

Recently, Kenneke et al., using rat liver microsomal preparations, provided evidence for a role of 11β-HSD1 in the metabolism of the fungicide triadimefon [167]. Conazoles are used in agriculture as fungicides and account for 20–25% of all fungicides used worldwide. In addition, they are widely used in medicine to treat fungal infections. Conazoles exert their effect by inhibition of the fungal lanosterol-14R-demethylase CYP51, thereby blocking the biosynthesis of ergosterol, an essential element of fungal cell membranes. Several toxic effects of conazoles have been observed in mammalian systems, including activation of nuclear receptors (CAR, PXR and PPAR) and induction of CYPs [168]. Kenneke et al. reported a dose-dependent inhibition of the conversion of triadimefon to triadimefol by glycyrrhetinic acid with an $IC_{50}$ of 31 nM [167]. Furthermore, cortisone was found to inhibit triadimefol formation, although with a relatively high $IC_{50}$ of 5.9 μM. In addition, the authors provided evidence that the efficient conversion of triadimefon to triadimefol is dependent on active glucose-6-phosphate transport through the ER membrane, and that an NADPH-regenerating system led to higher triadimefon reduction than simply addition of NADPH [169], in line with the assumption that 11β-HSD1/H6PDH is responsible for the observed reaction.

## 9. Outlook

11β-HSD1 has a wide substrate spectrum and in addition to glucocorticoids metabolizes other 11-oxysteroids, 7-oxysteroids as well as various structurally unrelated lipophilic carbonyl compounds. Besides its role in the regulation of intracellular glucocorticoid activation and modulation of energy metabolism and immune functions, 11β-HSD1 is involved in the first step of the metabolism of the food-derived oxidized cholesterol metabolite 7-ketocholesterol, and, based on *in vitro* experiments, plays a role in the regulation of the activity of 7-oxygenated neurosteroids as well as in the protection of intracellular membranes against toxic lipophilic carbonyl compounds. The current intense search for therapeutic 11β-HSD1 inhibitors has led to the identification of various highly potent and selective compounds that exert beneficial effects on several parameters of the metabolic syndrome, including decreased circulating levels of glucose, cholesterol, triglycerides and free fatty acids, improved insulin sensitivity and reduced weight gain. However, the potential interference of such inhibitors with the so-called alternative functions of 11β-HSD1 is mostly ignored, and suitable studies with normal, diseased and transgene animals addressing the potential toxicological consequences of coexposure to 11β-HSD1 inhibitors and xenobiotic substrates should be performed. The impact of impaired 11β-HSD1 function on 7-oxycholesterol and 7-oxysteroid metabolite pattern in tissues, blood and urine remains to be determined. Further investigations in animals and clinical studies are needed to elucidate the physiological relevance of these alternative functions. Such studies should be designed to assess the species- and tissue-specific functions of this multi-functional enzyme and will ultimately contribute to the safety assessment of therapeutic 11β-HSD1 inhibitors.

## Acknowledgements

This work was supported by the Swiss National Science Foundation (No. 31003A-124912). A.O. has a Chair of Molecular and Systems Toxicology by the Novartis Research Foundation.

## References

[1] R.M. Sapolsky, L.M. Romero, A.U. Munck, How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions, Endocr. Rev. 21 (2000) 55–89.
[2] T.J. Cole, J.A. Blendy, A.P. Monaghan, K. Krieglstein, W. Schmid, A. Aguzzi, G. Fantuzzi, E. Hummler, K. Unsicker, G. Schutz, Targeted disruption of the glucocorticoid receptor gene blocks adrenergic chromaffin cell development and severely retards lung maturation, Genes Dev. 9 (1995) 1608–1621.
[3] A. Odermatt, C. Gumy, A.G. Atanasov, A.A. Dzyakanchuk, Disruption of glucocorticoid action by environmental chemicals: potential mechanisms and relevance, J. Steroid Biochem. Mol. Biol. 102 (2006) 222–231.
[4] S. John, T.A. Johnson, M.H. Sung, S.C. Biddie, S. Trump, C.A. Koch-Paiz, S.R. Davis, R. Walker, P.S. Meltzer, G.L. Hager, Kinetic complexity of the global response to glucocorticoid receptor action, Endocrinology 150 (2009) 1766–1774.
[5] P.J. Fuller, J.W. Funder, Mineralocorticoid and glucocorticoid receptors in human kidney, Kidney Int. 10 (1976) 154–157.
[6] J.L. Arriza, C. Weinberger, G. Cerelli, T.M. Glaser, B.L. Handelin, D.E. Housman, R.M. Evans, Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor, Science 237 (1987) 268–275.
[7] J.W. Funder, P.T. Pearce, R. Smith, A.I. Smith, Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated, Science 242 (1988) 583–585.
[8] M.C. Zennaro, M.C. Keightley, Y. Kotelevtsev, G.S. Conway, F. Soubrier, P.J. Fuller, Human mineralocorticoid receptor genomic structure and identification of expressed isoforms, J. Biol. Chem. 270 (1995) 21016–21020.
[9] M.R. Yudt, J.A. Cidlowski, The glucocorticoid receptor: coding a diversity of proteins and responses through a single gene, Mol. Endocrinol. 16 (2002) 1719–1726.
[10] N.Z. Lu, J.A. Cidlowski, Glucocorticoid receptor isoforms generate transcription specificity, Trends Cell Biol. 16 (2006) 301–307.
[11] E. Charmandari, T. Kino, G.P. Chrousos, Glucocorticoids and their actions: an introduction, Ann. N.Y. Acad. Sci. 1024 (2004) 1–8.
[12] B.R. Walker, J.C. Campbell, R. Fraser, P.M. Stewart, C.R. Edwards, Mineralocorticoid excess and inhibition of 11β-hydroxysteroid dehydrogenase in patients with ectopic ACTH syndrome, Clin. Endocrinol. (Oxf.) 37 (1992) 483–492.
[13] T.G. Brien, Human corticosteroid binding globulin, Clin. Endocrinol. (Oxf.) 14 (1981) 193–212.
[14] B. Persson, Y. Kallberg, J.E. Bray, E. Bruford, S.L. Dellaporta, A.D. Favia, R.G. Duarte, H. Jornvall, K.L. Kavanagh, N. Kedishvili, M. Kisiela, E. Maser, R. Mindnich, S. Orchard, T.M. Penning, J.M. Thornton, J. Adamski, U. Oppermann, The SDR (short-chain dehydrogenase/reductase and related enzymes) nomenclature initiative, Chem. Biol. Interact. 178 (2009) 94–98.
[15] A.K. Agarwal, T. Mune, C. Monder, P.C. White, NAD(+)-dependent isoform of 11 beta-hydroxysteroid dehydrogenase. Cloning and characterization of cDNA from sheep kidney, J. Biol. Chem. 269 (1994) 25959–25962.
[16] A.L. Albiston, V.R. Obeyesekere, R.E. Smith, Z.S. Krozowski, Cloning and tissue distribution of the human 11β-hydroxysteroid dehydrogenase type 2 enzyme, Mol. Cell Endocrinol. 105 (1994) R11–17.
[17] A.K. Agarwal, C. Monder, B. Eckstein, P.C. White, Cloning and expression of rat cDNA encoding corticosteroid 11β-dehydrogenase, J. Biol. Chem. 264 (1989) 18939–18943.
[18] A.G. Atanasov, A. Odermatt, Readjusting the glucocorticoid balance: an opportunity for modulators of 11β-hydroxysteroid dehydrogenase type 1 activity? Endocr. Metabol. Immune Disord. 7 (2007) 125–140.
[19] A. Odermatt, A.G. Atanasov, Mineralocorticoid receptors: emerging complexity and functional diversity, Steroids 74 (2009) 163–171.
[20] J.W. Tomlinson, P.M. Stewart, Modulation of glucocorticoid action and the treatment of type-2 diabetes, Best Pract. Res. 21 (2007) 607–619.
[21] C.D. Boyle, T.J. Kowalski, 11β-hydroxysteroid dehydrogenase type 1 inhibitors: a review of recent patents, Expert Opin. Ther. Pat. 19 (2009) 801–825.
[22] C. Fotsch, M. Wang, Blockade of glucocorticoid excess at the tissue level: inhibitors of 11β-hydroxysteroid dehydrogenase type 1 as a therapy for type 2 diabetes, J. Med. Chem. 51 (2008) 4851–4857.
[23] D.J. St Jean Jr., M. Wang, C. Fotsch, Inhibitors of 11β-HSD1: a potential treatment for the metabolic syndrome, Curr. Top. Med. Chem. 8 (2008) 1508–1523.
[24] C.G. Schnackenberg, 11β-hydroxysteroid dehydrogenase type 1 inhibitors for metabolic syndrome, Curr. Opin. Invest. Drugs (London England) 9 (2008) 295–300.
[25] E. Saiah, The role of 11β-hydroxysteroid dehydrogenase in metabolic disease and therapeutic potential of 11beta-hsd1 inhibitors, Curr. Med. Chem. 15 (2008) 642–649.
[26] J.E. Bray, B.D. Marsden, U. Oppermann, The human short-chain dehydrogenase/reductase (SDR) superfamily: a bioinformatics summary, Chem. Biol. Interact. 178 (2009) 99–109.
[27] K.L. Kavanagh, H. Jornvall, B. Persson, U. Oppermann, Medium- and short-chain dehydrogenase/reductase gene and protein families: the SDR superfamily: functional and structural diversity within a family of metabolic and regulatory enzymes, Cell Mol. Life Sci. 65 (2008) 3895–3906.
[28] C. Filling, K.D. Berndt, J. Benach, S. Knapp, T. Prozorovski, E. Nordling, R. Ladenstein, H. Jornvall, U. Oppermann, Critical residues for structure and catalysis in short-chain dehydrogenases/reductases, J. Biol. Chem. 277 (2002) 25677–25684.

[29] V. Lakshmi, C. Monder, Purification and characterization of the corticosteroid 11β-dehydrogenase component of the rat liver 11β-hydroxysteroid dehydrogenase complex, Endocrinology 123 (1988) 2390–2398.
[30] G.M. Tannin, A.K. Agarwal, C. Monder, M.I. New, P.C. White, The human gene for 11β-hydroxysteroid dehydrogenase. Structure, tissue distribution, and chromosomal localization, J. Biol. Chem. 266 (1991) 16653–16658.
[31] A.K. Agarwal, M.T. Tusie-Luna, C. Monder, P.C. White, Expression of 11β-hydroxysteroid dehydrogenase using recombinant vaccinia virus, Mol. Endocrinol. 4 (1990) 1827–1832.
[32] C. Monder, V. Lakshmi, Y. Miroff, Kinetic studies on rat liver 11β-hydroxysteroid dehydrogenase, Biochim. Biophys. Acta 1115 (1991) 23–29.
[33] C.S. Nobel, F. Dunas, L.B. Abrahmsen, Purification of full-length recombinant human and rat type 1 11β-hydroxysteroid dehydrogenases with retained oxidoreductase activities, Protein Exp. Purif. 26 (2002) 349–356.
[34] E.A. Walker, A.M. Clark, M. Hewison, J.P. Ride, P.M. Stewart, Functional expression, characterization, and purification of the catalytic domain of human 11β-hydroxysteroid dehydrogenase type 1, J. Biol. Chem. 276 (2001) 21343–21350.
[35] A. Blum, E. Maser, The critical role of the N-terminus of 11β-hydroxysteroid dehydrogenase type 1, as being encoded by exon 1, for enzyme stabilization and activity, Chem. Biol. Interact. 143–144 (2003) 469–480.
[36] N. Shafqat, B. Elleby, S. Svensson, J. Shafqat, H. Jornvall, L. Abrahmsen, U. Oppermann, Comparative enzymology of 11β-hydroxysteroid dehydrogenase type 1 from glucocorticoid resistant (Guinea pig) versus sensitive (human) species, J. Biol. Chem. 278 (2003) 2030–2035.
[37] D.J. Hosfield, Y. Wu, R.J. Skene, M. Hilgers, A. Jennings, G.P. Snell, K. Aertgeerts, Conformational flexibility in crystal structures of human 11β-hydroxysteroid dehydrogenase type I provide insights into glucocorticoid interconversion and enzyme regulation, J. Biol. Chem. 280 (2005) 4639–4648.
[38] D. Ogg, B. Elleby, C. Norstrom, K. Stefansson, L. Abrahmsen, U. Oppermann, S. Svensson, The crystal structure of guinea pig 11β-hydroxysteroid dehydrogenase type 1 provides a model for enzyme-lipid bilayer interactions, J. Biol. Chem. 280 (2005) 3789–3794.
[39] J. Zhang, T.D. Osslund, M.H. Plant, C.L. Clogston, R.E. Nybo, F. Xiong, J.M. Delaney, S.R. Jordan, Crystal structure of murine 11β-hydroxysteroid dehydrogenase 1: an important therapeutic target for diabetes, Biochemistry 44 (2005) 6948–6957.
[40] H.M. Berman, J. Westbrook, Z. Feng, G. Gililand, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, The protein data bank, Nucl. Acid Res. 28 (2000) 235–242.
[41] E. Maser, B. Volker, J. Friebertshauser, 11β-hydroxysteroid dehydrogenase type 1 from human liver: dimerization and enzyme cooperativity support its postulated role as glucocorticoid reductase, Biochemistry 41 (2002) 2459–2465.
[42] C. Huang, B. Wan, B. Gao, S. Hexige, L. Yu, Isolation and characterization of novel human short-chain dehydrogenase/reductase SCDR10B which is highly expressed in the brain and acts as hydroxysteroid dehydrogenase, Acta Biochim. Polonica 56 (2009) 279–289.
[43] C. Frick, A.G. Atanasov, P. Arnold, J. Ozols, A. Odermatt, Appropriate function of 11β-hydroxysteroid dehydrogenase type 1 in the endoplasmic reticulum lumen is dependent on its N-terminal region sharing similar topological determinants with 50-kDa esterase, J. Biol. Chem. 279 (2004) 31131–31138.
[44] A. Odermatt, P. Arnold, A. Stauffer, B.M. Frey, F.J. Frey, The N-terminal anchor sequences of 11β-hydroxysteroid dehydrogenases determine their orientation in the endoplasmic reticulum membrane, J. Biol. Chem. 274 (1999) 28762–28770.
[45] C. Filling, E. Nordling, J. Benach, K.D. Berndt, R. Ladenstein, H. Jornvall, U. Oppermann, Structural role of conserved Asn179 in the short-chain dehydrogenase/reductase scaffold, Biochem. Biophys. Res. Commun. 289 (2001) 712–717.
[46] A. Blum, H.J. Martin, E. Maser, Human 11β-hydroxysteroid dehydrogenase type 1 is enzymatically active in its nonglycosylated form, Biochem. Biophys. Res. Commun. 276 (2000) 428–434.
[47] S. Arampatzis, B. Kadereit, D. Schuster, Z. Balazs, R.A. Schweizer, F.J. Frey, T. Langer, A. Odermatt, Comparative enzymology of 11β-hydroxysteroid dehydrogenase type 1 from six species, J. Mol. Endocrinol. 35 (2005) 89–101.
[48] A.K. Agarwal, T. Mune, C. Monder, P.C. White, Mutations in putative glycosylation sites of rat 11β-hydroxysteroid dehydrogenase affect enzymatic activity, Biochim. Biophys. Acta 1248 (1995) 70–74.
[49] N. Tanaka, T. Nonaka, T. Tanabe, T. Yoshimoto, D. Tsuru, Y. Mitsui, Crystal structures of the binary and ternary complexes of 7α-hydroxysteroid dehydrogenase from *Escherichia coli*, Biochemistry 35 (1996) 7715–7730.
[50] C. Mazza, R. Breton, D. Housset, J.C. Fontecilla-Camps, Unusual charge stabilization of NADP+ in 17β-hydroxysteroid dehydrogenase, J. Biol. Chem. 273 (1998) 8145–8152.
[51] P. Arnold, S. Tam, L. Yan, M.E. Baker, F.J. Frey, A. Odermatt, Glutamate-115 renders specificity of human 11β-hydroxysteroid dehydrogenase type 2 for the cofactor NAD(+), Mol. Cell Endocrinol. 201 (2003) 177–187.
[52] B. Sahni-Arya, M.J. Flynn, L. Bergeron, M.E. Salyan, D.L. Pedicord, R. Golla, Z. Ma, H. Wang, R. Seethala, S.C. Wu, J.J. Li, A. Nayeem, C. Gates, L.G. Hamann, D.A. Gordon, Y. Blat, Cofactor-specific modulation of 11β-hydroxysteroid dehydrogenase 1 inhibitor potency, Biochim. Biophys. Acta 1774 (2007) 1184–1191.
[53] E. Maser, G. Bannenberg, The purification of 11β-hydroxysteroid dehydrogenase from mouse liver microsomes, J. Steroid Biochem. Mol. Biol. 48 (1994) 257–263.
[54] J. Condon, M.L. Ricketts, C.B. Whorwood, P.M. Stewart, Ontogeny and sexual dimorphic expression of mouse type 2 11β-hydroxysteroid dehydrogenase, Mol. Cell Endocrinol. 127 (1997) 121–128.
[55] R.L. Veech, L.V. Eggleston, H.A. Krebs, The redox state of free nicotinamide-adenine dinucleotide phosphate in the cytoplasm of rat liver, Biochem. J. 115 (1969) 609–619.
[56] D.H. Williamson, P. Lund, H.A. Krebs, The redox state of free nicotinamide-adenine dinucleotide in the cytoplasm and mitochondria of rat liver, Biochem. J. 103 (1967) 514–527.
[57] N. Pollak, M. Niere, M. Ziegler, NAD Kinase Levels Control the NADPH Concentration in Human Cells, J. Biol. Chem. 282 (2007) 33562–33571.
[58] A.A. Dzyakanchuk, Z. Balazs, L.G. Nashev, K.E. Amrein, A. Odermatt, 11β-Hydroxysteroid dehydrogenase 1 reductase activity is dependent on a high ratio of NADPH/NADP(+) and is stimulated by extracellular glucose, Mol. Cell Endocrinol. 301 (2009) 137–141.
[59] A.K. Agarwal, R.J. Auchus, Minireview: cellular redox state regulates hydroxysteroid dehydrogenase activity and intracellular hormone potency, Endocrinology 146 (2005) 2531–2538.
[60] A.G. Atanasov, L.G. Nashev, R.A. Schweizer, C. Frick, A. Odermatt, Hexose-6-phosphate dehydrogenase determines the reaction direction of 11β-hydroxysteroid dehydrogenase type 1 as an oxoreductase, FEBS Lett. 571 (2004) 129–133.
[61] G. Banhegyi, A. Benedetti, R. Fulceri, S. Senesi, Cooperativity between 11β-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase in the lumen of the endoplasmic reticulum, J. Biol. Chem. 279 (2004) 27017–27021.
[62] I.J. Bujalska, N. Draper, Z. Michailidou, J.W. Tomlinson, P.C. White, K.E. Chapman, E.A. Walker, P.M. Stewart, Hexose-6-phosphate dehydrogenase confers oxo-reductase activity upon 11β-hydroxysteroid dehydrogenase type 1, J. Mol. Endocrinol. 34 (2005) 675–684.
[63] G.G. Lavery, E.A. Walker, N. Draper, P. Jeyasuria, J. Marcos, C.H. Shackleton, K.L. Parker, P.C. White, P.M. Stewart, Hexose-6-phosphate dehydrogenase knock-out mice lack 11β-hydroxysteroid dehydrogenase type 1-mediated glucocorticoid generation, J. Biol. Chem. 281 (2006) 6546–6551.
[64] I.J. Bujalska, E.A. Walker, M. Hewison, P.M. Stewart, A switch in dehydrogenase to reductase activity of 11β-hydroxysteroid dehydrogenase type 1 upon differentiation of human omental adipose stromal cells, J. Clin. Endocrinol. Metab. 87 (2002) 1205–1210.
[65] N. Draper, E.A. Walker, I.J. Bujalska, J.W. Tomlinson, S.M. Chalder, W. Arlt, G.G. Lavery, O. Bedendo, D.W. Ray, I. Laing, E. Malunowicz, P.C. White, M. Hewison, P.J. Mason, J.M. Connell, C.H. Shackleton, P.M. Stewart, Mutations in the genes encoding 11β-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase interact to cause cortisone reductase deficiency, Nat. Genet. 34 (2003) 434–439.
[66] R.A. De Sousa Peixoto, S. Turban, J.H. Battle, K.E. Chapman, J.R. Seckl, N.M. Morton, Preadipocyte 11β-hydroxysteroid dehydrogenase type 1 is a keto-reductase and contributes to diet-induced visceral obesity in vivo, Endocrinology 149 (2008) 1861–1868.
[67] P. Marcolongo, S. Senesi, B. Gava, R. Fulceri, V. Sorrentino, E. Margittai, B. Lizak, M. Csala, G. Banhegyi, A. Benedetti, Metyrapone prevents cortisone-induced preadipocyte differentiation by depleting luminal NADPH of the endoplasmic reticulum, Biochem. Pharmacol. 76 (2008) 382–390.
[68] M.S. Cooper, I. Bujalska, E. Rabbitt, E.A. Walker, R. Bland, M.C. Sheppard, M. Hewison, P.M. Stewart, Modulation of 11β-hydroxysteroid dehydrogenase isozymes by proinflammatory cytokines in osteoblasts: an autocrine switch from glucocorticoid inactivation to activation, J. Bone Miner. Res. 16 (2001) 1037–1044.
[69] E.P. Gomez-Sanchez, D.G. Romero, A.F. de Rodriguez, M.P. Warden, Z. Krozowski, C.E. Gomez-Sanchez, Hexose-6-phosphate dehydrogenase and 11β-hydroxysteroid dehydrogenase-1 tissue distribution in the rat, Endocrinology 149 (2008) 525–533.
[70] P.J. Mason, D. Stevens, A. Diez, S.W. Knight, D.A. Scopes, T.J. Vulliamy, Human hexose-6-phosphate dehydrogenase (glucose 1-dehydrogenase) encoded at 1p36: coding sequence and expression, Blood Cells Mol. Dis. 25 (1999) 30–37.
[71] J. Ozols, Isolation and the complete amino acid sequence of lumenal endoplasmic reticulum glucose-6-phosphate dehydrogenase, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 5302–5306.
[72] G. Banhegyi, M. Csala, A. Benedetti, Hexose-6-phosphate dehydrogenase: linking endocrinology and metabolism in the endoplasmic reticulum, J. Mol. Endocrinol. 42 (2009) 283–289.
[73] S. Piccirella, I. Czegle, B. Lizak, E. Margittai, S. Senesi, E. Papp, M. Csala, R. Fulceri, P. Csermely, J. Mandl, A. Benedetti, G. Banhegyi, Uncoupled redox systems in the lumen of the endoplasmic reticulum. Pyridine nucleotides stay reduced in an oxidative environment, J. Biol. Chem. 281 (2006) 4671–4677.
[74] S.E. Ferguson, Z. Pallikaros, A.E. Michael, B.A. Cooke, The effects of different culture media, glucose, pyridine nucleotides and adenosine on the activity of 11β-hydroxysteroid dehydrogenase in rat Leydig cells, Mol. Cell Endocrinol. 158 (1999) 37–44.
[75] R.S. Ge, M.P. Hardy, Initial predominance of the oxidative activity of type I 11β-hydroxysteroid dehydrogenase in primary rat Leydig cells and transfected cell lines, J. Androl. 21 (2000) 303–310.
[76] Y.L. Zhang, X. Zhong, Z. Gjoka, Y. Li, W. Stochaj, M. Stahl, R. Kriz, J.F. Tobin, D. Erbe, V. Suri, H6PDH interacts directly with 11β-HSD1: implications for determining the directionality of glucocorticoid catalysis, Arch. Biochem. Biophys. 483 (2009) 45–54.

[77] G.G. Lavery, D. Hauton, K.N. Hewitt, S.M. Brice, M. Sherlock, E.A. Walker, P.M. Stewart, Hypoglycemia with enhanced hepatic glycogen synthesis in recombinant mice lacking hexose-6-phosphate dehydrogenase, Endocrinology 148 (2007) 6100–6106.
[78] G.G. Lavery, E.A. Walker, N. Turan, D. Rogoff, J.W. Ryder, J.M. Shelton, J.A. Richardson, F. Falciani, P.C. White, P.M. Stewart, K.L. Parker, D.R. McMillan, Deletion of hexose-6-phosphate dehydrogenase activates the unfolded protein response pathway and induces skeletal myopathy, J. Biol. Chem. 283 (2008) 8453–8461.
[79] I.J. Bujalska, K.N. Hewitt, D. Hauton, G.G. Lavery, J.W. Tomlinson, E.A. Walker, P.M. Stewart, Lack of hexose-6-phosphate dehydrogenase impairs lipid mobilization from mouse adipose tissue, Endocrinology 149 (2008) 2584–2591.
[80] A.G. Atanasov, L.G. Nashev, L. Gelman, B. Legeza, R. Sack, R. Portmann, A. Odermatt, Direct protein–protein interaction of 11β-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase in the endoplasmic reticulum lumen, Biochim. Biophys. Acta 1783 (2008) 1536–1543.
[81] B.E. Murphy, Demonstration of novel compounds in human fetal tissues and a consideration of their possible role in parturition, Am. J. Obstet. Gynecol. 139 (1981) 353–358.
[82] G.W. Souness, S.A. Latif, J.L. Laurenzo, D.J. Morris, 11α- and 11β-hydroxyprogesterone, potent inhibitors of 11β-hydroxysteroid dehydrogenase (isoforms 1 and 2), confer marked mineralocorticoid activity on corticosterone in the ADX rat, Endocrinology 136 (1995) 1809–1812.
[83] D.J. Morris, G.W. Souness, Endogenous 11β-hydroxysteroid dehydrogenase inhibitors and their role in glucocorticoid Na+ retention and hypertension, Endocr. Res. 22 (1996) 793–801.
[84] A.S. Brem, R.B. Bina, T. King, D.J. Morris, 11β-OH-progesterone affects vascular glucocorticoid metabolism and contractile response, Hypertension 30 (1997) 449–454.
[85] M.D. Galigniana, G.P. Vicent, G. Burton, C.P. Lantos, Features of the shuttle pair 11 beta-hydroxyprogesterone-11-ketoprogesterone, Steroids 62 (1997) 358–364.
[86] G. Burton, M. Galigniana, S. De Lavallaz, A.L. Brachet-Cota, E.M. Sproviero, A.A. Ghini, C.P. Lantos, M.C. Damasco, Sodium-retaining activity of some natural and synthetic 21-deoxysteroids, Mol. Pharmacol. 47 (1995) 535–543.
[87] J. Honour, The possible involvement of intestinal bacteria in steroidal hypertension, Endocrinology 110 (1982) 285–287.
[88] V.D. Bokkenheuser, J. Winter, J.W. Honour, C.H. Shackleton, Reduction of aldosterone by anaerobic bacteria: origin of urinary 21-deoxy metabolites in man, J. Steroid Biochem. Mol. Biol. 11 (1979) 1145–1149.
[89] R.B. Heap, M. Holzbauer, H.M. Newport, Adrenal secretion rates of C-19 and C-21 steroids before and after hypophysectomy in the pig and the dog, J. Endocrinol. 36 (1966) 159–176.
[90] P.J. Burton, B.J. Waddell, Dual function of 11β-hydroxysteroid dehydrogenase in placenta: modulating placental glucocorticoid passage and local steroid action, Biol. Reprod. 60 (1999) 234–240.
[91] F.M. Ganis, L.R. Axelrod, L.L. Miller, The metabolism of hydrocortisone by kidney tissue in vitro, J. Biol. Chem. 218 (1956) 841–848.
[92] L. Kornel, Colocalization of 11β-hydroxysteroid dehydrogenase and mineralocorticoid receptors in cultured vascular smooth muscle cells, Am. J. Hypertens. 7 (1994) 100–103.
[93] D.J. Morris, S.A. Latif, M.P. Hardy, A.S. Brem, Endogenous inhibitors (GALFs) of 11β-hydroxysteroid dehydrogenase isoforms 1 and 2: derivatives of adrenally produced corticosterone and cortisol, J. Steroid Biochem. Mol. Biol. 104 (2007) 161–168.
[94] S.A. Latif, H.A. Pardo, M.P. Hardy, D.J. Morris, Endogenous selective inhibitors of 11β-hydroxysteroid dehydrogenase isoforms 1 and 2 of adrenal origin, Mol. Cell Endocrinol. 243 (2005) 43–50.
[95] G.M. Wang, R.S. Ge, S.A. Latif, D.J. Morris, M.P. Hardy, Expression of 11β-hydroxylase in rat Leydig cells, Endocrinology 143 (2002) 621–626.
[96] C.M. Leckie, L.A. Welberg, J.R. Seckl, 11β-hydroxysteroid dehydrogenase is a predominant reductase in intact rat Leydig cells, J. Endocrinol. 159 (1998) 233–238.
[97] H.B. Gao, R.S. Ge, V. Lakshmi, A. Marandici, M.P. Hardy, Hormonal regulation of oxidative and reductive activities of 11β-hydroxysteroid dehydrogenase in rat Leydig cells, Endocrinology 138 (1997) 156–161.
[98] H.B. Gao, L.X. Shan, C. Monder, M.P. Hardy, Suppression of endogenous corticosterone levels in vivo increases the steroidogenic capacity of purified rat Leydig cells in vitro, Endocrinology 137 (1996) 1714–1718.
[99] R. Claus, M. Lacorn, H. Welter, O. Lekhkota, N. Messe, A. Wagner, M. Bergmann, Expression of 11β-hydroxysteroid-dehydrogenase 2 in Sertoli cells of boar testes, Mol. Cell Endocrinol. 272 (2007) 86–92.
[100] Y. Honda, S. Ohno, S. Nakajin, Leydig cells from neonatal pig testis abundantly express 11β-hydroxysteroid dehydrogenase (11β-HSD) type 2 and effectively inactivate cortisol to cortisone, J. Steroid Biochem. Mol. Biol. 108 (2008) 91–101.
[101] R.S. Ge, Q. Dong, C.M. Sottas, S.A. Latif, D.J. Morris, M.P. Hardy, Stimulation of testosterone production in rat Leydig cells by aldosterone is mineralocorticoid receptor mediated, Mol. Cell Endocrinol. 243 (2005) 35–42.
[102] G.X. Hu, H. Lin, C.M. Sottas, D.J. Morris, M.P. Hardy, R.S. Ge, Inhibition of 11β-hydroxysteroid dehydrogenase enzymatic activities by glycyrrhetinic acid in vivo supports direct glucocorticoid-mediated suppression of steroidogenesis in Leydig cells, J. Androl. 29 (2008) 345–351.
[103] H.B. Gao, M.H. Tong, Y.Q. Hu, Q.S. Guo, R. Ge, M.P. Hardy, Glucocorticoid induces apoptosis in rat Leydig cells, Endocrinology 143 (2002) 130–138.
[104] C. Monder, Y. Miroff, A. Marandici, M.P. Hardy, 11β-Hydroxysteroid dehydrogenase alleviates glucocorticoid-mediated inhibition of steroidogenesis in rat Leydig cells, Endocrinology 134 (1994) 1199–1204.
[105] A.G. Rebuffat, S. Tam, A.R. Nawrocki, M.E. Baker, B.M. Frey, F.J. Frey, A. Odermatt, The 11-ketosteroid 11-ketodexamethasone is a glucocorticoid receptor agonist, Mol. Cell Endocrinol. 214 (2004) 27–37.
[106] R. Hampl, R. Morfin, L. Starka, Minireview 7-hydroxylated derivatives of dehydroepiandrosterone: what are they good for? Endocr. Regul. 31 (1997) 211–218.
[107] J.L. Yau, S. Rasmuson, R. Andrew, M. Graham, J. Noble, T. Olsson, E. Fuchs, R. Lathe, J.R. Seckl, Dehydroepiandrosterone 7-hydroxylase CYP7B: predominant expression in primate hippocampus and reduced expression in Alzheimer's disease, Neuroscience 121 (2003) 307–314.
[108] 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.
[109] A.R. Stiles, J.G. McDonald, D.R. Bauman, D.W. Russell, CYP7B1: one cytochrome P450, two human genetic diseases, and multiple physiological functions, J. Biol. Chem. 284 (2009) 28485–28489.
[110] J. Shi, S. Schulze, H.A. Lardy, The effect of 7-oxo-DHEA acetate on memory in young and old C57BL/6 mice, Steroids 65 (2000) 124–129.
[111] A.K. Pringle, W. Schmidt, J.K. Deans, E. Wulfert, K.G. Reymann, L.E. Sundstrom, 7-Hydroxylated epiandrosterone (7-OH-EPIA) reduces ischaemia-induced neuronal damage both in vivo and in vitro, Eur. J. Neurosci. 18 (2003) 117–124.
[112] S.B. Kim, M. Hill, Y.T. Kwak, R. Hampl, D.H. Jo, R. Morfin, Neurosteroids: cerebrospinal fluid levels for Alzheimer's disease and vascular dementia diagnostics, J. Clin. Endocrinol. Metab. 88 (2003) 5199–5206.
[113] S. Weill-Engerer, J.P. David, V. Sazdovitch, P. Liere, M. Schumacher, A. Delacourte, E.E. Baulieu, Y. Akwa, In vitro metabolism of dehydroepiandrosterone (DHEA) to 7alpha-hydroxy-DHEA and Delta5-androstene-3beta, 17beta-diol in specific regions of the aging brain from Alzheimer's and non-demented patients, Brain Res. 969 (2003) 117–125.
[114] R. Morfin, G. Courchay, Pregnenolone and dehydroepiandrosterone as precursors of native 7-hydroxylated metabolites which increase the immune response in mice, J. Steroid Biochem. Mol. Biol. 50 (1994) 91–100.
[115] J. Dulos, A.H. Boots, DHEA metabolism in arthritis: a role for the p450 enzyme Cyp7b at the immune-endocrine crossroad, Ann. N.Y. Acad. Sci. 1069 (2006) 401–413.
[116] H. Lardy, B. Partridge, N. Kneer, Y. Wei, Ergosteroids: induction of thermogenic enzymes in liver of rats treated with steroids derived from dehydroepiandrosterone, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 6617–6619.
[117] G. Ihler, H. Chami-Stemmann, 7-oxo-DHEA and Raynaud's phenomenon, Med. Hypotheses 60 (2003) 391–397.
[118] J. Sulcova, M. Hill, Z. Masek, R. Ceska, A. Novacek, R. Hampl, L. Starka, Effects of transdermal application of 7-oxo-DHEA on the levels of steroid hormones, gonadotropins and lipids in healthy men, Physiol. Res. 50 (2001) 9–18.
[119] S. Chalbot, R. Morfin, Human liver S9 fractions: metabolism of dehydroepiandrosterone, epiandrosterone, and related 7-hydroxylated derivatives, Drug Metab. Dispos. 33 (2005) 563–569.
[120] K.K. Miller, J. Cai, S.L. Ripp, W.M. Pierce Jr., T.H. Rushmore, R.A. Prough, Stereo- and regioselectivity account for the diversity of dehydroepiandrosterone (DHEA) metabolites produced by liver microsomal cytochromes P450, Drug Metab. Dispos. 32 (2004) 305–313.
[121] R. Lathe, Steroid and sterol 7-hydroxylation: ancient pathways, Steroids 67 (2002) 967–977.
[122] B. Robinzon, K.K. Michael, S.L. Ripp, S.J. Winters, R.A. Prough, Glucocorticoids inhibit interconversion of 7-hydroxy and 7-oxo metabolites of dehydroepiandrosterone: a role for 11β-hydroxysteroid dehydrogenases? Arch. Biochem. Biophys. 412 (2003) 251–258.
[123] B. Robinzon, R.A. Prough, Interactions between dehydroepiandrosterone and glucocorticoid metabolism in pig kidney: nuclear and microsomal 11β-hydroxysteroid dehydrogenases, Arch. Biochem. Biophys. 442 (2005) 33–40.
[124] M. Hult, B. Elleby, N. Shafqat, S. Svensson, A. Rane, H. Jornvall, L. Abrahmsen, U. Oppermann, Human and rodent type 1 11β-hydroxysteroid dehydrogenases are 7β-hydroxycholesterol dehydrogenases involved in oxysterol metabolism, Cell Mol. Life Sci. 61 (2004) 992–999.
[125] R.A. Schweizer, M. Zurcher, Z. Balazs, B. Dick, A. Odermatt, Rapid hepatic metabolism of 7-ketocholesterol by 11β-hydroxysteroid dehydrogenase type 1: species-specific differences between the rat, human, and hamster enzyme, J. Biol. Chem. 279 (2004) 18415–18424.
[126] L.G. Nashev, C. Chandsawangbhuwana, Z. Balazs, A.G. Atanasov, B. Dick, F.J. Frey, M.E. Baker, A. Odermatt, Hexose-6-phosphate Dehydrogenase Modulates 11β-Hydroxysteroid Dehydrogenase Type 1-Dependent Metabolism of 7-keto- and 7β-hydroxy-neurosteroids, PLoS One 2 (2007) e561.
[127] C. Muller, D. Pompon, P. Urban, R. Morfin, Inter-conversion of 7alpha- and 7beta-hydroxy-dehydroepiandrosterone by the human 11β-hydroxysteroid dehydrogenase type 1, J. Steroid Biochem. Mol. Biol. 99 (2006) 215–222.
[128] 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.
[129] O. Hennebert, C. Pernelle, C. Ferroud, R. Morfin, 7alpha- and 7beta-hydroxy-epiandrosterone as substrates and inhibitors for the human 11β-hydroxysteroid dehydrogenase type 1, J. Steroid Biochem. Mol. Biol. 105 (2007) 159–165.

[130] Z. Balazs, L.G. Nashev, C. Chandsawangbhuwana, M.E. Baker, A. Odermatt, Hexose-6-phosphate dehydrogenase modulates the effect of inhibitors and alternative substrates of 11β-hydroxysteroid dehydrogenase 1, Mol. Cell Endocrinol. 301 (2009) 117–122.
[131] W. Song, J. Chen, W.L. Dean, R.N. Redinger, R.A. Prough, Purification and characterization of hamster liver microsomal 7alpha-hydroxycholesterol dehydrogenase. Similarity to type I 11β-hydroxysteroid dehydrogenase, J. Biol. Chem. 273 (1998) 16223–16228.
[132] G.J. Schroepfer Jr., Oxysterols: modulators of cholesterol metabolism and other processes, Physiol. Rev. 80 (2000) 361–554.
[133] I. Bjorkhem, O. Andersson, U. Diczfalusy, B. Sevastik, R.J. Xiu, C. Duan, E. Lund, Atherosclerosis and sterol 27-hydroxylase: evidence for a role of this enzyme in elimination of cholesterol from human macrophages, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 8592–8596.
[134] A.J. Brown, S.L. Leong, R.T. Dean, W. Jessup, 7-Hydroperoxycholesterol and its products in oxidized low density lipoprotein and human atherosclerotic plaque, J. Lipid Res. 38 (1997) 1730–1745.
[135] M. Crisby, J. Nilsson, V. Kostulas, I. Bjorkhem, U. Diczfalusy, Localization of sterol 27-hydroxylase immuno-reactivity in human atherosclerotic plaques, Biochim. Biophys. Acta 1344 (1997) 278–285.
[136] L.M. Hulten, H. Lindmark, U. Diczfalusy, I. Bjorkhem, M. Ottosson, Y. Liu, G. Bondjers, O. Wiklund, Oxysterols present in atherosclerotic tissue decrease the expression of lipoprotein lipase messenger RNA in human monocyte-derived macrophages, J. Clin. Invest. 97 (1996) 461–468.
[137] T. Hitsumoto, M. Takahashi, T. Iizuka, K. Shirai, Clinical significance of serum 7-ketocholesterol concentrations in the progression of coronary atherosclerosis, J. Atheroscler. Thromb. Vasc. 16 (2009) 363–370.
[138] A.J. Brown, W. Jessup, Oxysterols and atherosclerosis, Atherosclerosis 142 (1999) 1–28.
[139] A. Hermanowski-Vosatka, J.M. Balkovec, K. Cheng, H.Y. Chen, M. Hernandez, G.C. Koo, C.B. Le Grand, Z. Li, J.M. Metzger, S.S. Mundt, H. Noonan, C.N. Nunes, S.H. Olson, B. Pikounis, N. Ren, N. Robertson, J.M. Schaeffer, K. Shah, M.S. Springer, A.M. Strack, M. Strowski, K. Wu, T. Wu, J. Xiao, B.B. Zhang, S.D. Wright, R. Thieringer, 11β-HSD1 inhibition ameliorates metabolic syndrome and prevents progression of atherosclerosis in mice, J. Exp. Med. 202 (2005) 517–527.
[140] A.M. Nuotio-Antar, D.L. Hachey, A.H. Hasty, Carbenoxolone treatment attenuates symptoms of metabolic syndrome and atherogenesis in obese, hyperlipidemic mice, Am. J. Physiol. 293 (2007) E1517–1528.
[141] N.M. Morton, M.C. Holmes, C. Fievet, B. Staels, A. Tailleux, J.J. Mullins, J.R. Seckl, Improved lipid and lipoprotein profile, hepatic insulin sensitivity, and glucose tolerance in 11β-hydroxysteroid dehydrogenase type 1 null mice, J. Biol. Chem. 276 (2001) 41293–41300.
[142] M. Wamil, R. Andrew, K.E. Chapman, J. Street, N.M. Morton, J.R. Seckl, 7-oxysterols modulate glucocorticoid activity in adipocytes through competition for 11β-hydroxysteroid dehydrogenase type, Endocrinology 149 (2008) 5909–5918.
[143] P.W. Hadoke, J. Iqbal, B.R. Walker, Therapeutic manipulation of glucocorticoid metabolism in cardiovascular disease, Br. J. Pharmacol. 156 (2009) 689–712.
[144] R.L. Felsted, N.R. Bachur, Mammalian carbonyl reductases, Drug Metab. Rev. 11 (1980) 1–60.
[145] U. Oppermann, Carbonyl reductases: the complex relationships of mammalian carbonyl- and quinone-reducing enzymes and their role in physiology, Ann. Rev. Pharmacol. Toxicol. 47 (2007) 293–322.
[146] F. Hoffmann, E. Maser, Carbonyl reductases and pluripotent hydroxysteroid dehydrogenases of the short-chain dehydrogenase/reductase superfamily, Drug Metab. Rev. 39 (2007) 87–144.
[147] E. Maser, K.J. Netter, Purification and properties of a metyrapone-reducing enzyme from mouse liver microsomes–this ketone is reduced by an aldehyde reductase, Biochem. Pharmacol. 38 (1989) 3049–3054.
[148] E. Maser, T. Gebel, K.J. Netter, Carbonyl reduction of metyrapone in human liver, Biochem. Pharmacol. 42 (Suppl.) (1991) S93–98.
[149] U.C. Oppermann, K.J. Netter, E. Maser, Cloning and primary structure of murine 11β-hydroxysteroid dehydrogenase/microsomal carbonyl reductase, Eur. J. Biochem. 227 (1995) 202–208.
[150] E. Maser, G. Bannenberg, 11β-hydroxysteroid dehydrogenase mediates reductive metabolism of xenobiotic carbonyl compounds, Biochem. Pharmacol. 47 (1994) 1805–1812.
[151] R. Sampath-Kumar, M. Yu, M.W. Khalil, K. Yang, Metyrapone is a competitive inhibitor of 11β-hydroxysteroid dehydrogenase type 1 reductase, J. Steroid Biochem. Mol. Biol. 62 (1997) 195–199.
[152] G. Bannenberg, H.J. Martin, I. Belai, E. Maser, 11β-Hydroxysteroid dehydrogenase type 1: tissue-specific expression and reductive metabolism of some anti-insect agent azole analogues of metyrapone, Chem. Biol. Interact. 143–144 (2003) 449–457.
[153] U.C. Oppermann, E. Maser, S.A. Mangoura, K.J. Netter, Heterogeneity of carbonyl reduction in subcellular fractions and different organs in rodents, Biochem. Pharmacol. 42 (Suppl.) (1991) S189–195.
[154] M. Hult, C.S. Nobel, L. Abrahmsen, D.A. Nicoll-Griffith, H. Jornvall, U.C. Oppermann, Novel enzymological profiles of human 11β-hydroxysteroid dehydrogenase type 1, Chem. Biol. Interact. 130–132 (2001) 805–814.
[155] V. Wsol, B. Szotakova, L. Skalova, E. Maser, Stereochemical aspects of carbonyl reduction of the original anticancer drug oracin by mouse liver microsomes and purified 11β-hydroxysteroid dehydrogenase type 1, Chem. Biol. Interact. 143–144 (2003) 459–468.
[156] B. Szotakova, L. Skalova, V. Wsol, E. Kvasnieckova, Reduction of the potential anticancer drug oracin in the rat liver in-vitro, J. Pharm. Pharmacol. 52 (2000) 495–500.
[157] V. Wsol, B. Szotakova, H.J. Martin, E. Maser, Aldo-keto reductases (AKR) from the AKR1C subfamily catalyze the carbonyl reduction of the novel anticancer drug oracin in man, Toxicology 238 (2007) 111–118.
[158] H.J. Martin, U. Breyer-Pfaff, V. Wsol, S. Venz, S. Block, E. Maser, Purification and characterization of akr1b10 from human liver: role in carbonyl reduction of xenobiotics, Drug Metab. Dispos. 34 (2006) 464–470.
[159] E. Maser, Significance of reductases in the detoxification of the tobacco-specific carcinogen NNK, Trends Pharmacol. Sci. 25 (2004) 235–237.
[160] E. Maser, J. Friebertshauser, B. Volker, Purification, characterization and NNK carbonyl reductase activities of 11β-hydroxysteroid dehydrogenase type 1 from human liver: enzyme cooperativity and significance in the detoxification of a tobacco-derived carcinogen, Chem. Biol. Interact. 143–144 (2003) 435–448.
[161] S.S. Hecht, Biochemistry, biology, and carcinogenicity of tobacco-specific N-nitrosamines, Chem. Res. Toxicol. 11 (1998) 559–603.
[162] U. Breyer-Pfaff, H.J. Martin, M. Ernst, E. Maser, Enantioselectivity of carbonyl reduction of 4-methylnitrosamino-1-(3-pyridyl)-1-butanone by tissue fractions from human and rat and by enzymes isolated from human liver, Drug Metab. Dispos. 32 (2004) 915–922.
[163] J.M. Jez, M.J. Bennett, B.P. Schlegel, M. Lewis, T.M. Penning, Comparative anatomy of the aldo-keto reductase superfamily, Biochem. J. 326 (Pt 3) (1997) 625–636.
[164] M. Soldan, G. Nagel, M. Losekam, M. Ernst, E. Maser, Interindividual variability in the expression and NNK carbonyl reductase activity of 11β-hydroxysteroid dehydrogenase 1 in human lung, Cancer Lett. 145 (1999) 49–56.
[165] S.A. Ahrendt, J.T. Chow, S.C. Yang, L. Wu, M.J. Zhang, J. Jen, D. Sidransky, Alcohol consumption and cigarette smoking increase the frequency of p53 mutations in non-small cell lung cancer, Cancer Res. 60 (2000) 3155–3159.
[166] R. Valentino, A.P. Tommaselli, S. Savastano, P.M. Stewart, M.R. Ghiggi, F. Galletti, P. Mariniello, G. Lombardi, C.R. Edwards, Alcohol inhibits 11β-hydroxysteroid dehydrogenase activity in rat kidney and liver, Horm. Res. 43 (1995) 176–180.
[167] J.F. Kenneke, C.S. Mazur, S.E. Ritger, T.J. Sack, Mechanistic investigation of the noncytochrome P450-mediated metabolism of triadimefon to triadimenol in hepatic microsomes, Chem. Res. Toxicol. 21 (2008) 1997–2004.
[168] W.O. Ward, D.A. Delker, S.D. Hester, S.F. Thai, D.C. Wolf, J.W. Allen, S. Nesnow, Transcriptional profiles in liver from mice treated with hepatotumorigenic and nonhepatotumorigenic triazole conazole fungicides: propiconazole, triadimefon, and myclobutanil, Toxicol. Pathol. 34 (2006) 863–878.
[169] C.S. Mazur, J.F. Kenneke, M.R. Goldsmith, C. Brown, Contrasting influence of NADPH and a NADPH-regenerating system on the metabolism of carbonyl-containing compounds in hepatic microsomes, Drug Metabol. Dispos. 37 (2009) 1801–1805.
[170] E. Maser, E. Richter, J. Friebertshauser, The identification of 11β-hydroxysteroid dehydrogenase as carbonyl reductase of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, Eur. J. Biochem./FEBS 238 (1996) 484–489.
[171] E. Maser, V. Wsol, H.J. Martin, 11β-hydroxysteroid dehydrogenase type 1: purification from human liver and characterization as carbonyl reductase of xenobiotics, Mol. Cell Endocrinol. 248 (2006) 34–37.
[172] V. Wsol, B. Szotakova, L. Skalova, E. Maser, The novel anticancer drug oracin: different stereospecificity and cooperativity for carbonyl reduction by purified human liver 11β-hydroxysteroid dehydrogenase type 1, Toxicology 197 (2004) 253–261.
[173] E. Maser, 11β-hydroxysteroid dehydrogenase responsible for carbonyl reduction of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in mouse lung microsomes, Cancer Res. 58 (1998) 2996–3003.