

display positive staining for the fibroblast marker FSP-1 in late stages of fibrosis, indicating possible transdifferentiation. Nadasdy and colleagues recently described the occurrence of interstitial cells with positivity for low-molecular-weight cytokeratin in sections from human end-stage renal disease [13]. Interestingly these cells were not atrophic but displayed high proliferative activity. Epithelial-mesenchymal transdifferentiation is known to occur during development but can also be induced in differentiated organs such as the thyroid [8]. Furthermore, loss of the basement membrane is frequently observed in end-stage renal failure and may facilitate the change in the state of differentiation, as studies in other epithelial cell systems indicate [8]. Thus the possibility of transdifferentiation of epithelial cells into fibroblasts in the mostly mesenchymally derived kidney is intriguing.

Conclusion

Renal fibroblasts are not simply undifferentiated mesenchymal cells but have to be regarded as differentiated interstitial cells. Active, matrix producing (myo)fibroblasts are in large part derived from a subpopulation of resident interstitial cells. In addition, however, they are possibly generated by the transdifferentiation of other cellular elements including tubular epithelial cells. Nevertheless, further studies are needed to determine if seemingly transdifferentiated epithelial cells are in fact participating in the production of extracellular matrix as they may do *in vitro*.

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11 β -hydroxysteroid dehydrogenase—why is it important for the nephrologist?

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Introduction

Adrenal steroids play a certain role in the genesis of hypertension. Apart from hyperaldosteronism and the rare adrenogenital syndrome, some novel findings focused attention on 11 β -hydroxysteroid dehydrogenase (11 β -OHSD). Abnormal activity of this enzyme not only accounts for some cases of congenital mineralocorticoid type of hypertension, but is also involved

in hypertension provoked by liquorice and other xenobiotics, and might even be implicated in hypertension in renal failure. The following comment is intended to give a brief summary of the current state of the art for the non-expert in this field.

Characteristics of 11 β -OHSD

11 β -OHSD is the enzyme accounting for the conversion of endogenous cortisol to cortisone or exogenous prednisolone to prednisone (Figure 1). Both cortisone and prednisone exhibit hardly any glucocorticoid

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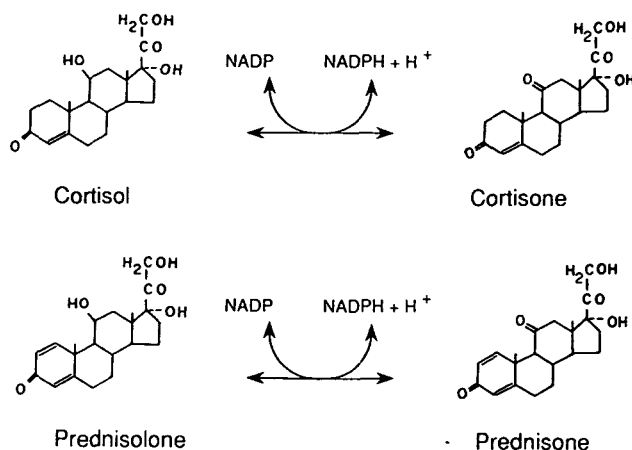


Fig. 1. Interconversion of cortisol to cortisone by 11 β -OHSD in the presence of NADP. The reaction is reversible. According to the structure homology between cortisol and prednisolone (one additional double bond in the A ring), it is assumed that the same mechanism occurs for the conversion of prednisolone to prednisone [38].

activity because they have negligible affinity to glucocorticoid receptors, whereas cortisol and prednisolone bind to glucocorticoid receptors and are thus biologically active glucocorticoids [1,2].

For a long time there was debate on whether one enzyme was catalysing the reaction in both directions or whether there was one enzyme catalysing the oxidation and another catalysing the reduction. In 1988, Monder purified a protein from rat liver that catalysed only oxidation [3]. This protein was used to find the corresponding cDNA [4]. When this cDNA was expressed in a toad-bladder cell line, it catalysed the reduction only [5]. When we expressed the same cDNA of 11 β -OHSD in COS-1 cells, which are devoid of spontaneous 11 β -OHSD activity, we found that the cDNA was encoding for a protein that could catalyse both reactions in the presence of appropriate cofactors [6]. The rate of oxidation in these cell extracts was higher than the rate of reduction [6]. In 1991 White and co-workers found on the basis of sequence homology with rat 11 β -OHSD a human NADP-dependent 11 β -OHSD with similar biological features to that found in rats [7].

The cDNA of 11 β -OHSD cloned by Monder and collaborators encodes for a 34-kDa NADP-dependent enzyme named now 11 β -OHSD₁ [4, 8]. In 1991 Krozowski found a short form of 11 β -OHSD₁ in the kidney which was missing the first 26 amino acids [9,10]. The biological relevance of this fragment is unknown since in the hands of the authors and ourselves using the expression system of COS-1 cells, no 11 β -OHSD activity was found. In an attempt to find a new 11 β -OHSD by expression cloning, Naray *et al.* injected mRNA from kidney collecting ducts cells into oocytes and described NAD-dependent 11 β -OHSD activity: the corresponding enzyme has not been sequenced until now [11]. In 1994 Krozowski found by means of expression cloning in CHOP-4 cells a

protein with 11 β -OHSD activity named 11 β -OHSD₂ [12]. For that purpose he used a human kidney cDNA library [12]. The affinity of 11 β -OHSD₂ for cortisol is in the nM range [12], whereas that of 11 β -OHSD₁ is in the μ M range [13]. Since cortisol and corticosterone concentrations in mammals are under physiological conditions in the nanomolar and not in the micromolar range, it appears that 11 β -OHSD₂ rather than 11 β -OHSD₁ is suitable for regulating steroids in the low physiological concentration range.

The intracellular localization of 11 β -OHSD₁ but not 11 β -OHSD₂ has been investigated. 11 β -OHSD₁ is mainly located in the microsomes [14]; however, there is also some evidence that it is present in the nuclei [15]. 11 β -OHSD activity has been found in most tissues [16]. The activity varies from organ to organ more than 40-fold, as we have demonstrated *in vivo* by assessing the ratios of prednisolone/prednisone in different organs from rats [17].

11 β -OHSD provides mineralocorticoid specificity

In-vitro studies with cloned mineralocorticoid receptors demonstrated that the mineralocorticoid receptor cannot distinguish between cortisol, corticosterone and aldosterone [18]. Cortisol circulates in a 100-fold molar excess to aldosterone *in vivo*. Thus cortisol rather than aldosterone should activate mineralocorticoid receptors *in vivo*, but this is not the case, as it is well established that *in vivo* only aldosterone is a potent mineralocorticoid hormone. Since mineralocorticoid-specific effects of aldosterone cannot be explained by receptor specificity, another mechanism has been proposed by Funder and co-workers [18] and Edwards and co-workers [19]. These authors hypothesized that 11 β -OH corticosteroids such as cortisol or corticosterone are inactivated locally into cortisone and dehydrocorticosterone by 11 β -OHSD, an enzyme which cannot inactivate aldosterone because the 11 β -OH group of aldosterone is protected by a covalent hemiacetal bond between C₁₁ and C₁₈ of the aldosterone molecule. By this mechanism only aldosterone has access to the mineralocorticoid receptor. This theory is supported by the following findings.

First, congenital deficiency of 11 β -OHSD causes a mineralocorticoid type of hypertension (apparent mineralocorticoid excess) with low aldosterone and low renin that can be cured by replacing the endogenous cortisol by glucocorticoids without an 11 β -hydroxy group [20].

Second, the inhibition of 11 β -OHSD *in vivo* by xenobiotics leads to a low renin, low aldosterone type of hypertension. This kind of hypertension has been observed in subjects taking carbenoxolone, an oral antacid agent, gossypol, a male contraceptive agent which could not be marketed due to the side effects, glycyrrhetic acid, the ingredient of liquorice and possibly various other less well defined endo- and xenobiotics [6,21–25].

Practically, the most relevant inhibitor of 11 β -OHSD

is certainly glycyrrhetic acid. This is a steroid molecule that has been said in many textbooks to exhibit mineralocorticoid activity by binding to the mineralocorticoid receptor. However careful *in-vitro* studies revealed that glycyrrhetic acid has no affinity for this receptor [26] and that glycyrrhetic acid cannot induce sodium retention and potassium excretion in adrenalectomized mammals. The mineralocorticoid effect of glycyrrhetic acid can only be restored in adrenalectomized animals by administering concomitantly glycyrrhetic acid with exogenous cortisol. The mechanism proposed for this observation is the inhibition of 11β -OHSD by glycyrrhetic acid which gives access of cortisol to the mineralocorticoid receptor. Thus in the presence of an inhibited 11β -OHSD, cortisol functions as a mineralocorticoid hormone.

In order to protect the mineralocorticoid receptor from promiscuous cortisol, 11β -OHSD has to fulfil some biochemical and anatomical prerequisites. First it has to have a high affinity for cortisol and corticosterone in the nanomolar range, second it has to favour the oxidation over the reduction, and third it has to colocalize with the mineralocorticoid receptors. These prerequisites match best, although not completely, with 11β -OHSD₂ [12].

11β -OHSD protects the fetus from high cortisol levels

Another biological relevance of 11β -OHSD is its role in protecting the fetus from maternal glucocorticoids. Most maternal cortisol crossing the human placenta is converted to cortisone [27]. By that mechanism, the fetus is protected from the growth-retarding effects of maternal glucocorticoids, which circulate at concentrations 5–10 times higher than those in the fetus [28].

Benediktsson *et al.* [29] proposed that glucocorticoid exposure *in utero* might be a predisposing factor for hypertension in the future adult life of the fetus. Epidemiological evidence revealed that hypertension is strongly predicted by the combination of low birthweight and large placenta in humans [30]. It was hypothesized that this association could be due to increased fetal exposure to maternal glucocorticoids. In rats placental 11β -OHSD activity was found to correlate positively with term fetal weight and negatively with placental weight. Rats treated during pregnancy with dexamethasone had offspring with lower birthweight and higher blood pressure than untreated rats [29]. Thus increased fetal glucocorticoid exposure secondary to attenuated placental 11β -OHSD activity might link low birthweight and high placental weight with hypertension. As a corollary, inhibition of 11β -OHSD in pregnant women might be hazardous, a hypothesis in line with the fact that many authorities discourage the use of frusemide—an inhibitor of 11β -OHSD—during pregnancy [6].

11β -OHSD alleviates glucocorticoid-mediated inhibition of testosterone synthesis in Leydig cells

Long-term exposure to elevated levels of circulating glucocorticoids in stress, Cushing's disease, or during immunosuppressive therapy lead to depressed plasma testosterone [31,32]. Leydig cells from adult rats exhibit a high activity of 11β -OHSD [33]. *In-vitro* studies using Leydig cells revealed that the inhibition of testosterone secretion by 11β -OHSD glucocorticosteroids was enhanced by the inhibition of 11β -OHSD. From these studies it was concluded that 11β -OHSD protects the Leydig cells from endogenous glucocorticoids [34,35]. This protection is absent in newborn rats. The developmental rise in intracellular 11β -OHSD in Leydig cells occurs in parallel with the increased capability of the Leydig cells to produce testosterone, suggesting that the absence of 11β -OHSD protects the organism in the early stage of life from unwarranted testosterone [35].

11β -OHSD protects glucocorticoid receptors from 11β -hydroxy glucocorticosteroids

The presence of a hydroxyl group at position 11 of the molecule is a prerequisite for steroid binding to the glucocorticoid receptors. Therefore cortisol and prednisolone bind to glucocorticoid receptors and are thus biologically active glucocorticoids, whereas cortisone and prednisone exhibit hardly any glucocorticoid activity (Figure 1). Local inactivation of 11β -hydroxy steroids into 11-keto steroids by 11β -OHSD might therefore modulate glucocorticoid access to the receptors. Such an effect has been shown in cell culture experiments and *in vivo* in humans.

Rat pituitary tumour GH3 cell lines express several glucocorticoid target genes, including prolactin. The potency of 11β -hydroxysteroids to inhibit the release of prolactin is enhanced by the addition *in vitro* of glycyrrhetic acid [36], an inhibitor of 11β -OHSD. Furthermore, *in vivo* glycyrrhetic acid potentiates the vasoconstrictor effect of cortisol but not that of dexamethasone, a steroid which is not an appropriate substrate for 11β -OHSD [37]. Thus 11β -OHSD modulates access of glucocorticoids to glucocorticoid receptors. Variable activity of 11β -OHSD may therefore at least partly explain intra- and interindividual differences in the expression of glucocorticoid effects in clinical practice.

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