display positive staining for the fibroblast marker FSP-1 in late stages of fibrosis, indicating possible transdifferentiation. Nadasdy and collegues 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*.

References

1. Cohnheim J. Über Entzündung und Eiterung. *Virchows Arch* 1867; 40: 1–79
2. Ross R, Everett NB, Tyler R. Wound healing and collagen formation. VI. The origin of the wound. Fibroblast studied in parabiosis. *J Cell Biol* 1970; 44: 645–654
3. Ekblom P, Weller A. Ontogeny of tubulointerstitial cells. *Kidney Int* 1991; 39: 394–400
4. Klein G, Langegger M, Goridis C, Ekblom P. Neural adhesion molecules during embryonic induction and development of the kidney. *Development* 1988; 102: 749–761
5. Strutz F, Okada H, Lo CW *et al.* Identification and characterization of fibroblast-specific protein 1 (FSP1). *J Cell Biol* (in press)
6. Kaissling B, Le Hir M. Characterization and distribution of interstitial cell types in the renal cortex of rats. *Kidney Int* 1994; 45: 709–720
7. Wiggins R, Goyal M, Merritt S, Killen PD. Vascular adventitial cell expression of collagen I mesenger ribonucleic acid in antiglomerular basement membrane antibody-induced crescentic nephritis in the rabbit. *Lab Invest* 1993; 68: 557–565
8. Hay ED. Extracellular matrix alters epithelial differentiation. *Curr Opin Cell Biol* 1993; 5: 1029–1035
9. Bachem MG, Meyer D, Schäfer W *et al.* The response of rat liver perisinusoidal lipocytes to polypeptide growth regulator changes with their transdifferentiation into myofibroblast-like cells in culture. *J Hepatol* 1993; 18: 40–52
10. Haverty TP, Kelly CJ, Hines WH *et al.* Characterization of a renal tubular epithelial cell line which secretes the autologous target antigen of autoimmune experimental interstitial nephritis. *J Cell Biol* 1988; 107: 1359–1367
11. Ziyadeh F, Snipes ER, Watanabe M, Alvarez RJ, Goldfarb S, Haverty TP. High glucose induces cell hypertrophy and stimulates collagen gene transcription in proximal tubule. *Am J Physiol* 1990; 259: F704-F714
12. Creely JJ, DiMari SJ, Howe AM, Haralson MA. Effects of transforming factor-β on collagen synthesis by normal rat kidney epithelial cells. *Am J Pathol* 1992; 140: 45–55
13. Nadasdy T, Laszik Z, Blick KE, Johnson DL, Silva FG. Tubular atrophy in the end-stage kidney: a lectin and immunohistochemical study. *Hum Pathol* 1994; 25: 22–28

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

G. Escher, B. M. Frey and F. J. Frey

Division of Nephrology, Department of Medicine, Universitätsspital Bern, Bern, Switzerland

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 11β-hydroxysteroid dehydrogenase (11β-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.

Correspondence and offprint requests to: Prof. F. J. Frey, Abteilung für Nephrologie, Departement Innere Medizin, Inselspital, 3010 Bern, Switzerland.

Characteristics of 11β-OHSD

11β-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

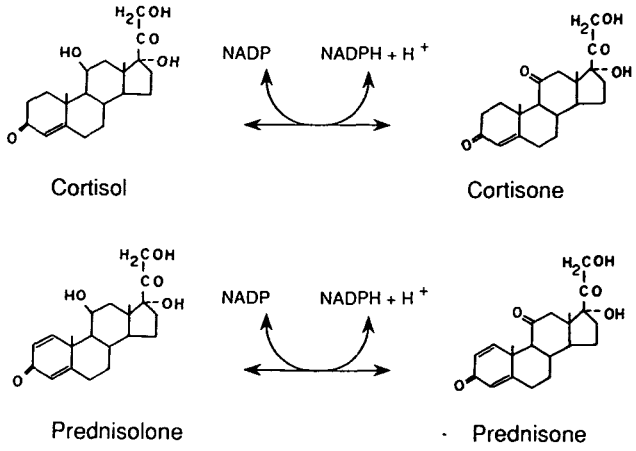

Fig. 1. Interconversion of cortisol to cortisone by 11β-OHSD in the presence of NADP. The reaction is reversible. According to the structure homology between cortisol and prednisolone (one additional double bond in the A ring), it is assumed that the same mechanism occurs for the conversion of prednisolone 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 11β-OHSD in COS-1 cells, which are devoid of spontaneous 11β-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 11β-OHSD a human NADP-dependent 11β-OHSD with similar biological features to that found in rats [7].

The cDNA of 11β-OHSD cloned by Monder and collaborators encodes for a 34-kDa NADP-dependent enzyme named now 11β-$OHSD_1$ [4, 8]. In 1991 Krozowski found a short form of 11β-$OHSD_1$ 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 11β-OHSD activity was found. In an attempt to find a new 11β-OHSD by expression cloning, Naray *et al.* injected mRNA from kidney collecting ducts cells into oocytes and described NAD-dependent 11β-OHSD activity: the corresponding enzyme has not been sequenced until know [11]. In 1994 Krozowski found by means of expression cloning in CHOP-4 cells a protein with 11β-OHSD activity named 11β-$OHSD_2$ [12]. For that purpose he used a human kidney cDNA library [12]. The affinity of 11β-$OHSD_2$ for cortisol is in the nM range [12], whereas that of 11β-$OHSD_1$ is in the μM range [13]. Since cortisol and corticosterone concentrations in mammalians are under physiological conditions in the nanomolar and not in the micromolar range, it appears that 11β-$OHSD_2$ rather than 11β-$OHSD_1$ is suitable for regulating steroids in the low physiological concentration range.

The intracellular localization of 11β-$OHSD_1$ but not 11β-$OHSD_2$ has been investigated. 11β-$OHSD_1$ is mainly located in the microsomes [14]; however, there is also some evidence that it is present in the nuclei [15]. 11β-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].

11β-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 11β-OH corticosteroids such as cortisol or corticosterone are inactivated locally into cortisone and dehydrocorticosterone by 11β-OHSD, an enzyme which cannot inactivate aldosterone because the 11β-OH group of aldosterone is protected by a covalent hemiacetal bond between C_{11} and C_{18} 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 11β-OHSD causes a mineralocorticoid type of hypertension (apparent mineralocorticoid exess) with low aldosterone and low renin that can be cured by replacing the endogenous cortisol by glucocorticoids without an 11β-hydroxy group [20].

Second, the inhibition of 11β-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, glycyrrhetinic acid, the ingredient of liquorice and possibly various other less well definied endo- and xenobiotics [6,21–25].

Practically, the most relevant inhibitor of 11β-OHSD

is certainly glycyrrhetinic 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 glycyrrhetinic acid has no affinity for this receptor [26] and that glycyrrhetinic acid cannot induce sodium retention and potassium excretion in adrenalectomized mammalians. The mineralocorticoid effect of glycyrrhetinic acid can only be restored in adrenalectomized animals by administering concomitantly glycyrrhetinic acid with exogenous cortisol. The mechanism proposed for this observation is the inhibition of 11β-OHSD by glycyrrhetinic 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 prerequisits. 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 prerequisits match best, although not completely, with 11β-$OHSD_2$ [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 gluocorticoids, which circlulate 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 glycyrrhetinic acid [36], an inhibitor of 11β-OHSD. Furthermore, *in vivo* glycyrrhetinic 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 glucococorticoid effects in clinical practice.

References

1. Monder C, White PC. 11β-hydroxysteroid dehydrogenase. *Vitam Horm* 1993; 46: 187–271
2. Frey FJ, Escher G, Frey BM. Pharmacology of 11β-hydroxysteroid dehydrogenase. *Steroids* 1994; 59: 74–79
3. Lakshmi V, Monder C. Purification and characterization of the corticosteroid 11β-dehydrogenase component of the rat liver 11β-hydroxysteroid dehydrogenase complex. *Endocrinology* 1988; 123: 2390–2398
4. Agarwal AK, Monder C, Eckstein B, White PC. Cloning

and expression of rat cDNA encoding corticosteroid 11β-hydroxysteroid dehydrogenase. *J Biol Chem* 1989; 264: 18939–18943
5. Duperrex H, Kenouch S, Gaeggeler HP *et al.* Rat liver 11β-hydroxysteroid dehydrogenase complementary deoxyribonucleic acid encodes oxoreductase activity in a mineralocorticoid-responsive toad bladder cell line. *Endocrinology* 1993; 132: 612–619
6. Escher G, Meyer KV, Vishwanath BS, Frey BM, Frey FJ. Furosemide inhibits 11β-hydroxysteroid dehydrogenase *in vitro* and *in vivo*. *Endocrinology* 1995; 136: 1759–1765
7. Tannin GM, Agarwal AK, Monder C, New MI, White PC. The human gene for 11β-hydroxysteroid dehydrogenase. *J Biol Chem* 1991; 266: 16653–16658.
8. Monder C, Lakshmi V. Corticosteroid 11β-hydroxysteroid dehydrogenase of rat tissues: immunological studies. *Endocrinology* 1990; 126: 2435–2443
9. Krozowski Z, Obeyesekere V, Smith R, Mercer W. Tissue-specific expression of an 11β-hydroxysteroid dehydrogenase with a truncated N-terminal domain. *J Biol Chem* 1992; 267: 2569–2574
10. Mercer W, Obeyesekere V, Smith R, Krozowski Z. Characterization of 11β-hydroxysteroid dehydrogenase 1B gene expression and enzymatic activity. *Mol Cell Endocrinol* 1993; 92: 247–251
11. Naray-Fejes-Toth A, Rusvai E, Denault DL, ST. Germain DL, Fejes-Toth G. Expression and characterization of a new species of 11β-hydroxysteroid dehydrogenase in Xenopus oocytes. *Am J Physiol* 1993; 265: F896–F900
12. Albiston AL, Obeyesekere VR, Smith RE, Krozowski Z. Cloning and tissue distribution of the human 11β-hydroxysteroid dehydrogenase type 2 enzyme. *Mol Cell Endocrinol* 1994; 105: R11–R17
13. Monder C, Lakshmi V, Miroff Y. Kinetic studies on rat liver 11β-hydroxysteroid dehydrogenase. *Biochim Biophys Acta* 1991; 1115: 23–29
14. Lakshmi V, Monder C. Extraction of 11β-hydroxysteroid dehydrogenase from rat liver microsomes by detergents. *J Steroid Biochem* 1985; 22: 331–340
15. Kobayashi N, Schulz W, Hierholzer K. Corticosteroid metabolism in rat kidney in vitro. *Pflügers Arch* 1987; 408: 46–53
16. Monder C. Heterogeneity of 11β-hydroxysteroid dehydrogenase in rat tissues. *J Steroid Biochem Molec Biol* 1991; 40: 533–536
17. Escher G, Frey FJ, Frey BM. 11β-hydroxysteroid dehydrogenase accounts for low prednisolone/prednisone ratios in the kidney. *Endocrinology* 1994; 135: 101–106
18. Funder JW, Pearce PT, Smith R, Smith AI. Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. *Science* 1988; 242: 583–585
19. Edwards CRW, Burt D, McIntyre MA *et al.* Localization of 11β-hydroxysteroid dehydrogenase–tissue specific protector of the mineralocorticoid receptor. *Lancet* 1988; 2: 986–989
20. Stewart PM, Corrie JET, Shackleton CHL, Edwards CRW. Syndrome of apparent mineralocorticoid excess. *J Clin Invest* 1988; 82: 340–349
21. Stewart PM, Wallace AM, Atherden SM, Shearing CH, Edwards CRW. Mineralocorticoid activity of carbenoxolone: contrasting effects of carbenoxolone and liquorice on 11β-hydroxysteroid dehydrogenase in man. *Clin Sci* 1990; 78: 49–54
22. Song D, Lorenzo B, Reidenberg MM. Inhibition of 11β-hydroxysteroid dehydrogenase by gossypol and bioflavonoids. *J Lab Clin Med* 1992; 120: 792–797
23. Monder C, Stewart PM, Lakshmi V, Valentino R, Burt D, Edwards CRW. Licorice inhibits corticosteroid 11β-hydroxysteroid dehydrogenase of rat kidney and liver: *in vivo* and *in vitro* studies. *Endocrinology* 1989; 125: 1046–1053
24. Conti M, Frey FJ, Escher G, Marone C, Frey BM. Renal handling of prednisolone/prednisone: effect of steroid dose and 11β-hydroxysteroid dehydrogenase. *Nephrol Dial Transplant* 1994; 9: 1622–1628
25. Perschel FH, Bühler H, Hierholzer K. Bile acids and their amidates inhibit 11β-hydroxysteroid dehydrogenase obtained from rat kidney. *Pflügers Arch* 1991; 418: 538–543
26. Armanini D, Karbowiak I, Funder JW. Affinity of liquorice derivatives for mineralocorticoid and glucocorticoid receptors. *Clin Endocrinol* 1983; 19: 609–619
27. Murphy BEP, Clark SJ, Donald IR. Conversion of maternal cortisol to cortisone during placental transfer to the human fetus. *Am J Obstet Gynecol* 1974; 118: 538–541
28. Edwards CRW, Benediktsson R, Lindsay RS, Seckl JR. Dysfunction of placental glucocorticoid barrier: link between fetal environment and adult hypertension? *Lancet* 1993; 341: 355–357
29. Benediktsson R, Lindsay RS, Noble J, Seckl JR, Edwards CRW. Glucocorticoid exposure *in utero*: new model for adult hypertension. *Lancet* 1993; 341: 339–341
30. Oka K, Hirano T, Homma M. Low-birthweight hypertension via enzyme and receptor imprintings of glucocorticoid. *Lancet* 1993; 342: 303–304
31. Monder C, Sakai RR, Blanchard RJ, Lakshmi V, Philipps DM, Hardy M. The mediation of testicular function by 11β-hydroxysteroid dehydrogenase. In: *Stress and Reproduction*. Raven Press, New York, 1992; 145–155
32. Cooke BA, Abayasekara DRE, Choi MSK, Dirami G, Phipp LH, West AP. The effects of stress-induced ligands on testosterone formation in Leydig cells. In: *Stress and Reproduction*. Raven Press, New York, 1992; 135–144
33. Phillips DM, Lakshmi V, Monder C. Corticosteroid 11β-hydroxysteroid dehydrogenase in rat testis. *Endocrinology* 1989; 125: 209–216
34. Monder C, Hardy MP, Blanchard RJ, Blanchard DC. Comparative aspects of 11β-hydroxysteroid dehydrogenase. Testicular 11β-hydroxysteroid dehydrogenase: development of a model for the mediation of Leydig cell function by corticosteroids. *Steroids* 1994; 59: 69–73
35. Monder C, Miroff Y, Marandici A, Hardy MP. 11β-hydroxysteroid dehydrogenase alleviates glucocorticoid-mediated inhibition of steroidogenesis in rat Leydig cells. *Endocrinology* 1994; 134: 1199–1204
36. Stewart PM, Whorwood CB. 11β-hydroxysteroid dehydrogenase activity and corticosteroid hormone action. *Steroids* 1994; 59: 90–95
37. Teelucksingh S, Mackie AD, Burt D, McIntyre MA, Brett L, Edwards CRW. Potentiation of hydrocortisone activity in skin by glycyrrhetinic acid. *Lancet* 1990; 335: 1060–1063
38. Frey FJ. Kinetics and dynamics of prednisolone/prednisone. *Endocrinology* 1987; 8: 453–473