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Evidence for Distinct Isoforms of 11β-Hydroxysteroid Dehydrogenase in the Ovine Liver and Kidney

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We have previously identified a unique 11β-hydroxysteroid dehydrogenase (11β-HSD) transcript in the ovine kidney. To examine whether this is indicative of a distinct isoform with respect to enzymatic activity, we studied and compared the characteristics of 11β-HSD activity in the ovine liver and kidney. 11β-HSD activity was determined by a radiometric conversion assay using cortisol and cortisone as physiological substrates. Although in both liver and kidney, the enzyme was localized by subcellular fractionation in the microsomes, the renal 11β-HSD displayed distinct characteristics in that it expressed only dehydrogenase activity and utilized almost exclusively NAD as cofactor (the respective activity in the presence of NAD and NADP was 190 ± 26 and 12 ± 2 pmol/min/mg protein). By contrast, the liver enzyme contained both dehydrogenase and reductase activities, and displayed preference for NADP and NADPH, respectively. Furthermore, with cortisol as substrate, the kidney 11β-HSD had a K_m of 68 ± 7 nM which was over 100 times lower than the hepatic enzyme (8 ± 1μM). In addition, the renal 11β-HSD activity was inhibited in a dose-dependent fashion by both carbenoxolone, a potent inhibitor of 11β-HSD, and the end product cortisone, whereas the liver enzyme showed little inhibition by either substance. In summary, these results provide strong evidence for the existence of distinct isoforms of 11β-HSD with respect to enzymatic activity in the ovine liver and kidney. In addition, the characteristics of the kidney enzyme closely resemble those of that described previously in the rabbit renal aldosterone target cells, and thus further demonstrating the presence of an isoform of 11β-HSD distinct from the NADP-dependent enzyme purified and cloned from the rat liver.

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INTRODUCTION

11β-Hydroxysteroid dehydrogenase (11β-HSD) is a microsomal enzyme responsible for the interconversion of active glucocorticoids (cortisol and corticosterone) and their inactive metabolites (cortisone and 11-dehydrocorticosterone) [1]. Therefore, 11β-HSD regulates the level of intracellular bioactive glucocorticoid [2, 3]. Although the activity of 11β-HSD is expressed ubiquitously in mammalian species [4], its biological significance in individual organs remains largely unknown [5]. In kidney, it has been proposed that 11β-HSD confers aldosterone specificity on the non-selective mineralocorticoid receptor by inactivating glucocorticoid locally [6, 7]. Thus, a defect in this enzyme activity, either congenital or acquired, leads to the syndrome of apparent mineralocorticoid excess in which cortisol acts as a mineralocorticoid causing hypertension and hypokalemia [8–10].

Although inhibition of this enzyme *in vivo* results in the loss of aldosterone specificity for the renal mineralocorticoid receptor [6, 7], immunohistochemical studies, using the antibody raised against rat liver enzyme, have failed to colocalize 11β-HSD and mineralocorticoid receptors in the rat kidney [11]. This has raised some doubts over the hypothesis that 11β-HSD confers aldosterone specificity on the renal mineralocorticoid receptor [12]. Attempts to reconcile this paradox have suggested that 11β-HSD may function in a paracrine fashion [11] or that the rat kidney may produce more than one isoform of 11β-HSD [13–19]. In support of the latter possibility, it has been shown that adult rat kidney contains four species of 11β-HSD

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mRNA [20]. Subsequently, a cDNA encoding a truncated liver form enzyme was cloned from rat kidney [16], but the expressed product in mammalian cells was enzymatically inactive [21]. Very recently, a new isoform of 11β-HSD with respect to its enzymatic characteristics has been described in the rabbit renal aldosterone target cells [19]. It is distinct from the reversible NADP-dependent rat liver enzyme in that it expresses only dehydrogenase activity and utilizes NAD as cofactor. In addition, it has over 100 fold higher affinity for endogenous glucocorticoid than the rat liver enzyme. Therefore, it has been suggested that it is this isoform that protects renal mineralocorticoid receptors from occupancy by endogenous glucocorticoids [19]. However, whether this isoform is present in kidneys of other mammalian species remains to be established.

We have previously identified a unique 11β-HSD transcript in the fetal and adult sheep kidney [22]. It is conceivable that this may be indicative of a kidney-specific isoform. In the present study, we therefore examined this possibility by determining whether the renal 11β-HSD is distinct from the liver enzyme with respect to enzymatic activity. We present evidence here to demonstrate that the ovine kidney contains a distinct isoform of 11β-HSD, as in the rabbit, which may represent the product of a gene distinct from 11β-HSD.

EXPERIMENTAL

Reagents and supplies

[1,2,6,7-^{3}H(N)]Cortisol (70.1 Ci/mmol) was purchased from Du Pont Canada Inc. (Markham, Ontario). Purity was improved regularly by thin layer chromatography. Non-radioactive steroids were obtained from Steraloids Inc. (Wilton, NH). Carbenoxolone and cofactors (NAD, NADH, NADP, and NADPH) were purchased from Sigma Chemicals (St Louis, MO). Polyester-backed thin layer chromatography (TLC) plates were obtained from Fisher Scientific Ltd. (Unionville, Ontario). All solvents used were OmniSolv grade from BDH Inc. (Toronto, Ontario).

Preparation of [1,2,6,7-^{3}H(N)]cortisone

[1,2,6,7-^{3}H(N)]Cortisone was prepared from [1,2,6,7-^{3}H(N)]cortisol by the method of Shaw and Quincey [23]. Briefly, the labelled cortisol (10 μCi) was incubated in 1 ml of 50% aqueous acetic acid containing 1% (w/v) chromium trioxide at room temperature for 20 min. The residue from the dichloromethane extract of the reaction products was chromatographed by TLC using chloroform–methanol (9:1, v/v) as solvent, and non-radioactive cortisol and cortisone as reference markers. The cortisone-containing region was scraped off and eluted with ethyl acetate.

Preparation of subcellular fractions

Subcellular fractions were prepared by a standard procedure [24, 25]. Briefly, liver and kidney tissues (4–5 g), obtained from pregnant ewes, were homogenized freshly in 5 vol of ice-cold 10 mM sodium phosphate buffer, pH 7.0, containing 0.25 M sucrose (Buffer A). All subsequent steps were performed at 0–4°C. The homogenates were centrifuged consecutively at 750 and 20,000 g for 30 min. The latter supernatant was centrifuged at 105,000 g for 60 min. The resultant pellets from all three centrifugations were saved, and were taken respectively as crude nuclear, mitochondrial and microsomal fractions. Individual pellets were then resuspended in appropriate amounts of Buffer A (5–10 mg/ml). The resuspension was either used immediately or stored in small aliquots at −70°C. In preliminary studies, it was found that both dehydrogenase and reductase activities were maintained for at least 2 weeks, and all the assays were conducted within this time.

Protein estimation

Protein concentration was determined by the Bradford method using a Bio-Rad (Mississauga, Ontario) protein assay kit with bovine serum albumin as standard.

Assay of 11β-dehydrogenase activity

11β-Dehydrogenase activity was determined by measuring the rate of conversion of cortisol to cortisone. The assay tubes contained approx. 100,000 cpm of the labelled cortisol, final concentrations of non-radioactive cortisol and cofactor (NAD or NADP) at 100 nM and 250 μM, respectively. Buffer B (0.1 M sodium phosphate buffer, pH 7.5) was added to bring the volume up to 0.4 ml. The tube was placed in a water bath at 37°C and shaken continuously for 10 min. The enzymatic reaction was started by the addition of 100 μl of samples of tissue fraction containing 20–30 μg protein, and was allowed to proceed for 5 min (preliminary studies indicated that the rate of reaction was linear with time from 2.5 to 10 min, and the amount of tissue fraction between 5–50 μg protein). Blanks containing all the assay components except that the buffer replaced enzyme preparation were included in all assays. The reaction was then arrested by transferring the tubes on ice rapidly, and by the addition of 4 ml ethyl acetate containing 40 μg mixture of non-radioactive cortisol and cortisone as carrier steroids. The extracts were dried, and the residues were resuspended in 100 μl methanol (the recovery rate of the extraction was determined by counting a fraction of the resuspension in liquid scintillation fluid, and was over 90%). A fraction of the resuspension was spotted on a TLC plate which was developed in chloroform–methanol (9:1, v/v). The bands containing the labelled cortisol and cortisone were identified by UV light of the cold carriers, cut out into scintillation vials and counted in liquid scintillation fluid. In all cases, the combined counts accounted for about 90% of the initial radioactivity. From the specific activity of the labelled

cortisol and the radioactivity of cortisone, the rate of cortisol to cortisone conversion was calculated, and expressed as the amount of cortisone (pmol) formed per min per mg protein.

Assay of 11-oxoreductase activity

11-Oxoreductase activity was determined similarly except that cortisone was used as substrate, NADH or NADPH as cofactor, and Buffer C (0.1 M phosphate buffer, pH 6.0) replaced Buffer B.

Kinetic analysis

For both liver and kidney, kinetic analyses were performed using the microsomal fraction as it contained the highest level of 11β-HSD activity (see Fig. 1). In each case, a rough estimate of K_m was first obtained by a direct linear plot of measurements of velocities at only two substrate concentrations, a high and low value giving the clearest intersection point. Conversion assays were then conducted using a fixed amount of cofactor (250 μM), enzyme preparation (20–30 μg protein), and reaction time (5 min), but with varying amounts of substrate ranging from $K_m/2$–5 K_m. The conditions were chosen so that the initial velocity was linear with the reaction time and the amount of enzyme preparation. Each experiment was done in duplicate. The data were plotted as a straight line of s/v against s according to the Michaelis-Menton Equation [26], and the K_m and V_{max} values were calculated from the intercepts of these plots as described previously [26].

Inhibition studies

Carbenoxolone, a well-known inhibitor of 11β-HSD [27], was used to determine to what extent it will inhibit 11β-HSD activity in the kidney and liver. The assays were performed using the microsomal fractions with cortisol as substrate in the absence and presence of carbenoxolone (at final concentrations of 50 and 100 nM), as described above. The degree of inhibition was expressed as percentage of the activity in the presence over that in the absence of carbenoxolone.

One of the notable differences between the rat liver 11β-HSD and the rabbit renal aldosterone target cell isoform is that only the latter exhibits the end product inhibition [19, 28]. To examine whether this is the case in the sheep, we carried out 11β-dehydrogenase activity assays using both liver and kidney microsomal fractions in the absence and presence of the end product cortisone, at final concentrations of 0.1, 1, and 10 μM, as described above. The results were presented as percentage of the activity over the control (in the absence of cortisone).

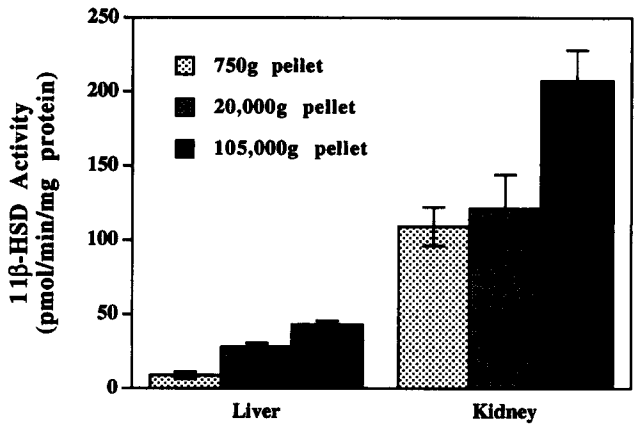

Fig. 1. Subcellular distribution of 11β-HSD activity in the ovine liver and kidney. 11β-Dehydrogenase activity, using cortisol as substrate at a final concentration of 100 nM, was determined in the presence of NADP (liver) or NAD (kidney) as described in the Experimental. The data are from four separate experiments, and presented as mean ± SEM.

RESULTS

Subcellular localization

The distribution of 11β-HSD activity in subcellular fractions prepared from both liver and kidney tissues is shown in Fig. 1. In both cases, although a substantial amount of 11β-HSD activity was present in all the three fractions, the majority of it was associated with the microsomal fraction. Therefore, all the subsequent experiments were conducted on microsomes.

Cofactor preference

In the liver, although 11β-dehydrogenase activity displayed a marginal preference for NADP, the reductase activity showed over 10-fold preference for NADPH (the respective activity in the presence of NADH and NADPH was 9 ± 1 and 101 ± 5 pmol/min/mg protein) [Fig. 2(A)]. By contrast, the renal 11β-dehydrogenase activity in the presence of NAD was 190 ± 26 pmol/min/mg protein, being almost 20 times higher than that in the presence of NADP (12 ± 2 pmol/min/mg protein). Furthermore, there was no detectable 11-oxoreductase activity regardless of which cofactor was present [Fig. 2(B)].

Kinetic data

With cortisol as physiological substrate, the renal 11β-HSD had a K_m of 69 ± 7 nM which was over 100 times lower than the hepatic enzyme (8 ± 1 μM) (Table 1). In marked contrast to the kidney where little reductase activity was found, there was a compatible amount of 11-oxoreductase activity in the liver with a K_m of 1 ± 0.2 μM and a V_{max} of 700 ± 62 pmol/min/mg protein (Table 1).

Inhibition by carbenoxolone and cortisone

As shown in Figs 3 and 4, both carbenoxolone and cortisone inhibited the renal 11β-HSD activity in a dose-dependent fashion with the highest inhibition (95%) by cortisone at a concentration of 10 μM. By

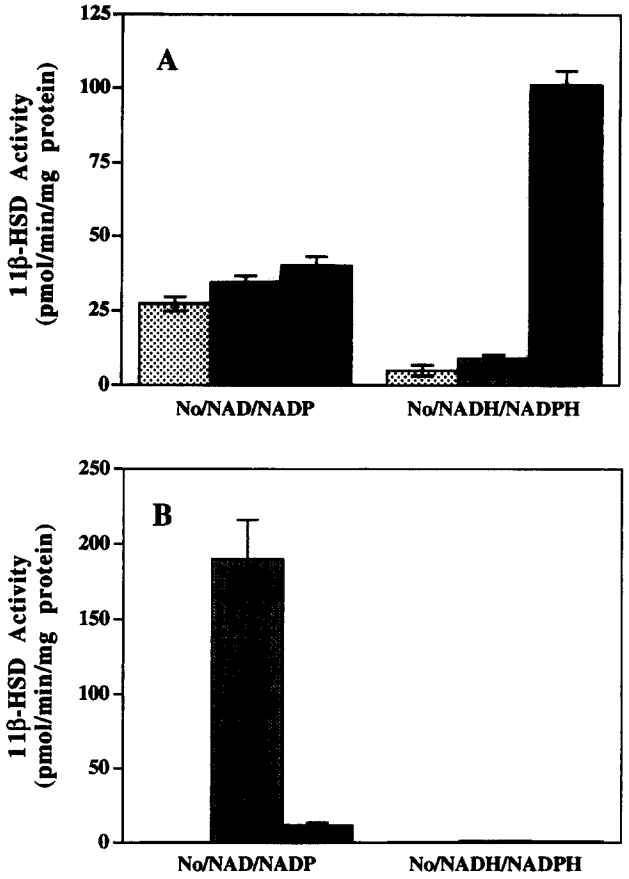

Fig. 2. Cofactor preference of 11β-HSD activity in the liver (A) and kidney (B). Microsomal fractions were incubated with 100 nM of cortisol or cortisone in the absence and presence of NAD/NADP or NADH/NADPH for determining the respective 11β-dehydrogenase and 11-oxoreductase activity, as described in the Experimental. Each bar represents mean $\pm$ SEM ($n = 4$). Similar results were obtained when 10 μM of cortisol (approximates to the K_m for the liver enzyme) was used with the liver microsomes (data not shown).

contrast, equal amounts of these two substances had little effect on the liver enzyme (Figs 3 and 4).

DISCUSSION

The discovery of a causal relationship between renal 11β-HSD deficiency and apparent mineralocorticoid

Table 1. *Kinetic parameters of kidney and liver 11β-HSD*

Tissue	K_m (nM)	V_{max} (pmol/min/mg protein)
Kidney dehydrogenase	69 $\pm$ 7	316 $\pm$ 35
Liver dehydrogenase	8000 $\pm$ 800	1970 $\pm$ 80
Liver reductase	950 $\pm$ 170	700 $\pm$ 62

Kinetic studies were carried out using the microsomal fraction as described in the Experimental. For kidney dehydrogenase, 25–400 nM of cortisol were used in the presence of NAD. 2–20 μM of cortisol in the presence of NADP and 0.5–4 μM of cortisone in the presence of NADPH were used for liver dehydrogenase and reductase, respectively. Values are mean $\pm$ SEM of four separate experiments.

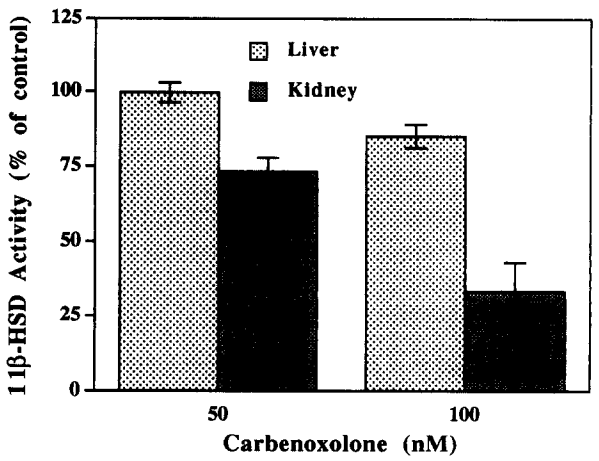

Fig. 3. Inhibition of 11β-HSD activity by carbenoxolone. 11β-HSD activity in microsomes were determined using 100 nM of cortisol as substrate in the absence and presence of carbenoxolone at concentrations of 50 and 100 nM, as described in the Experimental. Data are expressed as percent activity of the control (in the absence of carbenoxolone), and each bar represents mean $\pm$ SEM ($n = 4$). Similar results were obtained when 10 μM of cortisol (approximates to the K_m for the liver enzyme) was used with the liver microsomes (data not shown).

excess syndrome [8] has revived tremendous interests in the structure and function of 11β-HSD [29–31]. *In vivo*, aldosterone selectively binds to the renal mineralocorticoid receptor which has equal affinity for cortisol and aldosterone *in vitro* [32, 33], despite the circulating level of cortisol being at least 100 times higher than that of aldosterone. This suggests that 11β-HSD in kidney normally functions to confer aldosterone specificity on mineralocorticoid receptors

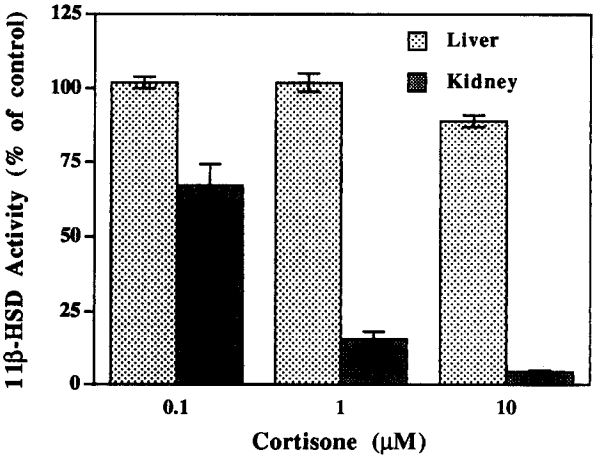

Fig. 4. Inhibition of 11β-HSD activity by cortisone. 11β-HSD activity in microsomes were determined using 100 nM of cortisol as substrate in the absence and presence of the end product cortisone at concentrations of 0.1, 1, and 10 μM. Data are expressed as percent activity of the control (in the absence of cortisone), and each bar represents mean $\pm$ SEM ($n = 4$). Similar results were obtained when 10 μM of cortisol (approximates to the K_m for the liver enzyme) was used with the liver microsomes (data not shown).

by converting glucocorticoids to their inactive metabolites [6, 7]. Although 11β-HSD has been purified and cloned from rat liver [29, 30], two lines of evidence have led to the hypothesis that an isoform of 11β-HSD distinct from the rat liver enzyme protects the renal mineralocorticoid receptor from occupancy by endogenous glucocorticoids. The first is the realization that the rat liver 11β-HSD can not function effectively in kidney because its K_m for endogenous glucocorticoids is at least 100 times higher than their normal circulating concentrations [29]. The second is the failure to localize 11β-HSD, by immunohistochemistry using an antibody raised against the rat liver enzyme, in the renal aldosterone target cells [11]. Indeed, such an isoform has been identified recently from the rabbit renal aldosterone target cells [19]. It is NAD-dependent and expresses only dehydrogenase activity. In addition, it has a K_m for corticosterone of 25.9 ± 2.4 nM, being within the physiological concentration of this hormone in circulation. However, its existence in kidneys of other mammalian species has not been reported. It should be noted that a NAD-dependent 11β-HSD with a similar affinity for endogenous glucocorticoid to the rabbit kidney enzyme has been partially purified from human placenta, but its reversibility was not reported [34]. In the present study, we addressed this important issue using the sheep as an experimental model, because we had demonstrated the presence of a unique 11β-HSD transcript in the fetal and adult sheep kidney [22]. Our present results clearly demonstrate that the ovine kidney 11β-HSD is distinct from the liver enzyme with respect to affinity for endogenous glucocorticoid, cofactor preference, reversibility of reaction, and inhibition by carbenoxolone and the end product cortisone. Furthermore, since the behaviours of ovine renal 11β-HSD closely resemble those of the newly identified 11β-HSD isoform from the rabbit renal aldosterone target cells [19], our results further demonstrate the presence and describe the activity of an isoform of 11β-HSD distinct from the well characterized rat liver enzyme.

Although the presence of the kidney 11β-HSD isoform has been reported previously in the rabbit [14, 19], this is the first study in which 11β-HSD activity was characterized concurrently in liver and kidney tissues from the same animals. This has allowed us to make direct comparisons, and our results demonstrate that 11β-HSD in the ovine liver is a reversible NADP-dependent enzyme. These findings are consistent with rat liver enzyme (both native and recombinant protein) [29, 30]. Furthermore, kinetic characteristics of the liver enzyme are also compatible with those described in the literature on other species [4, 29]. By contrast, the ovine kidney 11β-HSD expresses only dehydrogenase activity, and utilizes NAD as cofactor.

Another significant difference between the ovine liver and kidney 11β-HSD is that the latter has over 100 fold higher affinity for cortisol. In addition, unlike the liver enzyme, the renal 11β-HSD also exhibits profound inhibition by carbenoxolone, a well known inhibitor of 11β-HSD activity [6, 27], and by the end product cortisone. It should be emphasized that neither of the two significantly inhibited the ovine liver enzyme at concentrations tested. It is possible that they are capable of doing so but only at concentrations higher than those required to inhibit the renal 11β-HSD. Although it remains to be determined whether the distinct renal 11β-HSD activity described in the present study is localized in the renal aldosterone target cells, its affinity for cortisol is certainly within the physiological level of this hormone in circulation (52 ng/ml) [35]. This indicates that the renal 11β-HSD we described here is fitted for the role of the enzyme that is believed to confer aldosterone specificity on the mineralocorticoid receptor in kidney.

The sheep is unique in that a single kidney-specific 11β-HSD transcript was identified [22]. It is possible that the renal enzyme activity we described here is associated with the product of this kidney-specific transcript. Alternatively, it may represent a novel gene product. This dilemma will be resolved by cloning this kidney isoform, and by subsequent expression in mammalian cells. It is noteworthy that the size of the ovine kidney 11β-HSD mRNA (1.5 kb) is identical to one of the four identified in rat kidney [20]. It has also been demonstrated that the rat 1.5 kb transcript encodes a truncated liver-form protein [16], and is a consequence of transcriptional initiation up stream of exon 2 (within intron 1) in the rat 11β-HSD gene [18]. However, when Chinese hamster ovary cells transfected with this truncated cDNA, the expressed protein had no 11β-HSD activity [21]. Although it is possible that the truncated protein may be processed differently or is otherwise more stable, and thus may be functional in cells of the renal distal tubule and collecting duct, it seems more likely that the distinct 11β-HSD activity described in the present study and in previous studies using isolated rabbit collecting duct cells is associated with the product(s) of a gene distinct from 11β-HSD. Further support for this came from a recent study in which no defects in the 11β-HSD gene were found in patients with 11β-dehydrogenase or 11-oxoreductase deficiency [36].

In conclusion, our data demonstrate that ovine kidney 11β-HSD is distinct from the liver enzyme with respect to its enzymatic properties, but is similar/identical to that described previously in the rabbit renal aldosterone target cells, and thus providing further support for the notion that it is this isoform that confers aldosterone specificity on the renal mineralocorticoid receptor in mammals [19].

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REFERENCES

1. Monder C. and Shackleton C. H. L.: 11beta-Hydroxysteroid dehydrogenase: fact or fancy? *Steroids* **44** (1984) 383–417.
2. Stewart P. M. and Sheppard M. C.: Novel aspects of hormonal action: intracellular ligand supply and its control by a series of tissue specific enzymes. *Molec Cell. Endocr.* **83** (1992) C13–C18.
3. Whorwood C. B., Franklyn J. A., Sheppard M. C. and Stewart P. M.: Tissue localization of 11β-hydroxysteroid dehydrogenase and its relationship to the glucocorticoid receptor. *J. Steroid Biochem. Molec. Biol.* **41** (1992) 21–28.
4. Monder C. and Lakshmi V.: Corticosteroid 11 beta-hydroxysteroid dehydrogenase activities in vertebrate liver. *Steroids* **52** (1988) 515–528.
5. Monder C.: The forms and functions of 11 beta-hydroxysteroid dehydrogenase. *J. Steroid Biochem. Molec. Biol.* **45** (1993) 161–165.
6. Edwards C. R., Stewart P. M., Burt D., Brett L., McIntyre M. A., Sutanto W. S., de Kloet E. R. and Monder C.: Localisation of 11 beta-hydroxysteroid dehydrogenase-tissue specific protector of the mineralocorticoid receptor. *Lancet* **2** (1988) 986–989.
7. Funder J. W., Pearce P. T., Smith R. and Smith A. I.: Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. *Science* **242** (1988) 583–585.
8. Ulick S., Levine L. S., Gunczler P., Giovanni Z., Ramirez L. C., Rauh W., Rosler A., Bradlow H. L. and New M. I.: A syndrome of apparent mineralocorticoid excess associated with defects in the peripheral metabolism of cortisol. *J. Clin. Endocr. Metab.* **49** (1979) 757–764.
9. Stewart P. M., Wallace A. M., Valentino R., Burt D., Shackleton C. H. and Edwards C. R.: Mineralocorticoid activity of liquorice: 11-beta-hydroxysteroid dehydrogenase deficiency comes of age. *Lancet* **2** (1987) 821–824.
10. Stewart P. M., Corrie J. E., Shackleton C. H. and Edwards C. R.: Syndrome of apparent mineralocorticoid excess. A defect in the cortisol-cortisone shuttle. *J. Clin. Invest.* **82** (1988) 340–349.
11. Rundle S. E., Funder J. W., Lakshmi V. and Monder C.: The intrarenal localization of mineralocorticoid receptors and 11 beta-dehydrogenase: immunocytochemical studies. *Endocrinology* **125** (1989) 1700–1704.
12. Funder J. W.: 11 beta-hydroxysteroid dehydrogenase and the meaning of life. *Molec. Cell Endocr.* **68** (1990) C3–C5.
13. Monder C.: Heterogeneity of 11 beta-hydroxysteroid dehydrogenase in rat tissues. *J. Steroid Biochem. Molec. Biol.* **40** (1991) 533–536.
14. Naray Fejes Toth A., Watlington C. O. and Fejes Toth G.: 11 beta-Hydroxysteroid dehydrogenase activity in the renal target cells of aldosterone. *Endocrinology* **129** (1991) 17–21.
15. Stewart P. M., Whorwood C. B., Barber P., Gregory J., Monder C., Franklyn J. A. and Sheppard M. C.: Localization of renal 11 beta-dehydrogenase by *in situ* hybridization: autocrine not paracrine protector of the mineralocorticoid receptor. *Endocrinology* **128** (1991) 2129–2135.
16. Krozowski Z., Obeyesekere V., Smith R. and Mercer W.: Tissue-specific expression of an 11 beta-hydroxysteroid dehydrogenase with a truncated N-terminal domain. A potential mechanism for differential intracellular localization within mineralocorticoid target cells. *J. Biol. Chem.* **267** (1992) 2569–2574.
17. Mercer W. R. and Krozowski Z. S.: Localization of an 11 beta hydroxysteroid dehydrogenase activity to the distal nephron. Evidence for the existence of two species of dehydrogenase in the rat kidney. *Endocrinology* **130** (1992) 540–543.
18. Moisan M. P., Edwards C. R. and Seckl J. R.: Differential promoter usage by the rat 11 beta-hydroxysteroid dehydrogenase gene. *Molec. Endocr.* **6** (1992) 1082–1087.
19. Rusvai E. and Naray Fejes Toth A.: A new isoform of 11 beta-hydroxysteroid dehydrogenase in aldosterone target cells. *J. Biol. Chem.* **268** (1993) 10717–10720.
20. Krozowski Z., Stuchbery S., White P., Monder C. and Funder J. W.: Characterization of 11 beta-hydroxysteroid dehydrogenase gene expression: identification of multiple unique forms of messenger ribonucleic acid in the rat kidney. *Endocrinology* **127** (1990) 3009–3013.
21. Obeid J., Curnow K. M., Aisenberg J. and White P. C.: Transcripts originating in intron 1 of the HSD11 (11 beta-hydroxysteroid dehydrogenase) gene encode a truncated polypeptide that is enzymatically inactive. *Molec. Endocr.* **7** (1993) 154–160.
22. Yang K., Smith C. L., Dales D., Hammond G. L. and Challis J. R.: Cloning of an ovine 11 beta-hydroxysteroid dehydrogenase complementary deoxyribonucleic acid: tissue and temporal distribution of its messenger ribonucleic acid during fetal and neonatal development. *Endocrinology* **131** (1992) 2120–2126.
23. Shaw D. A. and Quincey R. V.: The preparation of tritium-labelled cortisol metabolites of high specific activity. *J. Endocr.* **26** (1963) 577–578.
24. Monder C. and Lakshmi V.: Evidence for kinetically distinct forms of corticosteroid 11 beta-dehydrogenase in rat liver microsomes. *J. Steroid Biochem.* **32** (1989) 77–83.
25. Lakshmi V., Nath N. and Muneyyirci Delale O.: Characterization of 11 beta-hydroxysteroid dehydrogenase of human placenta: evidence for the existence of two species of 11 beta-hydroxysteroid dehydrogenase. *J. Steroid Biochem. Molec. Biol.* **45** (1993) 391–397.
26. Henderson P. J. F.: Statistical analysis of enzyme kinetic data. In *Enzyme Assays: A Practical Approach* (Edited by R. Eisenthal and M. J. Danson) Oxford University Press, New York, (1992) pp. 277–316.
27. Monder C., Stewart P. M., Lakshmi V., Valentino R., Burt D. and Edwards C. R.: Licorice inhibits corticosteroid 11 beta-dehydrogenase of rat kidney and liver: *in vivo* and *in vitro* studies. *Endocrinology* **125** (1989) 1046–1053.
28. Monder C., Lakshmi V. and Miroff Y.: Kinetic studies on rat liver 11 beta-hydroxysteroid dehydrogenase. *Biochim. Biophys. Acta* **1115** (1991) 23–29.
29. Lakshmi V. and Monder C.: Purification and characterization of the corticosteroid 11 beta-dehydrogenase component of the rat liver 11 beta-hydroxysteroid dehydrogenase complex. *Endocrinology* **123** (1988) 2390–2398.
30. Agarwal A. K., Monder C., Eckstein B. and White P. C.: Cloning and expression of rat cDNA encoding corticosteroid 11β-dehydrogenase. *J. Biol. Chem.* **264** (1989) 18939–18943.
31. Tannin G. M., Agarwal A. K., Monder C., New M. I. and White P. C.: The human gene for 11β-Hydroxysteroid dehydrogenase. *J. Biol. Chem.* **266** (1991) 16653–16658.
32. Krozowski Z. and Funder J. W.: Renal mineralocorticoid receptors and hippocampal corticosterone-binding species have identical intrinsic steroid specificity. *Prtoc. Natn. Acad. Sci. U.S.A.* **80** (1983) 6056–6060.
33. Arriza J. L., Weinberger C., Cerelli G., Glaser T. M., Handelin B. L., Housman D. E. and Evans R. M.: Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor. *Science* **237** (1987) 268–275.
34. Brown R. W., Chapman K. E., Edwards C. R. and Seckl J. R.: Human placental 11 beta-hydroxysteroid dehydrogenase: evidence for and partial purification of a distinct NAD-dependent isoform. *Endocrinology* **132** (1993) 2614–2621.
35. Bettins I. Z., Kowarski A., Shermeta D. W., De Lemos R. A. and Migeon C. J.: Fetal and maternal secretion rate of cortisol in sheep: diffusion resistance of the placenta. *Pediat. Res.* **4** (1970) 129–134.
36. Nikkilä H., Tannin G. M., New M. I., Taylor N. F., Kalaitzoglou G., Monder C. and White P. C.: Defects in the HSD11 gene encoding 11β-hydroxysteroid dehydrogenase are not found in patients with apparent mineralocorticoid excess or 11-oxoreductase deficiency. *J. Clin. Endocr. Metab.* **77** (1993) 687–691.