CLINICAL STUDY

Expression of renal 11 β -hydroxysteroid dehydrogenase type 2 is decreased in patients with impaired renal function

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Abstract

Objective: Renal 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) enables selective access of aldosterone to the mineralocorticoid receptor (MR). Impaired 11 β -HSD2 activity has been suggested in patients with hypertension as well as in patients with renal disease, where it may contribute to sodium retention, oedema and hypertension. To date, these studies have relied upon urinary cortisol (F) metabolite levels as surrogate markers of renal 11 β -HSD2 activity.

Methods: We have directly analysed renal 11 β -HSD2 mRNA expression in 95 patients undergoing kidney biopsy using TaqMan real-time PCR. Serum and 24-h urine samples were used to document underlying renal function and endocrine parameters. Urinary F and cortisone (E) metabolites were analysed using gas chromatography/mass spectrometry.

Results: Expression of 11 β -HSD2 did not correlate with blood pressure or urinary Na/K ratio, but a significant positive correlation with creatinine clearance was observed ($r = 0.284$; $P < 0.01$). Immunofluorescence and confocal laser microscopy confirmed decreased 11 β -HSD2 expression in patients with impaired renal function. For the first time, we showed that 11 β -HSD2 mRNA expression correlated negatively with the urinary free (UF) F/E (UFF/UFE) ratio ($r = 0.276$; $P < 0.05$) as well as with the urinary tetrahydrocortisol + 5 α -tetrahydrocortisol/tetrahydrocortisone ((THF + α THF)/THE) ratio ($r = 0.256$; $P < 0.05$). No difference in 11 β -HSD2 mRNA expression or in the UFF/UFE ratio was found between groups with no proteinuria, microalbuminuria, moderate or severe proteinuria. In contrast, the urinary (THF + α THF)/THE ratio increased significantly ($P < 0.05$) in patients with severe albuminuria, suggesting increased hepatic 11 β -HSD1 in those patients.

Conclusions: These data suggest that renal 11 β -HSD2 expression may be represented only marginally better, if at all, by the UFF/UFE than by the (THF + α THF)/THE ratio. Reduced renal 11 β -HSD2 expression may lead to occupancy of the MR by glucocorticoids such as cortisol and may contribute to the increased sodium retention seen in patients with impaired renal function.

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Introduction

Two distinct isozymes of 11 β -hydroxysteroid dehydrogenase (11 β -HSD) catalyse the interconversion of hormonally active cortisol (F) and inactive cortisone (E) (1–3). In human tissues the type 1 enzyme (11 β -HSD1) is widely distributed but most abundant in liver and adipose tissue. It functions mainly as an oxoreductase converting E to F. Conversely, in adult tissues the type 2 isozyme (11 β -HSD2) is found predominantly in mineralocorticoid receptor (MR) target tissues, kidney, colon and salivary gland, where it serves to protect the MR from glucocorticoid excess. The MR has the same affinity for cortisol and aldosterone *in vitro* (4), and the inactivation of F to E by 11 β -HSD2 at the site of the MR enables aldosterone

to bind to this receptor *in vivo* (5, 6). Mutations in the gene encoding 11 β -HSD2 (HSD11B2) account for an inherited form of hypertension, the syndrome of Apparent Mineralocorticoid Excess (AME) where F induces hypertension and hypokalaemia (7–11). A similar clinical picture to AME occurs following the ingestion of liquorice and carbenoxolone, which are competitive inhibitors of 11 β -HSD2 (12). Increased sensitivity to salt is a forerunner to 'essential' hypertension and polymorphic variability in the HSD11B2 gene with an impaired 11 β -HSD2 activity in part determines salt sensitivity (11, 13, 14). Evidence suggests impaired 11 β -HSD2 activity in some patients with hypertension but also in patients with renal disease where it may contribute to sodium retention, oedema and hypertension. To date, these studies have relied upon

urinary F metabolite analyses as markers of renal 11 β -HSD2 activity (15, 16). For the first time, we have directly assessed human renal 11 β -HSD2 mRNA expression and correlated this to urinary F metabolite ratios. We also investigated renal 11 β -HSD2 mRNA expression and urinary F metabolite ratios in patients with impaired renal function and proteinuria.

Materials and methods

Subjects

All patients ($n = 95$) were recruited from the Department of Nephrology, Queen Elizabeth Hospital, Birmingham, UK, and underwent kidney biopsies because of suspected underlying renal disease having presented with unexplained protein- and/or haematuria and/or a rise in serum creatinine concentrations. Patients with acute renal failure were excluded as were those taking spironolactone or prednisolone. Other forms of secondary hypertension, e.g. renal artery stenosis, were excluded. The study had the approval of the local research ethics committee, and all patients had given written informed consent prior to inclusion in the study.

Blood pressure was measured three times before the kidney biopsy and the average was calculated. Height and weight were recorded for body mass index (BMI) calculations. Basic demographic data, information on associated diseases, and current medication were obtained for all subjects. For renal biopsies we used a standard 16-gauge needle with a semi-automatic gun delivering a tissue biopsy cylinder measuring approximately 1.5 to 2 cm in length and 1 mm in width. Renal biopsy material was simultaneously analysed for histology and tissue was rapidly frozen at -80°C for further processing. Histological diagnoses were made on the formalin-saline and glutaraldehyde fixed biopsy by established methods using light microscopy, immunohistochemistry, and electron microscopy.

Serum and urine sampling

Urea, creatinine, albumin, electrolytes (sodium, potassium, chloride, magnesium), haemoglobin, platelets, bilirubin and aspartate aminotransferase (AST) were measured in serum samples using standard laboratory methods (Instrumentation Laboratory, Birchwood Warrington, UK). Creatinine clearance (CCR) was calculated by the Cockcroft-Gault's formula (17): $\text{CCR (ml/min)} = [1.23 \text{ (for men) or } 1.04 \text{ (for women)} \times (140/\text{age}) \times \text{weight (kg)}] / \text{serum creatinine } (\mu\text{mol/l})$. Additional calculations were performed using the Modification of Diet in Renal Disease (MDRD) study equation (glomerular filtration rate (GFR) in $\text{ml/min}/1.73 \text{ m}^2 = 170 [\text{plasma-creatinine in mg/dl}]^{-0.989} \times [\text{age}]^{-0.176} \times [0.762 \text{ if patient is female}] \times [1.18 \text{ if patient is black}] \times [\text{serum urea}$

$\text{nitrogen in mg/dl}]^{-0.17} \times [\text{serum albumin in g/dl}]^{+0.318}$) (18). This estimate of renal function by the MDRD study equation did not significantly alter our observations using the Cockcroft-Gault's formula.

Twenty-four-hour urine sampling was performed at least one day prior to kidney biopsies. Creatinine, albumin and electrolytes were measured in urine samples.

Urinary steroid analysis

Urinary free cortisol (UFF), free cortisone (UFE), tetrahydrocortisol (THF), 5 α -tetrahydrocortisol (α THF) and tetrahydrocortisone (THE) were analysed using gas chromatography/mass spectrometry as previously described (19).

RNA extraction and RT

Kidney biopsies were homogenised on ice using a metal Ultra-Turrax homogeniser treated with RNase Zap (Ambion Ltd., Huntingdon, Cambridgeshire, UK). After homogenisation, samples were centrifuged at 12 000 g for 10 min at 4°C . Total RNA was extracted from supernatants using the Tri Reagent extraction method (Sigma, UK). RNA integrity was assessed by electrophoresis on 1% agarose gels and the quantity determined spectrophotometrically at OD₂₆₀. RNA was processed into cDNA as previously described (20).

Quantitative RT-PCR

Expression of 11 β -HSD2 mRNA was analysed using an ABI Prism 7700 sequence detection system (Perkin-Elmer Applied Biosystems, Beaconsfield Bucks, UK) that employs TaqMan chemistry for highly accurate quantification of mRNA levels as previously described (21). Reactions were performed in 25- μl volumes on 96-well plates, in buffer containing TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and 25 ng cDNA template. All reactions were multiplexed with the housekeeping gene 18S (Perkin-Elmer). Reactions were as follows: 50°C for 2 min, 95°C for 10 min, and then 44 cycles of 95°C for 15 s and 60°C for 1 min. Oligonucleotide primers and a TaqMan probe for 11 β -HSD2 were as follows: forward, 5'-GGGCTATGGA-ACCTCAA-3'; reverse 5'-GACCCACGTTTCTCACTGACTCT-3'; probe 5'-CCGTGGCGCTACTCATGGACACA-3'. The probe was produced by Applied Biosystems (Applied Biosystems UK, Woolston, Warrington). According to the manufacturer's guidelines, data were expressed as CT values (the cycle number at which logarithmic PCR plots cross a calculated threshold line) and used to determine ΔCT values ($\Delta\text{CT} = \text{CT of the target gene minus CT of the housekeeping gene}$; high ΔCT values represent low levels of expression). Fold changes in expression were calculated according to the transformation: $\text{fold increase} = 2^{-\text{difference in } \Delta\text{CT}}$. The CT values of 11 β -HSD2 expression measured by real-time PCR varied

between 21 and 38 cycles with an overall mean of 31 cycles, and the CT values of 18S expression varied between 12 and 25 cycles with an overall mean of 19 cycles.

Immunofluorescence and confocal laser microscopy

Immunofluorescence and confocal laser microscopy for 11 β -HSD2 were performed as previously described (22). Briefly, the processed sections were incubated with a 1:25 dilution of 11 β -HSD2 antiserum for 60 min, washed in PBS for 10 min, and then incubated with a 1:25 dilution of an antidonkey sheep fluorescent conjugate (Binding Site, Birmingham, UK) for 30 min. Sections were washed in PBS for 60 min and then counterstained with the nuclear-specific propidium iodide and rinsed in PBS. Sections were mounted in glycerol containing 1,4-diazabicyclo(2.2.2) octane and examined under a Zeiss Universal fluorescent microscope as previously reported (23). A Bio-Rad 500 laser scanning confocal system (Bio-Rad, Richmond, CA, USA) attached to a Leitz SM-Lux microscope (Leitz, Rockleigh, NJ, USA) was used to analyse the sections through an optical section of approximately 0.1 μ m, as described previously (22, 24). Biopsies from 10 patients were analysed in a blinded fashion by two independent investigators. Five 'normal' biopsies were from patients with microscopic haematuria, normal protein excretion and normal renal function, the other 5 biopsies were from patients with impaired renal function (creatinine clearance < 60 ml/min (25)). For each patient, five randomly selected microscopic fields were used to determine the area of 11 β -HSD2 positive-stained cells compared with the total remaining renal tissue.

Statistical analysis

Data are expressed as means $\pm$ S.D. unless otherwise stated. Statistical analysis on real-time PCR data were performed on mean Δ CT values (and not on fold changes) to exclude potential bias owing to averaging data that had been transformed through the equation $2^{-\Delta\Delta\text{CT}}$. Statistical analysis of comparisons between groups was undertaken using paired and unpaired *t*-tests where appropriate, otherwise the Mann-Whitney Rank Sum Test was used. For correlations, a stepwise multiple regression analysis, including corrections for multiple comparisons, was performed using the SPSS software package (SPSS Inc., Chicago, IL, USA) as well as Pearson's correlations.

Results

Clinical data

Fifty-three patients were men, and forty-two were women. Seventy-six patients were of Caucasian origin,

twelve of Asian and seven of African-Caribbean origin. The main diagnoses are shown in Table 1. The most frequent diagnosis was thin glomerular basement disease. Those patients received a biopsy mostly due to persistent microscopic haematuria. They showed very thin basement membranes on electron microscopy and usually had a good prognosis if there were no additional risk factors present such as hypertension. The second most frequent diagnosis was chronic ischaemic renal damage, including hypertensive damage, which is common in renal biopsy specimens from middle aged and old people. In our population IgA nephropathy was the third most common diagnosis. These specimens were taken to investigate macroscopic haematuria and showed immunohistochemical deposition of IgA. The rest of our population consisted of a very broad spectrum of different diagnoses reflecting referral to the specialised nephrology services at a UK university hospital. Relevant clinical data (median, range) were as follows: age 49.3 years (18–83 years), BMI 26.8 kg/m² (16.6–44.5 kg/m²), systolic blood pressure 134 mmHg (90–171 mmHg), diastolic pressure 74 mmHg (45–96 mmHg), and mean arterial pressure 94 mmHg (60–117 mmHg). Clinically relevant laboratory results (median, range) were as follows: serum albumin 38.3 g/l (10–50 g/l), urea 10.0 mmol/l (2.2–35.6 mmol/l), haemoglobin 7.8 mmol/l (= 12.6 g/dl) (3.8–11 mol/l = 6.1–17.7 g/dl), platelets 258 $\times 10^6$ /ml (121–614 $\times 10^6$ /ml), serum creatinine 177 μ mol/l (67–1110 μ mol/l), creatinine clearance 68.6 ml/min (7.4–150.6 ml/min), urinary albumin excretion 1.25 g/24 h (0–18.0 g/24 h). Men presented with higher systolic (139.5 $\pm$ 15.6 mmHg vs 127.6 $\pm$ 18.8 mmHg; *P* < 0.005) and diastolic (76.6 $\pm$ 8.8 mmHg vs 71.4 $\pm$ 12.9 mmHg; *P* < 0.05) blood pressure than women, and with a lower creatinine clearance than women (59.7 $\pm$ 40.0 ml/min vs 78.3 $\pm$ 30.1 ml/min; *P* < 0.05). Forty-four patients had an underlying diagnosis of hypertension. In total, 48 patients received anti-hypertensive therapy due to

Table 1 Principal histological diagnosis of patients (*n* = 95) who underwent renal biopsy.

Thin glomerular basement membrane disease	23
Chronic ischaemic renal damage	22
IgA nephropathy	18
Focal segmental sclerosing GN	8
Diabetic glomerulopathy	5
Lupus nephritis	3
Mild IgM mesangial GN	3
Vasculitis	3
Nodular light chain glomerulopathy with myeloma	3
Amyloidosis	2
Minimal change nephropathy	2
Membranous nephropathy with renal vasculitis	1
Interstitial nephritis	1
Metastatic carcinoma in kidney	1

GN, glomerulonephritis.

hypertension and/or proteinuria: 14 single therapy, 22 with double therapy, 6 with three medications and 6 with four medications; 18 of them received furosemide, 14 angiotensin converting enzyme (ACE) inhibitors, 5 thiazides given either as single therapy or in combination among each other or in combination with Ca-antagonists or β -blockers.

Urinary cortisol metabolites and 11 β -HSD2 mRNA expression

Using a cut-off of 60 ml/min for creatinine clearance (25), 58 patients had good or slightly impaired renal function and 37 patients moderately or severely impaired renal function. Patients with decreased renal function had higher systolic (142.7 ± 15.6 mmHg vs 128.9 ± 17.4 mmHg; $P < 0.001$) and diastolic (77.3 ± 11.0 mmHg vs 72.5 ± 10.7 mmHg; $P < 0.05$) blood pressure. Patients with decreased renal function showed decreased 11 β -HSD2 mRNA expression (Δ CT: 13.5 ± 3.0 vs 11.6 ± 2.5 ; $P < 0.005$). Accordingly, 11 β -HSD2 mRNA expression correlated significantly ($r = 0.284$; $P < 0.01$) with renal function (Fig. 1). Patients with decreased renal function had significantly higher UFF/UFE (1.12 ± 0.54 vs 0.89 ± 0.48 ; $P < 0.05$) (Fig. 2A) and (THF + α THF)/THE ratios (1.81 ± 0.80 vs 1.24 ± 0.66 ; $P < 0.001$) (Fig. 2B). We did not find a significant correlation between total amount of cortisol metabolites and renal function. 11 β -HSD2 mRNA expression correlated significantly with the UFF/UFE ratio ($r = 0.276$; $P < 0.05$) (Fig. 3A) as well as with the urinary (THF + α THF)/THE ratio ($r = 0.256$; $P < 0.05$) (Fig. 3B).

After correction for age and renal function no gender difference was found in 11 β -HSD2 mRNA expression or in urinary cortisol metabolites. No correlation was found between systolic or diastolic blood pressure, urinary Na/K ratio and 11 β -HSD2 mRNA expression or urinary cortisol metabolites ratios.

We analysed the data with regard to urinary albumin excretion (26, 27): patients with no albuminuria (< 30 mg/24 h, $n = 30$), with microalbuminuria (30–300 mg/24 h, $n = 26$), with moderate albuminuria (300 mg–2 g/24 h, $n = 17$) and with severe

Figure 1 Tissue 11 β -HSD2 mRNA expression (in Δ CT values) in renal biopsies from 95 patients in correlation to their renal function (creatinine clearance). High Δ CT values represent low levels of expression and vice versa.

Figure 2 Correlation between renal function (creatinine clearance) and (A) the urinary free cortisol/urinary free cortisone (UFF/UFE) ratio, and (B) the urinary (tetrahydrocortisol (THF) + α THF)/tetrahydrocortisone (THE) ratio in 95 patients receiving renal biopsy.

Figure 3 Correlation between tissue 11 β -HSD2 mRNA expression (in Δ CT values) and (A) the urinary free cortisol/urinary free cortisone (UFF/UFE) ratio, and (B) the urinary (tetrahydrocortisol (THF) + α THF)/tetrahydrocortisone (THE) ratio in 95 patients receiving renal biopsy. High Δ CT values represent low levels of expression and vice versa.

albuminuria (>2 g/24 h, $n = 14$). As predicted, patients with no albuminuria had the best renal function (creatinine clearance: 88.3 ± 29.1 ml/min vs microalbuminuria: 59.5 ± 40.1 ml/min ($P < 0.005$), moderate albuminuria: 64.8 ± 36.5 ml/min ($P < 0.05$), and severe albuminuria: 50.0 ± 31.7 ml/min ($P < 0.001$), the lowest systolic (127.6 ± 18.4 mmHg vs 135.3 ± 15.2 mmHg (not significant (n.s.)), 143.1 ± 14.1 mmHg ($P < 0.005$), 144.0 ± 17.1 mmHg ($P < 0.05$) respectively) and lowest diastolic (71.0 ± 10.4 mmHg vs 74.2 ± 11.0 mmHg (n.s.), 79.1 ± 8.6 mmHg ($P < 0.01$), 78.1 ± 10.1 mmHg (n.s.) respectively) blood pressure. We found no significant difference in 11 β -HSD2 mRNA expression (Fig. 4) or UFF/UFE ratio (Fig. 5) between the groups with different severity of albuminuria, but patients with severe proteinuria had significantly increased urinary (THF + α THF)/THE ratio (2.05 ± 0.83 vs no albuminuria: 1.37 ± 0.83 ($P < 0.05$), microalbuminuria: 1.58 ± 1.15 (n.s.), moderate albuminuria: 1.36 ± 0.65 ($P < 0.05$)) (Fig. 5). No change in the urinary THF/ α THF ratio reflecting 5 α -reductase activity was found between the groups (Fig. 5). No significant difference in 11 β -HSD2 mRNA expression, and urinary UFF/UFE and THF/ α THF ratios was found between the groups of diagnosis. We could not detect a difference in 11 β -HSD2 expression between patients who received ACE inhibitors and/or thiazides and/or furosemide, compared with those on no medication after adjustment for age and renal function.

Immunofluorescence

Compared with the intense expression of 11 β -HSD2 immunoreactive protein in collecting ducts in kidneys from two patients with 'normal' renal function (Fig. 6A and B), a much weaker 11 β -HSD2 immunofluorescence signal was found in renal biopsies from

Figure 4 Fold increase in 11 β -HSD2 mRNA expression in renal biopsies from 95 patients in relation to the severity of their proteinuria. 11 β -HSD2 mRNA expression in patients with no proteinuria (<30 mg/24 h) was set at 1.

Figure 5 Urinary free cortisol/urinary free cortisone (UFF/UFE) ratio, urinary (tetrahydrocortisol(THF) + α THF)/tetrahydrocortisone (THE) ratio and urinary THF/ α THF ratio in 95 patients in relation to the severity of their proteinuria.

four patients with an impaired renal function (creatinine clearance <60 ml/min (25)) (Fig. 6C to F). Semi-quantitative analysis of 11 β -HSD2 immunofluorescent-positive cells revealed significantly more positive staining in correlation to the remaining renal tissue in biopsies from patients with 'normal' renal function ($n = 5$) than from patients with impaired renal function ($n = 5$) (Fig. 7).

Discussion

In the human kidney, 11 β -HSD2 is expressed in cortical and medullary collecting ducts (22) as well as in the glomerulus (28), and is responsible for the conversion of cortisol to hormonally inactive cortisone thus conferring the *in vivo* specificity of the MR for aldosterone (5, 6).

At the present time, cortisol and its urinary metabolites (cortisone, THF, α THF and THE) are measured as surrogate markers for the *in vivo* assessment of 11 β -HSD1 and -2 activities. The urinary (THF + α THF)/THE ratio has historically been used to diagnose 11 β -HSD2-deficient states, such as AME (3, 29, 30), but has also been used to estimate hepatic 11 β -HSD1 activity (31–34). The ratio probably provides an index of 'global' 11 β -HSD activity within the body, i.e. principally 11 β -HSD1 in the liver and fat and 11 β -HSD2 in the kidney. Changes in 11 β -HSD1 activity may be reflected by the urinary (THF + α THF)/THE ratio, but only if 11 β -HSD2 and 5 α - and 5 β -reductase activities are not altered (the latter assessed by the THF/ α THF ratio). In contrast, we and others have suggested that renal 11 β -HSD2 activity may be better reflected by the urinary free cortisol/free cortisone (UFF/UFE) ratio than by the (THF + α THF)/THE ratio (19, 35, 36). Normal subjects excrete two- to threefold more urinary free cortisone than free cortisol, indicative of renal 11 β -HSD2-mediated conversion of cortisol to cortisone (37). In AME, UFE

Figure 6 Confocal laser microscopy of 11 β -HSD2 immunofluorescence in kidney biopsies. The images depict the distribution of immunofluorescence signal as scanned through an optical section of approximately 0.1 μ m. The merged images of epithelial cells in collecting ducts stained with 11 β -HSD2 anti-serum (green signal) and propidium iodide (red signal) to highlight nuclei are shown from two normal biopsies (A, B) and four biopsies from patients with renal disease and loss of renal function (C-F).

excretion is virtually undetectable (19) resulting in a high UFF/UFE ratio. Serum cortisol and cortisone concentrations have been used in a number of studies (20, 38–41). Whitworth *et al.* documented reduced plasma cortisone concentrations in patients with loss of renal function (37), which was confirmed by other investigators (39–41).

For the first time, we were able to measure 11 β -HSD2 mRNA expression in renal biopsies of 95 patients. We have to emphasise that the results of our 11 β -HSD2 mRNA expression data have to be interpreted carefully due to the fact that the kidney is a very heterogenous organ and, in renal biopsies, the ratios between various anatomical structures might vary considerably. In addition, our data are difficult to interpret due to the additional facts of interindividual differences in salt intake, the broad spectrum of

different histological diagnoses and the number of antihypertensive drugs prescribed.

We correlated the 11 β -HSD2 mRNA expression data to urinary cortisol metabolite ratios. We observed that the UFF/UFE ratio was significantly negatively correlated with renal 11 β -HSD2 mRNA expression; a low UFF/UFE ratio corresponded to a high 11 β -HSD2 mRNA expression. The urinary (THF + α THF)/THE ratio was also negatively correlated with renal 11 β -HSD2 mRNA expression. However, in specific situations the urinary (THF + α THF)/THE ratio may be misleading if used as the sole evaluation of 11 β -HSD2 activity. Thus, when we divided our patients into groups with no proteinuria, microalbuminuria, moderate and severe proteinuria, although there was no change in 11 β -HSD2 mRNA expression or the UFF/UFE ratio between the groups, the urinary (THF + α THF)/THE ratio was increased in patients with severe proteinuria. We suggest that this increased urinary (THF + α THF)/THE ratio is likely to reflect increased hepatic 11 β -HSD1. In patients with nephrotic syndrome, an increase in the urinary (THF + α THF)/THE ratio was observed by Vogt *et al.* (15), but interpreted as an impaired 11 β -HSD2 activity, yet neither UFF nor UFE were measured. In an additional study by Vogt *et al.* (42) using rats with puromycin aminonucleoside (PAN)-induced proteinuria and with adriamycin nephrosis, the authors showed an increased urinary TH-corticosterone + α TH-corticosterone)/11-dehydro-TH-corticosterone ratio which is in accordance with our finding in patients with nephrotic syndrome. In contrast to Vogt *et al.*, we believe our evidence suggests that this altered ratio is due to increased hepatic 11 β -HSD1 activity rather than decreased 11 β -HSD2 activity, because 11 β -HSD2 mRNA expression and the UFF/UFE ratio were not altered in this group.

Figure 7 Area of 11 β -HSD2 immunofluorescent positive cells in relation to the remaining renal tissue in kidney biopsies from 5 patients with normal renal function and 5 patients with renal insufficiency. Results are means $\pm$ S.E.M.

The cause of this putative upregulation of hepatic 11 β -HSD1 activity proposed by us remains to be elucidated.

Due to our findings, we suggest that human renal 11 β -HSD2 mRNA expression decreases with impairment of renal function and this is correlated with the UFF/UFE and the (THF + α THF)/THE ratio. In addition, we performed immunofluorescence and confocal laser microscopy for 11 β -HSD2 in a smaller number of renal biopsies and found that protein expression was also diminished in patients with loss of renal function. But it must be taken into account that our protein expression data is semi-quantitative at best and needs to be interpreted with care. Nevertheless, our data are supported by earlier clinical studies: patients with chronic renal failure have a prolonged plasma cortisol half-life (2.9 h compared with 2.1 h in controls) (43), and plasma cortisone concentrations have been shown to be inversely correlated with plasma creatinine concentrations (37, 39, 40, 44).

In our study, the reason for the diminished 11 β -HSD2 expression in renal disease is unknown. Zaehner *et al.* (45) screened 587 subjects, including 260 patients with end-stage renal disease, for mutations in exons 2 through 5 of the HSD11B2 gene, and found a high prevalence of single-nucleotide polymorphisms (SNPs) but no exonic mutations. It is unknown if these might result in altered 11 β -HSD2 mRNA expression. A further factor may be hypoxia, which is known to be associated with several renal tubular diseases. Hypoxia has been shown to significantly decrease 11 β -HSD2 in ovine fetal kidney (46). Heiniger *et al.* (47) have documented decreased renal 11 β -HSD2 mRNA levels after induction of unilateral renal ischaemia in rats, thought to be mediated by MAP kinase-dependent upregulation of the early growth response gene (Egr-1). Besides tubular expression, 11 β -HSD2 is reported to be found in the glomerulus (28), but its specific glomerular function is not known. It might regulate the access of cortisol to glomerular glucocorticoid and/or mineralocorticoid receptors, and its dysregulation might be involved in glomerular disease.

Despite the limitations of our study and the difficulties of interpretation, as pointed out above, we suggest that the defective renal cortisol metabolism with consequent access of cortisol to the MR could contribute to some of the consequences of renal disease, such as hypertension and sodium retention, or may modify others, such as hyperkalaemia and decreased glomerular filtration rate. Several authors have also suggested that impaired 11 β -HSD2 activity may be involved in the pathogenesis of essential hypertension (48–50). However, in our study involving patients with a diversity of renal diseases, no relationship was found between the UFF/UFE ratio or 11 β -HSD2 mRNA expression and electrolytes or blood pressure.

Taking the limitations of our study into account, we think to have shown reduced expression of renal 11 β -HSD2 in patients with impaired renal function. Our data indicate that this is not influenced by underlying proteinuria or a diagnosis of hypertension but may be directly correlated with kidney function as reflected by creatinine clearance. In addition to secondary hyperaldosteronism as a consequence of renal hypoperfusion, impaired 11 β -HSD2 expression may lead to occupancy of the MR by glucocorticoids such as cortisol and may contribute to the increased sodium retention seen in patients with impaired renal function. In addition, this study suggests that the UFF/UFE ratio directly correlates with renal 11 β -HSD2 expression and activity and that the UFF/UFE ratio is only marginally better, if at all, than the urinary (THF + α THF)/THE ratio.

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