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11β-HYDROXYSTEROID DEHYDROGENASE MEDIATES REDUCTIVE METABOLISM OF XENOBIOTIC CARBONYL COMPOUNDS

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Abstract—The enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) is considered to confer mineralocorticoid specificity on the non-selective Type I adrenocorticoid receptor by converting active 11-hydroxyglucocorticoids to receptor-inactive 11-oxo metabolites, in mineralocorticoid target tissues like the kidney. However, 11β-HSD is also present in the liver, where it may regulate steroid exposure to the glucocorticoid Type II receptor. Because of the much higher activities compared to that in kidney, liver 11β-HSD possibly has additional functions besides the metabolism of glucocorticoids. In the present investigation we have isolated 11β-HSD from mouse liver microsomes and demonstrate that the homogeneously purified enzyme is also capable of catalyzing the reductive metabolism of xenobiotic carbonyl compounds such as metyrapone, *p*-nitroacetophenone and *p*-nitrobenzaldehyde. Enzyme kinetic studies revealed that, in addition to $NADP^+$, mouse liver 11β-HSD also accepts NAD^+ as cosubstrate for glucocorticoid 11β-dehydrogenation. NADH as cosubstrate for 11-oxoreduction plays only a minor role compared to that with NADPH, a fact which is also true for xenobiotic carbonyl reduction. Inhibition experiments revealed strong sensitivity of xenobiotic carbonyl reduction to glucocorticoids. The competitive nature of this inhibition suggests that both glucocorticoids and xenobiotic carbonyl substances bind to the same catalytically active site of 11β-HSD. High enzyme activities were also found in microsomal fractions of the ovary and adrenal gland but, although expressing considerable glucocorticoid 11-dehydrogenation activity (one third that of liver), almost no carbonyl reduction was detectable in kidney microsomes. Immunoblot analysis with polyclonal antibodies directed against the liver 11β-HSD did not yield an immunological crossreaction in the same tissues. In conclusion, corresponding to the cytosolic aldo-keto reductases, microsomal 11β-HSD of liver may be considered to play a role in the phase I biotransformation of pharmacologically relevant carbonyl substances as well as protecting organisms against toxic carbonyl compounds by converting them to less lipophilic and more soluble and conjugatable metabolites. Discrepancies in bioactivity together with the lack of response to anti-liver 11β-HSD antibodies strongly indicate the existence of distinct forms of 11β-HSD to be present in kidney, adrenal gland and ovary. The ability of xenobiotic carbonyl reduction might be another distinguishing feature among the various 11β-HSD isozymes.

Key words: 11β-hydroxysteroid dehydrogenase; glucocorticoid metabolism; carbonyl reduction; phase I drug metabolism; detoxification; metyrapone

11β-HSD† (EC 1.1.1.146) is a 34 kDa microsomal enzyme responsible for the interconversion of the active 11-hydroxyglucocorticoids cortisol and corticosterone to the inactive 11-oxo forms cortisone and dehydrocorticosterone [1]. A physiological role for 11β-HSD has recently been demonstrated in the kidney, where it protects mineralocorticoid receptors from exposure to active glucocorticoid, thereby allowing preferential access for aldosterone [2, 3]. Diminished 11β-HSD activity has been associated with the clinical syndrome of apparent mineralocorticoid excess, which is characterized by sodium retention, potassium wasting and hypertension, without measurable increases in aldosterone [4, 5]. Similar effects have been observed in individuals after ingestion of large quantities of liquorice containing glycyrrhetinic acid [6], a potent inhibitor of 11β-HSD [2]. However, 11β-HSD activity is also found in glucocorticoid target tissues, notably the liver [7, 8], where it may regulate steroid exposure to the glucocorticoid receptor. Because of the much higher activities of 11β-HSD in the liver compared to that in the kidney, the liver enzyme possibly has additional functions besides the metabolism of glucocorticoids. The conversion of 11β-hydroxycorticosteroids to 11-oxocorticosteroids is reversible *in vivo* under normal circumstances and it has long been discussed whether 11β-dehydrogenation and 11-oxoreduction are catalyzed by a single bidirectional enzyme or by two distinct enzymes [9, 10]. Purified 11β-HSD from rat liver microsomes did not show any 11-oxoreducing activity [11], supporting the two enzyme hypothesis, but, in contrast, expression of the 11β-HSD cDNA in CHO cells resulted in an enzyme with both activities, dehydrogenase and reductase [12], casting doubt on the two enzyme theory.

In previous investigations we established the

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† Abbreviations: 11β-HSD, 11β-hydroxysteroid dehydrogenase; 3α-HSD, 3α-hydroxysteroid dehydrogenase; SCAD, short chain alcohol dehydrogenases.

existence of a 34 kDa enzyme in mouse liver microsomes, capable of reducing the carbonyl group of the cytochrome P450 inhibitor metyrapone [13]. According to its subcellular localization in the endoplasmic reticulum and its insensitivity towards the diagnostic carbonyl reductase inhibitor quercitrin, this microsomal enzyme differed from the common characteristics of the cytosolic carbonyl reductases [14]. Surprisingly, N-terminal amino acid sequence analysis revealed an approximate homology of this protein of 54% to rat liver 11β-HSD. We then could demonstrate that the homogeneous enzyme is indeed involved in the reversible 11β-oxidoreduction of glucocorticoids [15]. In addition, upon improving the purification procedure, homogeneous mouse liver 11β-HSD retains both 11-dehydrogenation as well as 11-oxoreducing activities of glucocorticoid metabolism, thus disproving the two enzyme theory of 11β-HSD [15].

The intention of the present study was to answer the question of whether the microsomal metyrapone reducing activity and the glucocorticoid 11β-oxidoreducing activity in fact reside within the same protein, which would mean that these reactions are mediated by the same enzyme. Based on the copurification of glucocorticoid 11β-dehydrogenation, glucocorticoid 11-oxoreducing and xenobiotic carbonyl reducing activities, as well as on kinetic studies with the homogeneously purified enzyme, including competitive inhibition experiments, we provide evidence that mouse liver 11β-HSD is indeed able to catalyze the reductive metabolism of xenobiotic carbonyl compounds. Thus, 11β-HSD may be considered to play a significant role in the phase I biotransformation of pharmacologically relevant carbonyl substances. Moreover, concluding from immunoblot analysis with an antibody specific against the liver 11β-HSD, together with differences in the functional behaviour, distinct isozyme forms of 11β-HSD seem to be present in kidney, adrenal gland and ovary.

MATERIALS AND METHODS

Animals. Livers, kidneys, ovaries and adrenal glands of female NMRI mice (25–30 g) were used throughout the study.

Chemicals. Glucocorticoids (cortisol, cortisone, corticosterone and 11-dehydrocorticosterone) were purchased from Sigma Chemie GmbH (Munich, Germany) and metyrapone from Fluka AG (Buchs, Switzerland). Cofactors NAD^+, NADH, $NADP^+$ and NADPH were from Boehringer Mannheim (Mannheim, Germany). Enzyme purification was carried out with octyl-sepharose CL-4B and red-sepharose A from Pharmacia Fine Chemicals (Uppsala, Sweden) and DEAE-cellulose from Sigma Chemie GmbH (Munich, Germany). Emulgen 913 was supplied by Kao-Atlas Co. (Tokyo, Japan) and sodium cholate by E. Merck (Darmstadt, Germany). Peroxidase conjugated anti-rabbit IgG antibodies were from Dakopatts (Hamburg, Germany). All other chemicals used in the experiments were reagent grade and were obtained from commercial suppliers.

Preparation of microsomes. The animals were killed by means of dislocation of the cervical spinal cord. Livers were perfused with ice-cold 1.15% KCl and homogenized in 4 volumes of 20 mM Tris–HCl buffer, pH 7.4, containing 250 mM sucrose and 1 mM EDTA using a glass–Teflon Potter–Elvehjem homogenizer. Kidneys, ovaries and adrenal glands were prepared in the same way except for perfusion. Ovaries and adrenal glands of six mice, respectively, were pooled per experiment. The homogenates were centrifuged at 600 *g* for 10 min and at 10,000 *g* for 10 min to sediment nuclei, cell debris and mitochondria. The supernatant at this stage was centrifuged at 170,000 *g* for 1 hr to sediment microsomes. The microsomal pellet was resuspended in the Tris/sucrose buffer finally yielding a protein concentration of 3–5 mg/mL (adrenal glands and ovaries) or 15–20 mg/mL (liver and kidneys). All preparations were carried out at 4°.

Purification of liver 11β-*HSD.* For solubilization of membrane associated proteins the microsomal suspension was diluted with an equal volume of a 10 mM sodium phosphate buffer, pH 7.2, containing 1 mM EDTA, 1 M NaCl, 40% glycerol (w/v) and 0.4% (w/v) of the nonionic detergent Emulgen 913. The solution was gently stirred for 45 min and subsequently centrifuged at 210,000 *g* for 60 min. The supernatant was adjusted to 0.4% (w/v) of sodium cholate before being applied to the octyl-sepharose CL-4B column.

For hydrophobic interaction chromatography on octyl-sepharose CL-4B the following buffers were used. Buffer A: 10 mM sodium phosphate, 1 mM EDTA, 500 mM NaCl, 20% (w/v) glycerol, 0.5% (w/v) sodium desoxycholate, pH 7.4; Buffer B: 10 mM sodium phosphate, 1 mM EDTA, 400 mM NaCl, 20% (w/v) glycerol, 0.4% (w/v) sodium cholate, 0.1% (w/v) Emulgen 913, pH 7.4; Buffer C: 10 mM sodium phosphate, 1 mM EDTA, 20% (w/v) glycerol, 2% (w/v) Emulgen 913, pH 7.4.

Solubilized microsomes (maximum 24 mL) were applied to the octyl-sepharose CL-4B column (1.8 × 25 cm) previously equilibrated with 300 mL of buffer A. Elution was performed with buffer A until the end of peak 2, then with buffer B until the end of peak 3 followed by buffer C finally eluting peak 4. The elution profile was monitored measuring the absorbance of the fractions at the wavelength of 417 nm. The column flow rate was 84 mL/hr and the volume per fraction 5 mL. Enzyme activity coincided only with peak 3, the fractions of which were collected, concentrated through an Amicon PM-10 membrane to about 20 mL and dialysed overnight against 5 mM sodium phosphate buffer, pH 7.4.

The dialysed enzyme solution was applied to a column (1.6 × 20 cm) packed with DEAE-cellulose and previously equilibrated with 5 mM sodium phosphate buffer, pH 7.4. The column was washed with the equilibration buffer and the adsorbed enzyme was then eluted with a 40 mM phosphate buffer, pH 7.4, at a flow rate of 36 mL/hr and fraction volumes of 3 mL. Enzymatically active fractions were pooled, concentrated through an Amicon PM-10 membrane to about 2–3 mL and supplemented with glycerol to a final concentration of 30%.

Fractions from DEAE-cellulose chromatography were directly applied to a red sepharose A column

(1.2 × 5 cm), previously equilibrated with a 10 mM phosphate buffer, pH 7.4. The column was rinsed successively with the equilibration buffer, then with the equilibration buffer containing 0.8 mM NaCl and 2 mM NADP and then with the same buffer containing 1 M NaCl and 2 mM NADP. The enzyme was finally eluted with a 10 mM phosphate buffer, pH 7.4, containing 1 M NaCl, 2 mM NADP and 0.1% Emulgen 913. The fractions with high enzyme activity were pooled, concentrated through an Amicon PM-10 membrane to about 2–3 mL and stored in 0.2 mL aliquots at −70°.

Throughout the purification the temperature was kept at 4°.

Enzyme assays. Assay of 11β-dehydrogenation activity was performed by preincubating 20 μL of 50 mM sodium phosphate buffer, pH 9, 10 μL of $NADP^+$ (final concentration 3.2 mM) and 10 μL of 11-hydroxyglucocorticoids (final concentration 1 mM) for 3 min at 37°. Glucocorticoids were dissolved in 50% ethanol. Control velocities were determined in the presence of various quantities of the solvent which did not influence enzyme activity up to an ethanol concentration of 10%. The reaction was started by adding 10 μL of enzyme solution (0.2–0.5 mg of protein). After 30 min incubation time the reaction was stopped and metabolites were extracted by adding 150 μL of ice cold acetonitrile. The samples were centrifuged for 6 min at 8000 *g* in the cold and 20 μL of the supernatant served for the determination of glucocorticoids by HPLC analysis. Specific activities are expressed as nanomoles 11-oxidized glucocorticoids formed per mg of protein within 30 min.

Assay of 11-oxoreducing activity was performed by preincubating 20 μL of 50 mM sodium phosphate buffer, pH 7.4, 10 μL of NADPH (final concentration 3.2 mM) and 10 μL of 11-oxoglucocorticoids (final concentration 1 mM). Incubation conditions and metabolite extraction were the same as described under 11β-dehydrogenation activity, except that incubation periods were 2 hr. Specific activities are expressed as nanomoles of 11-reduced glucocorticoids formed per mg of protein within 30 min.

Assay of carbonyl reducing activity was performed by preincubating 20 μL of 50 mM sodium phosphate buffer, pH 7.4, 10 μL of NADPH (final concentration 3.2 mM) or a NADPH-regenerating system (final concentrations 0.8 mM $NADP^+$, 6 mM G-6-P, 0.35 U G-6-P-DH, 3 mM $MgCl_2$) and 10 μL of xenobiotic carbonyl substrate solution (final concentration 1 mM) for 3 min at 37°. Incubation conditions and metabolite extraction were performed as described under 11β-dehydrogenation activity.

Values of pH 9 for oxidation and pH 7.4 for reduction were chosen according to the pH optima of the respective enzymatic reaction. In all cases control experiments without biological material were performed to determine non-enzymatic substrate conversions.

Kinetic parameter estimations. The determination of purified 11β-HSD enzyme kinetics was performed with substrate concentrations between 0.0125 mM and 10 mM. Time and enzyme protein concentrations were chosen so that reaction velocities were time linear which was found to be true at least for 4 hr. Kinetic parameter estimations were made using the GraphPad InPlot kinetic computer software.

Metabolite determination by HPLC. After enzymic conversion oxidized or reduced metabolites were detected on a BioRad (Munich, Germany) reversed phase HPLC system, with an Octadecyl-Si 100 polyol (Serva, Heidelberg, Germany) matrix column (4.5 mm × 25 cm), a UV monitor and HRLC integration software (BioRad, Munich, Germany). Using an eluent of 30% acetonitrile (v/v) in 0.1% acetic acid, pH 7.4 (flow rate: 1 mL/min) the following retention times were achieved: metyrapol, 6.4 min; metyrapone, 10.3 min; *p*-nitrophenylmethylcarbinol, 11.3 min; *p*-nitroacetophenone, 18.8 min; *p*-nitrobenzalcohol, 8.2 min; *p*-nitrobenzaldehyde, 14.8 min. Substances were monitored at 254 nm.

HPLC separation of glucocorticoids was achieved using a methanol/H_2O (58:42) eluent and a flow rate of 0.5 mL/min. Under these conditions glucocorticoids elute as follows: cortisone, 13.6 min; cortisol, 16.7 min; corticosterone, 25.5 min; dehydrocorticosterone, 15.9 min. Glucocorticoids were monitored at 262 nm.

SDS–PAGE. SDS–PAGE was carried out as described by Laemmli [16] using 10% acrylamide in the separating gel. For immunochemical comparisons, equal amounts of protein (15 μg) were applied per lane.

Immunization and preparation of antisera. One milligram of the enzyme preparation was dissolved in 62.5 mM Tris–HCl, 0.1% (w/v) SDS, 0.5 mM EDTA, pH 6.8 and emulsified with 1 vol. complete Freund's adjuvant. For immunization, a female rabbit was injected subcutaneously (0.9 mL emulsion containing about 0.3 mg protein (2×) or 0.2 mg protein (2×) per injection) four times on the following days: 1/3/5/36, the latter being the booster. Antibody titer determination in the rabbit serum was performed by the immunoblot technique and the antiserum was collected at day 63.

Immunoblot. Electrophoretically separated proteins were transferred to nitrocellulose sheets. Antisera against liver 11β-HSD were diluted 1:1000 and incubated with protein saturated nitrocellulose sheets. Antigen–antibody complexes were detected by peroxidase conjugated secondary antibodies specific for rabbit IgG (dilution 1:1000). These complexes were visualized by the peroxidase reaction (chloronaphthol method).

Protein determination. Protein concentration was determined by the methods of Lowry *et al.* [17] or Bradford [18] using bovine serum albumin as standard.

RESULTS

Table 1 summarizes the purification procedure of 11β-HSD with corticosterone and dehydrocorticosterone as substrates for 11β-dehydrogenation and 11-oxoreduction of glucocorticoids, respectively, as well as with metyrapone as the substrate for xenobiotic carbonyl reduction. The mere solubilization of microsomes already resulted in an increase in specific activity, thus pointing to the phenomenon of enzyme latency. Although 11β-

Table 1. Glucocorticoid 11β-dehydrogenation (11-DH), 11-oxoreduction (11-OR) and xenobiotic carbonyl reduction (CR) catalyzed by mouse liver 11β-HSD during purification

Step	11-DH (μmol/mg/30 min)	11-OR (nmol/mg/30 min)	CR (nmol/mg/30 min)
Microsomes	0.059	4.74	0.016
Solubilized microsomes*	0.169	5.99	0.081
Octyl-sepharose CL-4B	2.959	141.22	1.350
DEAE-cellulose	7.686	307.86	2.670
Red-sepharose A	9.880	481.00	3.640

Enzyme activities were assayed in 50 mM sodium phosphate buffer (pH 9, with 3.2 mM $NADP^+$ and 1 mM corticosterone as substrate for 11β-dehydrogenation; pH 7.4, with a NADPH-regenerating system and 1 mM dehydrocorticosterone as substrate for 11-oxoreduction; pH 7.4, with a NADPH-regenerating system and 1 mM metyrapone as substrate for xenobiotic carbonyl reduction, respectively).

* The solubilized microsomal suspension used in this reaction mixture did not contain sodium cholate.

Table 2. Kinetic properties of purified mouse liver 11β-HSD

Substrate	Cosubstrate	V_{max}	K_m	V_{max}/K_m
Corticosterone	$NADP^+$	9.88	0.66	14.97
	NAD^+	13.42	1.00	13.42
Cortisol	$NADP^+$	3.24	0.34	9.53
	NAD^+	3.27	0.73	4.48
Dehydrocorticosterone	NADPH	0.160	0.22	0.73
	NADH	0.097	0.44	0.22
Cortisone	NADPH	0.149	0.184	0.81
	NADH	0.029	0.239	0.12
Metyrapone	NADPH	0.33	0.50	0.66
	NADH	0.24	1.02	0.24
p-Nitrobenzaldehyde	NADPH	0.79	1.19	0.66
	NADH	0.06	7.40	0.008
p-Nitroacetophenone	NADPH	ND	1.67	ND
	NADH	—	—	—

Kinetic parameters of purified 11β-HSD were calculated from experiments performed with cosubstrate concentrations of 3.2 mM and substrate concentrations between 0.0125 mM and 10 mM. Time and enzyme protein concentrations were chosen so that reaction velocities were time linear. pH values for glucocorticoid conversion were pH 9 for cortisol and corticosterone oxidation, and pH 7.4 for cortisone, dehydrocorticosterone and xenobiotic carbonyl reduction. Kinetic parameter estimations were made using the GraphPad InPlot kinetic computer software. V_{max}: (μmol/30 min/mg); K_m: (mM); V_{max}/K_m: (intrinsic clearance values); values were mean of four individual experiments with less variation than 10% in the case of oxidation and 20% in the case of reduction.
— = No detectable activity; ND = not determined.

HSD latency was significantly higher in the case of xenobiotic carbonyl reduction (factor 5.0) compared to that of glucocorticoid 11-oxoreduction (factor 1.3) and 11-dehydrogenation (factor 2.9), throughout the subsequent purification procedure specific activities of the three reactions almost coincidently increased, indicating that they are mediated by the same enzyme.

The kinetic properties of homogeneously purified 11β-HSD together with the estimated intrinsic clearance values of the respective substrates are presented in Table 2. These data also show that both glucocorticoid 11-oxidoreduction as well as xenobiotic carbonyl reduction can be catalyzed by purified 11β-HSD. Whereas glucocorticoid 11β-dehydrogenation is favoured over glucocorticoid 11-oxoreduction, the latter has approximately equal intrinsic clearance values as xenobiotic carbonyl reduction. However, in contrast to the reversibility of glucocorticoid oxidoreduction, no dehydrogenation of xenobiotic alcohol metabolites could be detected. Comparing the intrinsic clearance values estimated with both pyridine nucleotide forms NADP(H) or NAD(H) it turns out that, in the case of glucocorticoid 11-dehydrogenation, both pyridine nucleotides can function as cosubstrates, whereas for glucocorticoid 11-oxoreduction only weak values were obtained with NADH compared to those with

Table 3. Inhibition of 11β-HSD mediated carbonyl reduction of metyrapone by glucocorticoids

Glucocorticoid	Relative enzyme activity (%)
Cortisone	0
Dehydrocorticosterone	0
Corticosterone	10
Cortisol	26

Metyrapone reducing activities were assayed in sodium phosphate buffer, pH 7.4, in the presence of 0.5 mM metyrapone as substrate, 3.2 mM NADPH as cosubstrate and 0.5 mM glucocorticoid as inhibitor. Percentages were calculated from uninhibited control experiments. Values were mean of three to four individual experiments with no more than 10% variation.

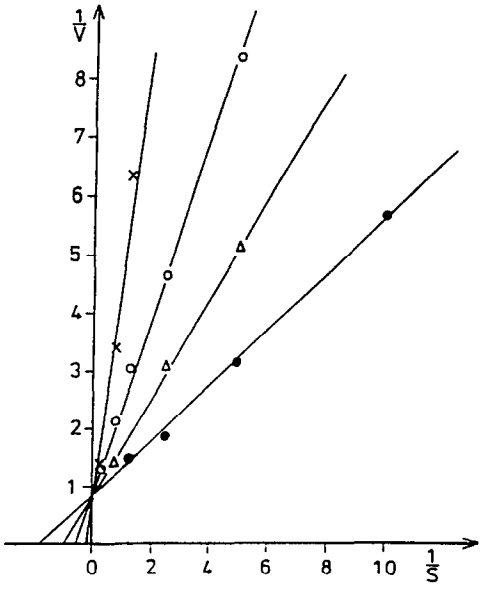

Fig. 1. Competitive inhibition of metyrapone reduction by corticosterone. Activity of purified 11β-HSD was assayed in the presence of substrate concentrations between 6 μM and 1.6 mM metyrapone and inhibitor concentrations of 0.125 mM, 0.25 mM and 0.5 mM corticosterone. (v = μmol/mg/30 min; s = mM.)

NADPH, a fact which is also true for xenobiotic carbonyl reduction.

Table 3 illustrates that glucocorticoids are potent inhibitors of xenobiotic carbonyl reduction. Whereas the 11-hydroxy forms corticosterone and cortisol dramatically decreased xenobiotic carbonyl reduction (90 and 74%, respectively), even complete inhibition (100%) of metyrapone reduction was achieved with the 11-oxoglucocorticoids cortisone and dehydrocorticosterone. The competitive nature of inhibition of metyrapone reduction by corticosterone (Fig. 1) suggests that both glucocorticoids and xenobiotic carbonyl substances bind to the catalytically active site of 11β-HSD and that both are substrates of this enzyme.

Microsomes of mouse tissues with considerable glucocorticoid metabolizing activity were also tested for xenobiotic carbonyl reduction. Table 4 shows that the liver had by far the highest glucocorticoid 11-dehydrogenation activity, followed by the adrenal gland, kidney and ovary. Surprisingly, the adrenal gland and ovary had almost 3-fold higher carbonyl-reducing activities than the liver, whereas that in kidney is hardly detectable. Although significant glucocorticoid 11-dehydrogenation activity was measured in kidney (one third that of liver) almost no metyrapone reduction could be observed. Accordingly, kidney 11β-HSD seems not to be able to mediate xenobiotic carbonyl reduction.

Antibodies directed against purified liver 11β-HSD were raised in rabbits to investigate the expression of the liver type 11β-HSD isozyme in ovary, adrenal gland and kidney. Figure 2 shows the immunoblot analysis of the respective mouse tissues. Although expressing glucocorticoid 11-dehydrogenation activity (Table 4), no immunological crossreaction could be detected in microsomes of ovary, adrenal gland and kidney with the antibody directed against liver type 11β-HSD. These findings suggest the existence of distinct 11β-HSD isozymes within these organs, at least with respect to their immunological homologies and the capability for xenobiotic carbonyl reduction.

DISCUSSION

Carbonyl reduction of biologically and pharmacologically active xenobiotic carbonyl compounds to the corresponding alcohols is generally mediated by cytosolic NADPH-dependent enzymes like carbonyl reductase (EC 1.1.1.184), aldehyde reductase (EC 1.1.1.2) and aldose reductase (EC 1.1.1.21) [19]. Based on similar functional properties, these three enzymes constitute the enzyme family of the aldo-keto reductases, although recent investigations revealed no structural homology between carbonyl reductase and the two other enzymes [20, 21]. According to their affinity for certain steroids, several members of the aldo-keto reductase family were reported to be identical to cytosolic 3α- or 3(17β)-hydroxysteroid dehydrogenases, which have been isolated mostly from hepatic tissues [22–25].

However, carbonyl reduction also takes place in the endoplasmic reticulum of the cell, but nothing is known about the physiological function or the grouping of respective microsomal carbonyl reductases into already existing enzyme families, although several proteins involved in this reaction have been purified and described [26–28].

In previous investigations we isolated a 34 kDa enzyme from mouse liver microsomes capable of reducing the carbonyl group of the cytochrome P450 inhibitor metyrapone [13]. Due to its subcellular localization in the endoplasmic reticulum and its insensitivity towards the diagnostic carbonyl reductase inhibitor quercitrin, this reductase differed from common patterns of the cytosolic aldo-keto reductase family [14]. N-terminal amino acid

Table 4. 11β-HSD activity in various mouse tissue microsomes

Tissue	Cortisol oxidation	Metyrapone reduction	Immunoreactivity
Liver	14.6	16.0	++
Adrenal	5.8	46.2	—
Kidney	4.8	0.1	—
Ovary	3.4	42.3	—

Enzyme activities were assayed in 50 mM sodium phosphate buffer containing 1 mM substrate and 1 mM cosubstrate. Activities are expressed as nmol/mg·30 min. Values were means of four to six individual experiments with no more than 20% variation.

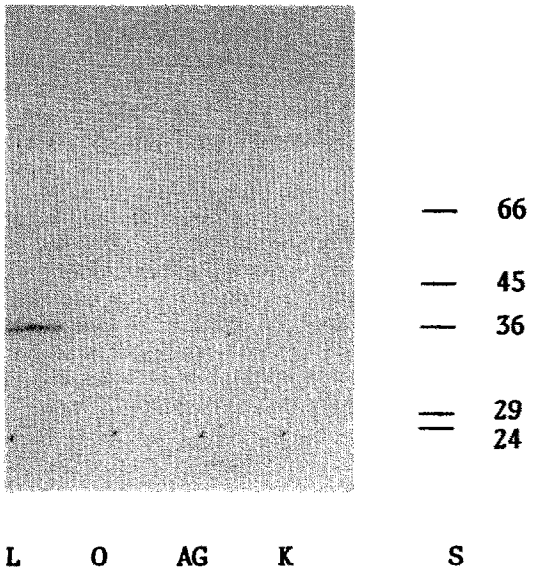

Fig. 2. Immunological expression of 11β-HSD in NMRI mouse liver (L), ovary (O), adrenal gland (AG) and kidney (K) microsomes. For immunochemical comparison, equal amounts of protein (15 μg) were applied per track on the SDS gel. Polyclonal antibodies raised in rabbits against liver 11β-HSD were incubated as primary antibody. Antigen–antibody complexes were identified by the peroxidase reaction of the secondary conjugated antibody specific for rabbit IgG. (S = molecular mass standard; albumin, bovine = 66 kDa; albumin, egg = 45 kDa; glyceraldehyde-3-phosphate dehydrogenase = 36 kDa; carboanhydrase = 29 kDa; trypsinogen = 24 kDa.)

sequence analysis revealed structural homologies to 11β-HSD from rat liver, the physiological function of which is the interconversion of the active 11-hydroxyglucocorticoids cortisol and corticosterone to the inactive 11-oxo forms cortisone and dehydrocorticosterone, and which belongs to the enzyme family of the short chain alcohol dehydrogenases (SCAD) [29]. Accordingly, our enzyme seems to be the 11β-HSD representative in the mouse liver. Antibodies, raised in rabbits against the mouse liver 11β-HSD, crossreacted specifically with 3α-hydroxysteroid dehydrogenase (3α-HSD) from *Pseudomonas testosteroni* [30], which was also shown to be a member of the SCAD family [31]. Based on N-terminal amino acid sequence composition, mouse liver 11β-HSD and 3α-HSD from *Pseudomonas testosteroni* likewise showed structural homologies [31], thus again confirming the grouping of our enzyme into the SCAD family. We could then demonstrate that the mouse liver enzyme is indeed capable of mediating glucocorticoid 11-oxidoreduction [32]. Moreover, upon improving the purification procedure, mouse liver 11β-HSD retained both 11-dehydrogenation as well as 11-oxoreducing activity, thus disproving the two enzyme theory of 11β-HSD [15].

The intention of this study was to demonstrate that the carbonyl reducing activity of the previously purified metyrapone reductase and the glucocorticoid 11-oxidoreducing activity of 11β-HSD from mouse liver microsomes both reside within the same protein and that both are in fact identical enzymes. Evidence of a homogeneous enzyme preparation was given by yielding a single band after SDS–PAGE and subsequent silver staining, as well as by N-terminal amino acid sequence analysis, a procedure which affords pure protein preparations.

Copurification of the three activities, glucocorticoid 11β-dehydrogenation, glucocorticoid 11-oxoreduction and xenobiotic carbonyl reduction, revealed a coincidently increasing specific activity, although enzyme latency upon solubilization of microsomes was higher in the case of xenobiotic carbonyl reduction than that of glucocorticoid metabolism. The phenomenon of enzyme latency was already described for 11β-HSD by Monder and Lakshmi [33] and for microsomal carbonyl reduction by Sawada *et al.*[27], who attributed the increasing specific enzyme activity upon solubilization to the inhibitory action of the surrounding phospholipid bilayer of the endoplasmic reticulum which, in the native state, restricts substrate access to the active site of the enzyme. The solubilization by detergents thus simultaneously amounts to a delipidation effect of the respective membrane-associated enzyme. It is likely that the reductase component of rat liver 11β-HSD is more labile than dehydrogenase, resulting in a complete loss of glucocorticoid 11-oxoreducing activity of the purified enzyme [11]. Our purification method may possibly afford a gentle membrane solubilization as well as providing a favourable detergent surrounding during the various chromatographic steps, resulting in an enzyme preparation containing both glucocorticoid 11-oxoreducing as well as 11-dehydrogenation activities

[15]. However, during the course of 11β-HSD purification the subsequent chromatographic steps gave corresponding yields, indicating that the three reactions are mediated by the same enzyme.

Enzyme kinetic studies confirmed that, in addition to glucocorticoid 11-oxidoreduction, purified 11β-HSD is capable of reducing xenobiotic carbonyl compounds, such as metyrapone, *p*-nitroacetophenone and *p*-nitrobenzaldehyde. But in contrast to the reversibility of glucocorticoid oxidoreduction, no dehydrogenation of xenobiotic alcohol metabolites could be detected. Comparing the intrinsic clearance values of glucocorticoid 11-oxidoreduction, it becomes obvious that oxidation is favoured over reduction. Whereas $NADP^+$ is described to be the preferred cosubstrate of rat liver 11β-HSD, the mouse liver enzyme also accepts NAD^+ as cosubstrate for glucocorticoid 11β-dehydrogenation. NADH as cosubstrate for 11-oxoreduction plays only a minor role compared to that with NADPH, a fact which is also true for xenobiotic carbonyl reduction. As expected, corticosterone, the predominant glucocorticoid in rats and mice, has higher intrinsic clearance values than cortisol, which is the active glucocorticoid in man.

Inhibition experiments revealed strong sensitivity of xenobiotic carbonyl reduction to glucocorticoids. Whereas the 11-hydroxyglucocorticoids corticosterone and cortisol dramatically decreased metyrapone reduction, the 11-oxoglucocorticoids cortisone and dehydrocorticosterone completely abolished it. The competitive nature of this inhibition by corticosterone suggests that both glucocorticoids and xenobiotic carbonyl substances bind to the same catalytically active site of 11β-HSD and that both are substrates of this enzyme.

The capability for xenobiotic carbonyl reduction seems not to be true for the kidney 11β-HSD isozyme. Although expressing considerable glucocorticoid 11-dehydrogenation activity (one third that of liver) almost no carbonyl reducing activity was detectable in kidney microsomes. This finding, together with the failure of an immunological crossreaction of the kidney 11β-HSD with the antibody directed against the liver 11β-HSD, suggests a different isozyme form of 11β-HSD to be present in the kidney. A similar inconsistency was already reported [34], where northern blot analysis of the rat kidney with a cDNA probe corresponding to rat liver 11β-HSD reveals a strong mRNA signal but western blot analysis shows only a poor immunological crossreaction with the antibody against liver 11β-HSD. The ability of xenobiotic carbonyl reduction, which is true for the liver 11β-HSD but not for the kidney 11β-HSD, as shown in this study, possibly signifies another distinguishing feature between the two isoforms.

Carbonyl-reducing enzymes are considered to be of significance in the bioreduction of pharmacologically relevant carbonyl substances or in the protection of organisms against toxic carbonyl compounds by converting them to less lipophilic and more soluble and more readily conjugated metabolites [35]. Therefore, our results suggest that 11β-HSD plays an important role in the phase I metabolism of these carbonyl compounds. Until now, several steroid dehydrogenases, which catalyze the hydroxy-oxidation or oxo-reduction at C-3 and/or C-17 of the steroid nucleus are described to be also involved in reductive xenobiotic carbonyl metabolism [22, 24, 30, 36–38]. This report provides evidence that, in addition to these 3α-, 3β- and 17β-hydroxysteroid dehydrogenases, another group of hydroxysteroid dehydrogenases is capable of xenobiotic carbonyl reduction, the physiological function of which is the oxidoreduction at C-11 of the steroid nucleus.

In conclusion, our data demonstrate that mouse liver 11β-HSD, the physiological function of which is glucocorticoid 11β-oxidoreduction, can act as a carbonyl reductase for xenobiotics. Thus it shows properties similar to the previously described 3α-, 3β-, and 17β-hydroxysteroid dehydrogenases and contributes to an expanding list of pluripotent enzymes which are involved in reductive xenobiotic carbonyl metabolism as well as being specific towards their physiological steroid substrates. Oxidation and reduction of functional groups of steroids is an important mechanism for regulating the actions of these hormones in multicellular organisms. The additional ability of xenobiotic carbonyl reduction possibly traces back to the original functions of these enzymes in procaryonts which could have been the metabolism of steroids and other carbonyl compounds as a source of carbon and energy.

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