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HETEROGENEITY OF CARBONYL REDUCTION IN SUBCELLULAR FRACTIONS AND DIFFERENT ORGANS IN RODENTS

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Abstract—The pattern and distribution of carbonyl reduction in liver, kidney and adrenal gland subcellular fractions of NMRI mice, Wistar rats and Hartley guinea pigs were examined using the ketone compound metyrapone (2-methyl-1,2-di(3-pyridyl)1-propanone) commonly used as a diagnostic cytochrome P450 inhibitor. A direct HPLC method for alcohol metabolite determination instead of the indirect spectrophotometric recording of pyridine nucleotide oxidation at 340 nm was applied. All the tissues examined in these species rapidly reduced the employed compound but at the subcellular level no general distribution scheme of specific activity was found, although in all fractions metyrapol formation could be attributed to aldo-keto reductases. Cytosolic and microsomal metyrapone reducing enzymes are distinguished by their inhibitor sensitivity to phenobarbitone and quercitrin and thus can be characterized as aldehyde and ketone reductases according to the inhibitor subclassification of the aldo-keto reductase family. Moreover, the enzymes also differ with respect to their immunological cross-reactivity to anti-microsomal mouse liver metyrapone reductase antibodies. Immunological homologies were found between metyrapone reductases of liver microsomes from all species and kidney and adrenal gland microsomes from guinea pig. However, the protein of all the cytosolic fractions as well as that of kidney and adrenal gland microsomes from mouse and rat did not cross-react with the antibodies, indicating the absence of common antigenic determinants. From catalytic properties and functional data it is concluded that hydroxysteroid dehydrogenases present in the suspected subcellular fractions form a structurally and functionally related enzyme family which may have been conserved during evolution.

Carbonyl reduction of xenobiotic or endogenous compounds is a universal reaction; it has been found to occur in every tissue and organism so far examined [1]. In this process different NADPH (NADH) linked oxidoreductases are involved, which include for example alcohol dehydrogenases, steroid dehydrogenases and aldo-keto reductases, depending on the substrate [1]. Carbonyl reducing enzymes are considered to protect the cell against toxic carbonyl metabolites and convert them to less lipophilic, more soluble and more easily conjugated metabolites, or they are involved in the metabolic pathways of certain transmitters (e.g. biogenic amines), hormones (steroids), mediators (prostaglandins) and sugars [1–3].

High activities are found in liver, kidney, lung, heart, intestine and brain [1]. Mainly cytosolic fractions express high specific activities but membraneous fractions also contain considerable amounts of carbonyl reductase activity [1, 4]. As regards classification, these low molecular weight enzymes (Fig. 1) are divided into aldehyde and ketone reductases, according to substrate specificity and inhibitor sensitivity, using the diagnostic inhibitors quercitrin (inhibition of ketone reductases) and phenobarbitone (inhibition of aldehyde reductases) [1]. Aldehyde reductases catalyse the reduction of aldehyde compounds whereas ketone reductases can reduce both aldehyde and ketone compounds, due probably to the greater chemical reactivity of aldehydes. Exceptions to this rule are reported as being daunorubicin reductase [5] from rat liver cytosol and microsomal mouse liver metyrapone reductase [4] which are classified as aldehyde

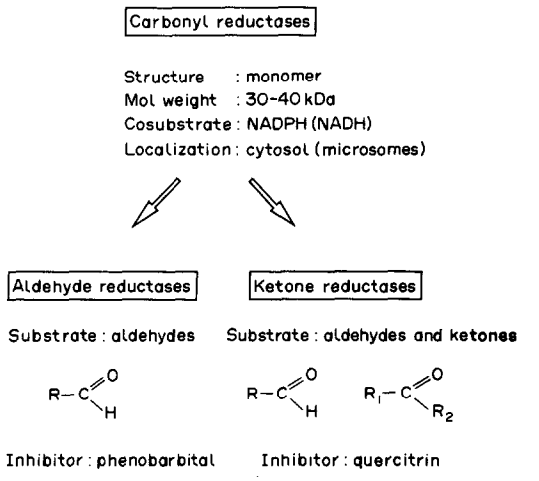

Fig. 1. Scheme of the general characterization and subclassification of the carbonyl reductases.

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reductases (although reducing a ketone compound) with respect to inhibitor sensitivity.

Structural homologies between certain members in this aldo-keto reductase family were first suggested in the early 80s by Felsted and Bachur [1] by comparison of Metzger indices of some purified enzymes, and recently demonstrated by comparing the amino acid and nucleotide sequences of certain steroid dehydrogenases [6] and aldo-keto reductases [7, 8] of eucaryontic and procaryontic origins.

We used the ketone compound metyrapone (the diagnostic cytochrome P450 inhibitor) as substrate for carbonyl reduction to the corresponding alcohol metabolite metyrapol. Reduction at the carbonyl function is the first and most important step in the metabolism of this substance *in vivo* [9, 10].

In previous investigations we found that in the liver of NMRI mice metyrapone is reduced by two different enzymes which differ in cofactor requirement, inhibitor sensitivity, intracellular localization and immunological homology. The cytoplasmic enzyme turned out to be a ketone reductase (inhibited by quercitrin) strictly requiring NADPH as cofactor and showing no cross-reaction with antibodies raised against the microsomal mouse liver metyrapone reductase (MLMR*). In contrast, the microsomal enzyme turned out to be an aldehyde reductase (inhibited by phenobarbitone) able to utilize either NADH or NADPH as cofactor and competitively inhibited by 3-oxosteroids [4, 11].

In this study we examined the extent and type of carbonyl reduction of metyrapone in liver, kidney and adrenal gland from NMRI mice, Wistar rats and Hartley guinea pigs. The immunological homologies of the involved enzymes were tested by using anti-MLMR antibodies with the immunoblot technique.

A preliminary account of this work has been presented [12].

MATERIAL AND METHODS

Animals. NMRI mice (25–30 g body wt), Wistar rats (200–300 g body wt) and guinea pigs (400–600 g body wt) of the Hartley strain of both sexes were used throughout the study.

Chemicals. Metyrapone was purchased from Fluka AG (Buchs, Switzerland). Enzymatic tests were performed using NADH, NADPH, glucose-6-phosphate and glucose-6-phosphate dehydrogenase from Boehringer Mannheim (Mannheim, F.R.G.). For HPLC acetonitrile of HPLC grade from E. Merck (Darmstadt, F.R.G.) was used.

For inhibitor studies quercitrin was purchased from Roth (Karlsruhe, F.R.G.) and phenobarbitone from Fluka (Neu-Ulm, F.R.G.).

Low molecular weight markers were obtained from Bio Rad (Munich, F.R.G.), SDS from Fluka and acrylamide, and methylene-bisacrylamide from E. Merck.

Nitrocellulose was purchased from Schleicher & Schuell (Dassel, F.R.G.), peroxidase-conjugated anti-rabbit IgG antibodies from Dakopatts (Hamburg, F.R.G.) and 4-chloro-1-naphthol, and H_2O_2 from E. Merck.

Octyl-Sepharose CL 4B and Protein A-Sepharose were from Pharmacia LKB (Freiburg, F.R.G.) and DEAE-Cellulose was from E. Merck. Renex 679 was from Kao Atlas (Tokyo, Japan). All other chemicals used were of the highest grade commercially available.

SDS–PAGE. SDS–PAGE was carried out as described by Laemmli [13] using an acrylamide concentration of 10% in the separating gel. Protein bands were visualized by staining with Coomassie blue.

Immunoblotting. Immobilization and transfer to nitrocellulose sheets from SDS–polyacrylamide gels were achieved by applying the semi-dry Western Blot technique, described by Kyhse-Anderson [14]. Immunoreactive bands were visualized using peroxidase-conjugated second antibodies with 4-chloro-1-naphthol and H_2O_2 as substrates [15].

Protein determination. Protein determination was carried out according to the method of Bradford [16] or Lowry *et al.* [17] using bovine serum albumin as standard.

Preparation of organs and subcellular fractions. 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 a TRIS/Saccharose buffer (10 mM/250 mM, respectively, pH 7.4). Kidneys and adrenal glands were prepared in the same way except for perfusion. Mitochondrial and nuclear fractions were removed by centrifugation. Cytosolic supernatant and microsomal pellet were obtained by ultracentrifugation at 105,000 *g* for 1 hr. The microsomal pellet was washed and resuspended in Tris/Saccharose buffer. For each experiment 5–6 animals were used. Preparations were carried out at 4°.

Preparation of polyclonal antibodies against microsomal mouse liver metyrapone reductase. Purification of MLMR was achieved by a procedure similar to that described by Maser and Netter [4]. In brief: after solubilization of mouse liver microsomes with the non-ionic detergent Renex 679 and subsequent centrifugation at 150,000 *g*, MLMR was purified by hydrophobic interaction chromatography on Octyl-Sepharose CL 4B and following ion exchange chromatography on DEAE-Cellulose. The resulting dominant protein band in the 34 kDa region in SDS–PAGE was cut out of a preparative SDS–polyacrylamide gel and eluted by a method described by Gerton *et al.* [18] in an "ELUSTAR" elution apparatus from Phase GmbH (Moelln, F.R.G.). Homogeneity was checked after removal of SDS [19] which was found to be complete [20]. Isoelectric focusing in a pH gradient from 3–10 resulted in a single band with a pI of 5.9. Furthermore, one single N-terminal amino acid sequence of eight amino acid residues was determined by use of an automated sequencer (Applied Biosystems, Weiterstadt, F.R.G.) [21]. A female rabbit was immunized with $4 \times 150\ \mu g$ of purified antigen on days 1, 3, 6 and 36. Antiserum was collected on day 56. IgG antibodies were purified on Protein A-Sepharose and antigen specific

* Abbreviations: MLMR, microsomal mouse liver metyrapone reductase; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; IgG, immunoglobulin class G.

antibodies were obtained by affinity purification with immobilized antigen on nitrocellulose [22].

Metyrapone reduction assay. In a 1.5 mL Eppendorf cup, 100 μL protein solution, 10 μL cofactor solution, 10 μL 30 mM NaOH or inhibitor solution and 20 μL 50 mM sodium phosphate buffer pH 7.4 were pipetted and incubated for 3 min at 37°. The reaction was started with 10 μL of a 30 mM metyrapone solution. Inhibitor was dissolved in 30 mM NaOH. Inhibitor concentration was 1 mM in the final incubation mixture. NADH or NADPH concentration was 1.5 mM. The NADPH regenerating system consisted of 2 mg $NADP^+$, 6 mg glucose-6-phosphate, 0.1 mL 50 mM sodium phosphate buffer pH 7.4, 0.1 mL 0.1 mM $MgCl_2$ and 5 μL glucose-6-phosphate dehydrogenase from yeast. Aliquots were taken at 15, 30 and 60 min and the reaction was stopped by adding the 3-fold volume of ice-cold acetonitrile. After centrifugation for 5 min at 1000 rpm in an Eppendorf centrifuge the supernatant was examined for metyrapol on a reversed phase HPLC system, using an Octadecyl-Si 100 (Serva, Heidelberg, F.R.G.) matrix (column: 4.5 mm × 25 cm) and an eluent of 50 mM phosphate buffer and 30% acetonitrile (v/v). Metyrapol elutes at about 6.5 min and metyrapone at 10 min in this HPLC system (flow rate: 1.0 mL/min), and they are monitored at 254 nm in an UV monitor. Quercitrin and phenobarbitone could be separated from metyrapol by the use of two serially connected columns (50 cm altogether in length). Peak calculations were made by using a Bio Rad HRLC interface, PC and integration software.

RESULTS

Specific activity

Figure 2 shows the tissue distribution and cofactor requirement of metyrapone reduction in cytosolic and microsomal fractions of liver, kidney and adrenal gland from rodent species. In addition to liver subcellular fractions a heterogenic specific activity of carbonyl reduction occurs in kidney and adrenal gland, depending on the species and cofactor substitution. In all fractions tested a linear relation between alcohol metabolite forming and time over the first 30 min was observed. Highest cytosolic activities were found in the kidney of mouse and guinea pig and adrenal gland of rat. In the microsomal fractions comparable specific activities were detected in the kidney of guinea pig and adrenal gland of mouse and guinea pig. Cytosolic metyrapone reducing enzymes seemed to utilize almost exclusively NADPH as cofactor whereas mouse and rat microsomal liver enzymes also showed a considerable activity with NADH. In general, highest activities were obtained with a NADPH-regenerating system, except for microsomal metyrapol formation in guinea pig liver and kidney where higher specific activities were measured by addition of 1.0 mM NADPH. Very low or no activity of carbonyl reduction of metyrapone was found in kidney microsomes of mouse and rat, adrenal gland microsomes of rat and adrenal gland cytosol of guinea pig.

Inhibitor profile

Figure 3 shows the inhibitor profile of the tested subcellular fractions. According to the inhibitor classification of the aldo-keto reductase enzyme family [1], phenobarbitone and quercitrin were used as classical inhibitors of aldehyde and ketone reductases, respectively. Cytosolic metyrapone reductases were inhibited predominantly by the plant flavonoid quercitrin (except in the case of guinea pig adrenal gland cytosol) and can therefore be characterized as ketone reductases. In contrast, the involved microsomal enzymes of mouse and rat tissues were affected mainly by phenobarbitone and to a lesser degree by quercitrin, and can therefore be classified as aldehyde reductases. This pattern of inhibition became very distinct in adrenal microsomes of guinea pig. However, metyrapone reducing enzymes of guinea pig liver and kidney microsomes are scarcely inhibited by phenobarbitone, and quercitrin, and therefore do not follow this generalized inhibitor profile.

Immunoblotting

Figures 4–6 show the protein pattern after SDS–PAGE and subsequent immunoblotting of all tested fractions. Incubations were carried out with affinity purified anti-MLMR IgG antibodies. In liver microsomes of mouse and rat the antibodies cross-reacted with the homologous antigen in the 34 kDa molecular weight region. In guinea pig liver microsomes the respective protein seemed to be slightly smaller, showing a molecular weight of 32 kDa. Immunoreactivity with a protein band of 32 kDa also occurred in guinea pig kidney and adrenal gland microsomes. However, in kidney and adrenal gland microsomes of mouse and rat, as well as in all cytosolic fractions, no cross-reactivity with the antibodies could be observed indicating the absence of common antigenic determinants.

DISCUSSION

The objective of this study was to examine the pattern of carbonyl reduction using the carbonyl compound metyrapone (2-methyl-1,2-di(3-pyridyl)1-propanone) in liver, kidney and adrenal gland subcellular fractions of mouse, rat and guinea pig. All examined tissues can rapidly reduce the carbonyl compound to its respective alcohol metabolite metyrapol but the activities could be assigned to different carbonyl reducing enzymes of the subcellular fractions. The nature of these enzymes seems not to follow a general rule but some conclusions can be drawn: according to substrate specificity, inhibitor susceptibility and cosubstrate dependency they all belong to the family of aldo-keto reductases. Cytosolic enzymes seem to utilize exclusively NADPH as cofactor (see upper panel, Fig. 2). In contrast, mouse and rat liver microsomal metyrapone reduction occurs also to a considerable degree with NADH (see open bars, Fig. 2). Cytosolic enzymes are more affected by the flavonoid quercitrin and, therefore, can be classified as ketone reductases. In contrast, microsomal mouse and rat metyrapone reducing enzymes of all fractions are inhibited more strongly by phenobarbitone than by quercitrin and therefore are better grouped as aldehyde reductases, considering the inhibitor subclassification of the

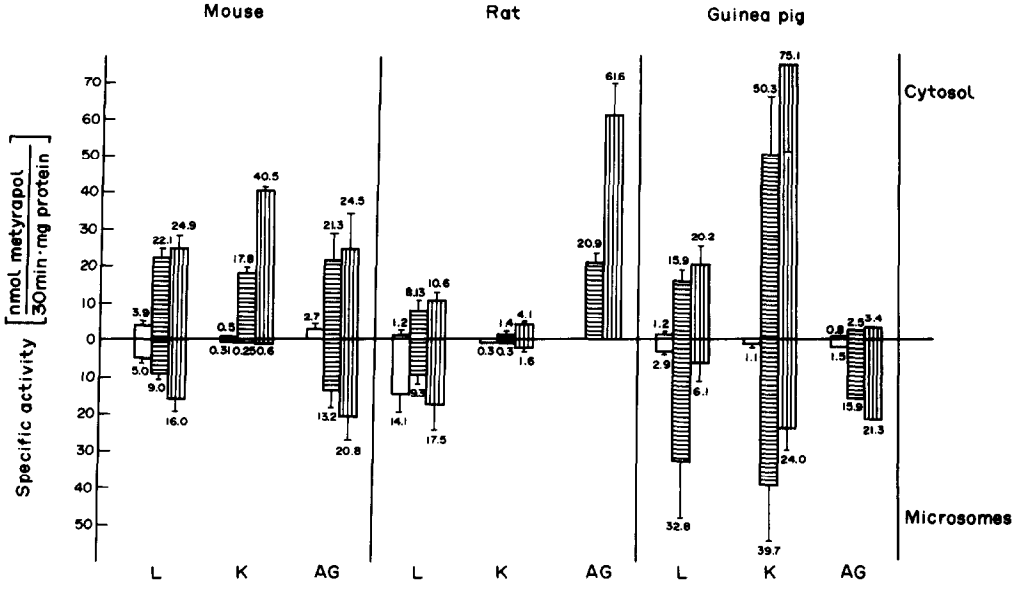

Fig. 2. Comparison of specific activities and cofactor requirements of cytosolic and microsomal metyrapone reductases in liver (L), kidney (K) and adrenal gland (AG) from rodent species. The specific activity was assayed in the standard reaction mixture containing 50 mM sodium phosphate buffer pH 7.4, 2 mM metyrapone and either 1.5 mM NADH (□), 1.5 mM NADPH (▤) or a NADPH-regenerating system (▥) (cf. Materials and Methods), respectively. Activity is expressed as nmol metyrapol formed in 30 min/mg protein; N = 5–6.

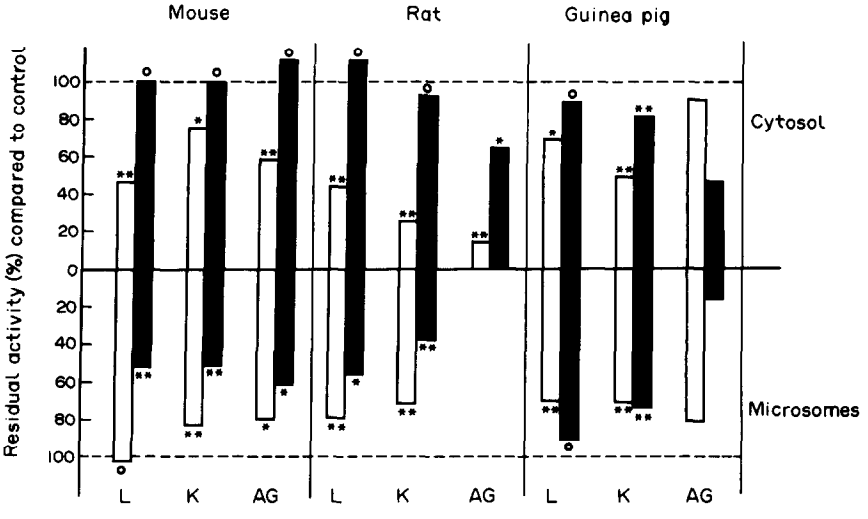

Fig. 3. Inhibitor profile of cytosolic and microsomal metyrapone reductases in liver (L), kidney (K) and adrenal gland (AG) from rodent species. The activity was assayed in the standard reaction mixture containing 50 mM sodium phosphate buffer pH 7.4, 2 mM metyrapone, a NADPH-regenerating system (cf. Materials and Methods) and 1 mM inhibitor. Quercitrin (□); phenobarbitone (■). The percentages are calculated from uninhibited control experiments. Statistical calculations were performed using the Students *t*-test. (○), Not significant; *$P \leq 0.05$; ** $P \leq 0.01$; N = 5–6.

aldo-keto reductase family. In liver and kidney microsomes of guinea pig no clear distinction between aldehyde and ketone reductases can be made because neither of the two inhibitors had any convincing effect. In guinea pig adrenal microsomes metyrapone reduction was inhibited strongly by phenobarbitone but scarcely by quercitrin, and thus resembles the situation in mouse and rat microsomal fractions. These observations are visualized in the summary graphic of Fig. 3.

MLMR is an enzyme species with a M_r of 34 kDa and is considered to be a 3α-hydroxysteroid

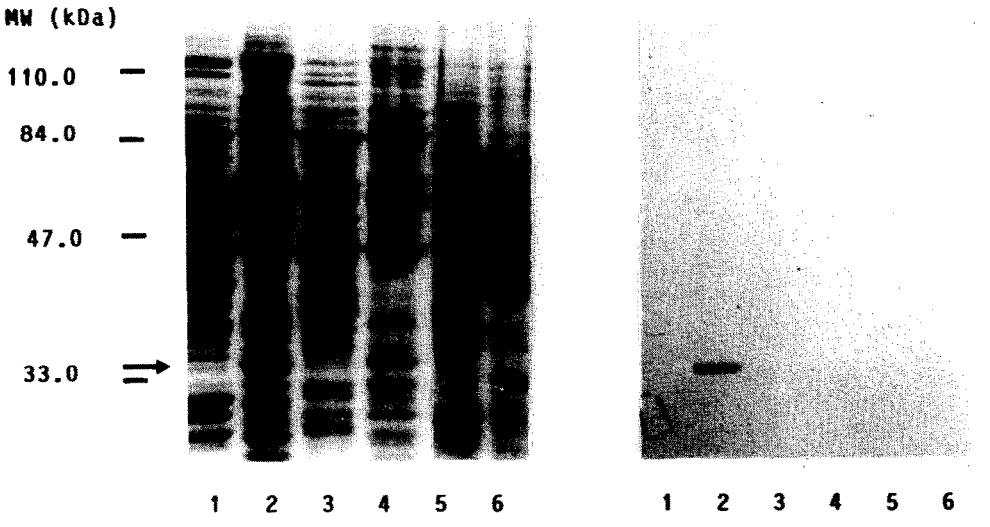

Fig. 4. Immunological comparison of cytosolic and microsomal metyrapone reductases in liver, kidney and adrenal gland from NMRI mice. Left: electrophoretically separated proteins stained with Coomassie blue. Right: immunoblot of the same fractions after SDS–PAGE using affinity purified MLMR IgG antibodies. Twenty-five micrograms protein applied per lane. Lanes of panels correspond to each other. Lane 1: liver, cytosol; lane 2: liver, microsomes; lane 3: kidney, cytosol; lane 4: kidney, microsomes; lane 5: adrenal gland, cytosol; lane 6: adrenal gland, microsomes.

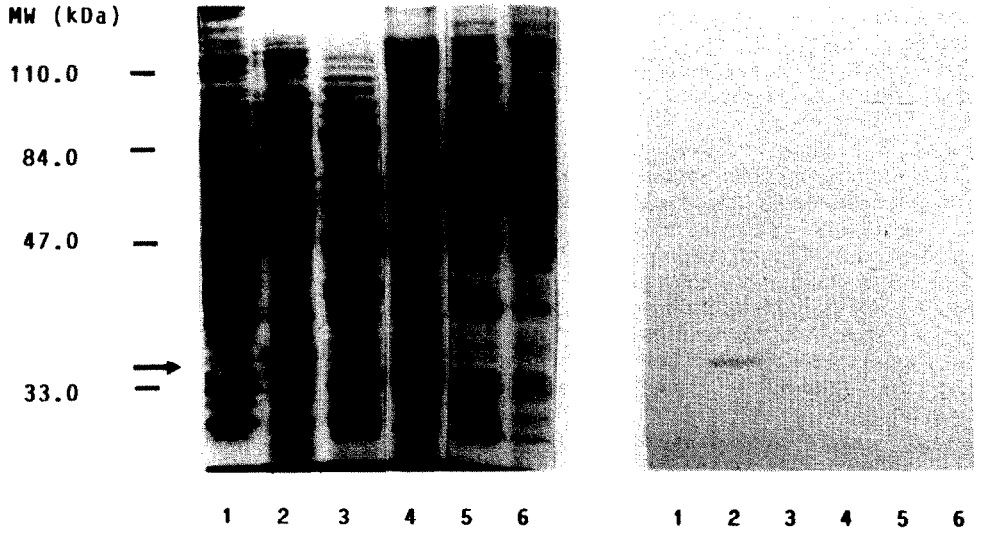

Fig. 5. Immunological comparison of cytosolic and microsomal metyrapone reductases in liver, kidney and adrenal gland from Wistar rats. Left: electrophoretically separated proteins stained with Coomassie blue. Right: immunoblot of the same fractions after SDS–PAGE using affinity purified MLMR IgG antibodies. Thirty micrograms protein applied per lane. Lanes of panels correspond to each other. Lane 1: liver, cytosol; lane 2: liver, microsomes; lane 3: kidney, cytosol; lane 4: kidney, microsomes; lane 5: adrenal gland, cytosol; lane 6: adrenal gland, microsomes.

dehydrogenase [4]. Up to now, several 3α-hydroxysteroid dehydrogenases in liver microsomes of rat and guinea pig have been described: four isozymes were detected in microsomes of rat liver [23–26], two of them having M_rs of 54 and 32 kDa as revealed by gel filtration chromatography. The molecular weights of the two others are still unknown. One form of guinea pig liver microsomal 3α-hydroxysteroid dehydrogenase was described as having an M_r of 34 kDa [27] and another as being a tetramer with subunits of M_r 32 kDa [27].

As revealed by the immunoblot experiments the

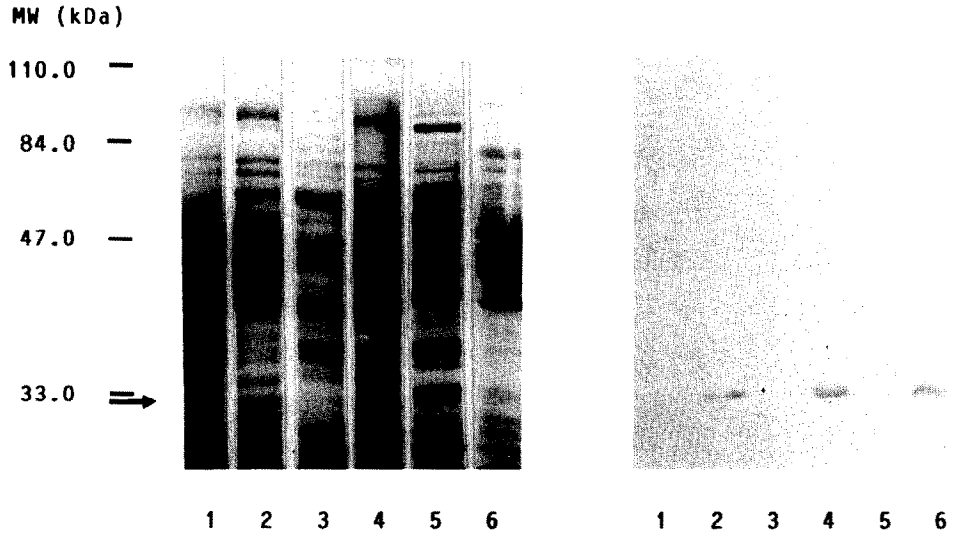

Fig. 6. Immunological comparison of cytosolic and microsomal metyrapone reductases in liver, kidney and adrenal gland from Hartley guinea pigs. Left: electrophoretically separated proteins stained with Coomassie blue. Right: immunoblot of the same fractions after SDS–PAGE using affinity purified MLMR IgG antibodies. Twenty-five micrograms protein applied per lane. Lanes of both panels correspond to each other. Lane 1: liver, cytosol; lane 2: liver, microsomes; lane 3: kidney, cytosol; lane 4: kidney, microsomes; lane 5: adrenal gland, cytosol; lane 6: adrenal gland, microsomes.

liver microsomal metyrapone reductases of all species and those of guinea pig kidney and adrenal gland microsomes seem to be structurally related due to the common specific cross-reaction with antibodies against the metyrapone reductase from mouse liver microsomes. No immunoreactivity was found in kidney and adrenal gland microsomes of mouse and rat which parallels the absence or extremely low level of metyrapone reducing activity in these fractions. The occurrence of metyrapone reduction in mouse adrenal gland microsomes possibly points to an immunologically different enzyme protein. However, all cytosolic fractions of the three tissues of all three species were devoid of immunoreactivity indicating the absence of common antigenic determinants.

Therefore, liver microsomal metyrapone reductases, at least, of all the species tested can be distinguished as a structurally related class from other carbonyl reductases which were present in all the cytosolic fractions tested, as well as in the mouse adrenal gland microsomal fraction. Guinea pig microsomal kidney and adrenal gland enzyme activities have a corresponding immunoreactivity.

The finding that metyrapone reduction can be titrated with anti-MLMR antibodies in immunoreactive liver fractions to the same extent as in mouse liver microsomes (30, 25 and 23% inhibition in mouse, rat and guinea pig liver microsomes, respectively, compared to the same amount of control serum antibodies) shows that there are structural and functional relationships between the enzymes involved within these fractions. The weak inhibition of the antibodies might be the result of an immunization with completely denatured antigen. Possibly the polyclonal antibodies recognize mainly surface epitopes of the denatured antigen (as revealed by Western Blot analysis) and only slightly the functional site of the active enzyme. This is confirmed by the high antibody titer of the antiserum when used in Western Blot or ELISA.

These results together with the finding that anti-guinea pig liver hydroxysteroid dehydrogenase (32 kDa protein) antibodies cross-react with a kidney microsome protein from the same species in immunodiffusion assay [27] suggest that at least one related enzyme species, whose endogenous substrates seem to be steroids of the androgen class, is involved in the reduction of xenobiotic carbonyl compounds in liver microsomes. The relationship of MLMR to a procaryontic enzyme from *Pseudomonas* species with the same catalytic properties has already been shown [11]. Structural homology of 17β-hydroxysteroid dehydrogenase from human placenta with several procaryontic enzymes with carbonyl reducing capability has also been reported [6]. Carbonyl and aldehyde reductases from several other sources were also shown to be related to prostaglandin F synthetase and frog lens ε-crystallin [7, 8].

Together with our findings it is concluded that the carbonyl reductase enzyme family consists of different proteins whose members are structurally and functionally related, and evolutionarily conserved, although the exact role in normal cell physiology is not fully understood. An important function of carbonyl metabolizing enzymes with hydroxysteroid dehydrogenase activity is the metabolism of carcinogenic compounds, a role which has already been shown for liver cytosolic dihydrodiol dehydrogenase [28–30]. Similar features for microsomal steroid dehydrogenases in detoxification processes remain to be determined.

REFERENCES

1. Felsted RL and Bachur N, Mammalian carbonyl reductase. *Drug Metab Rev* **11**: 1–60, 1980.
2. Hayashi H, Fujii Y, Watanabe K, Urade Y and Hayaishi O, Enzymatic conversion of prostaglandin H2 to prostaglandin F2α by aldehyde reductase from human liver. In: *Enzymology and Molecular Biology of Carbonyl Metabolism* (Eds. Weiner H and Flynn TG), Vol. 2, pp. 365–379. A. R. Liss, New York, 1989.
3. Sawada H, Hara A, Hayashibara M and Nakayama T, Guinea pig liver aromatic aldehyde-ketone reductases identical with 17β-hydroxysteroid dehydrogenase isozymes. *J Biochem* **86**: 883–892, 1979.
4. Maser E and Netter KJ, Purification and properties of a metyrapone reducing enzyme from mouse liver microsomes. *Biochem Pharmacol* **38**: 3049–3054, 1989.
5. Felsted RL, Richter D and Bachur NR, Rat liver aldehyde reductase. *Biochem Pharmacol* **26**: 1117–1124, 1977.
6. Baker ME, Human placental 17β hydroxy steroid dehydrogenase is homologous to NodG Protein from *Rhizobium meliloti*. *Mol Endocrinol* **3**: 881–884, 1989.
7. Bohren KM, Bullock B, Wermuth B and Gabbay KH, The aldo-keto reductase superfamily. *J Biol Chem* **264**: 9547–9551, 1989.
8. Watanabe K, Fujii Y, Nakayama K, Ohlubo H, Kuramitsu S, Kagamiyama H, Nakanishi S and Hayaishi O, Structural similarity of bovine prostaglandin F synthetase to lens ε-crystallin of the European common frog. *Proc Natl Acad Sci USA* **85**: 11–15, 1988.
9. Maser E and Legrum W, Alteration of the inhibitory effect of metyrapone by reduction to metyrapol during the metabolism of methacetin *in vivo* in mice. *Naunyn Schmiedebergs Arch Pharmacol* **331**: 283–289, 1989.
10. Usansky JI and Damani LA, The *in vivo* metabolism of metyrapone in the rat. *Drug Metab Dispos* **9**: 231–235, 1985.
11. Maser E, Oppermann U and Netter KJ, Immunological homologies between carbonyl reducing enzymes from procaryonts and mammalian liver microsomes. *Eur J Pharmacol* **183**: 1366, 1990.
12. Oppermann U and Maser E, Cofactor requirement and inhibitor profile of carbonyl reducing enzymes in kidney of rodents. *Naunyn Schmiedebergs Arch Pharmacol* **341**(Suppl): R8, 1990.
13. Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacterophage T4. *Nature* **227**: 680–685, 1970.
14. Kyhse-Anderson J, Electroblotting of multiple gels. A simple apparatus without buffer tank for rapid transfer of proteins from polyacrylamide gels to nitrocellulose. *J Biochem Biophys Methods* **10**: 203–209, 1984.
15. Hawkes R, Niday E and Gordon J, A dot immunobinding assay for monoclonal and other antibodies. *Anal Biochem* **119**: 142–147, 1982.
16. Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248–254, 1976.
17. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
18. Gerton GL, Wardrip NJ and Hendrick JL, A gel eluter for the recovery of proteins separated by polyacrylamide gel electrophoresis. *Anal Biochem* **126**: 116–121, 1982.
19. Henderson LE, Oroszlan S and Konigsberg W, A micromethod for complete removal of dodecyl sulfate from proteins by ion-pair extraction. *Anal Biochem* **93**: 153–157, 1979.
20. Sokoloff RL and Frigon RP, Rapid spectrophotometric assay of dodecyl sulfate using acridine orange. *Anal Biochem* **118**: 138–141, 1981.
21. Edman P and Begg G, A protein sequenator. *Eur J Biochem* **1**: 80–91, 1967.
22. Kreutzfeldt C and Potthast M, Homologies between yeast ribosomal protein L2 and rat liver ribosomal protein L4 and L24. *Curr Genet* **13**: 235–239, 1988.
23. Golf SW and Graef V, Isolation of a NAD 3$\beta(\alpha)$ hydroxy-5β-androstane dehydrogenase from rat liver microsomes. *J Steroid Biochem* **10**: 201–205, 1979.
24. Golf SW, Graef V and Nowotny E, Solubilisierung und Anreicherung einer 3α-Hydroxysteroid-Dehydrogenase aus Rattenlebermikrosomen. *Hoppe Seylers Z Physiol Chem* **357**: 35–40, 1976.
25. Golf SW and Graef V, Isolation of a 3-equatorial-hydroxysteroid dehydrogenase from rat liver microsomes. *FEBS Lett* **64**: 315–318, 1976.
26. Hara A, Usui S, Hayashibara M, Horiuchi T, Nakayama T and Sawada H, Microsomal carbonyl reductase in rat liver. Sex difference, hormonal regulation and characterization. In: *Enzymology and Molecular Biology of Carbonyl Metabolism* (Eds. Weiner H and Flynn TG), pp. 401–414. A. R. Liss, New York, 1987.
27. Usui S, Hara A, Nakayama T and Sawada H, Purification and characterization of two forms of microsomal carbonyl reductase in guinea pig liver. *Biochem J* **223**: 697–705, 1984.
28. Sawada H, Hara A, Nakayama T, Nakagawa M, Inoue Y, Hasebe K and Zhang YP. Mouse liver dihydrodiol dehydrogenases. *Biochem Pharmacol* **37**: 453–458, 1988.
29. Glatt HR, Bentley P, Platt KL and Oesch F, Reduction of benzo(*a*)pyrene mutagenicity by dihydrodiol dehydrogenase. *Nature* **277**: 319–320, 1979.
30. Penning TM, Smithgall TE, Askonas LJ and Sharp RB, Rat liver 3α hydroxy steroid dehydrogenase. *Steroids* **47**: 221–247, 1986.