Physiol. Res. 40: 81–86, 1991

N-demethylation Activity of Renal and Hepatic Subcellular Fractions: An Interspecies Comparison

F. TREJTNAR[1], O. JINDROVÁ, H. LIŠKOVÁ, J. ŠIROKÁ, J. KVĚTINA

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Charles University, Hradec Králové and [1]Institute of Experimental Biopharmaceutics, Czechoslovak Academy of Sciences, Hradec Králové

Received February 19, 1990
Accepted September 5, 1990

Summary

The enzymatic activity of the mixed-function oxidase system in the kidney and liver was evaluated by means of an *in vitro* N-demethylation activity assay with aminopyrine as the substrate. Renal and hepatic demethylation activity of 9000 x g supernatant fraction was determined in the rat, rabbit, and guinea-pig. In terms of interspecies comparison, the renal tissue demethylation activities were on a similar level with a slight increase in the order guinea-pig, rabbit and rat. In relation to hepatic activity, these relative demethylation activities of renal tissue had the same values in the rat and rabbit, whereas that in the guinea pig was significantly lower.

The distribution of demethylation activity in the kidney was determined by comparing the cortex and medullary activity in relation to the total kidney tissue activity in the rabbit and guinea-pig. Although the higher demethylation activities were obtained in rabbit renal preparations and low demethylation activity was detected in the guinea-pig renal medulla only, no significant interspecies differences were found by the statistical evaluation. It may be concluded that the mixed-function oxidase system responsible for renal demethylation activity seems to be concentrated in the renal cortex and its distribution coincides in the rabbit and guinea-pig kidney.

Key words:
Kidney – Demethylation activity – MFO-system

Introduction

Oxidative demethylation mediated by the mixed-function oxidase system (MFO-system) is a frequent pathway in the xenobiotic biotransformation process. A considerable renal aminopyrine-N-demethylase activity has been demonstrated in the sheep (Larrieu and Galtier, 1988), in the rabbit (Davis *et al.* 1981), in the rat

(Cojocel *et al.* 1988, Litterst *et al.* 1975), in the mouse, in the hamster, and in the guinea pig (Litterst *et al.* 1975). Demethylation of p-chloro-N-methylaniline has been observed in a 9 000 x g supernatant fraction of the rat kidney tissue homogenate (Navran and Louis-Ferdinand 1975). Aminopyrine-N-demethylase activity was detected in the microsome preparation of the renal cortex, the microsome preparation of the renal medulla was inactive in the rabbit (Davis *et al.* 1981) and in the rat (Cojocel *et al.* 1988).

A comparison of the results from MFO-system investigations is complicated by the variability of experimental conditions, and by a lack of uniformity in the presentation of results by individual authors. An attempt has been made in this paper to compare the renal and hepatic demethylation activity in three common laboratory animal species, and to estimate the distribution of demethylation activity in the kidney by means of an improved *in vitro* method for demethylation activity screening in the tissues.

Material and Methods

Chemicals

Acetylacetone was obtained from International Enzymes Ltd. (Windsor, England), glucose-6-phosphate (disodium salt) and nicotinamide-adenine dinucleotide phosphate (NADP) from Reanal (Budapest, Hungary). All other chemicals used were purchased from Lachema (Brno, Czechoslovakia); all chemicals were of analytical grade.

Animals

Adult males of the following species of laboratory animals were used in all experiments: the Wistar rat (180–220 g), English short-haired guinea-pig (350–400 g), and grey chinchilla rabbit (3 500–3 900 g). Animals were kept in conventional conditions and allowed food (diets DOS 2b for rats and MOK for rabbits and guinea-pigs from Velaz, Prague, Czechoslovakia) and water *ad libitum*. They fasted for 12 h before the experiment.

Preparation of 9 000 x g supernatant fraction

Animals were killed by exsanguination, the kidney and the right medial lobe of the liver were rapidly removed and transferred into ice-cold physiological saline. All subsequent operations were carried out at 4°C. The kidney was decapsulated and the renal pelvis was removed. Samples of total kidney tissue were obtained by transversal section of the kidney in all animal species used. Cortical and medullary parts were carefully separated in the case of the rabbit and guinea-pig. Tissues were minced, and then homogenized in 3 volumes of 20 mmol/l TRIS-HCl buffer (pH 7.4) containing 150 mmol/l KCl in a glass homogenizer with a Teflon pestle (MLW, Engelsdorf, GDR). The homogenates were centrifuged at 9 000 x g for 20 min at 4 °C. The supernatants were separated, protein concentrations were determined by the standard kit (Lachema, Brno, Czechoslovakia), and supernatants were diluted to the concentration of 25 mg of protein per ml.

Demethylation activity assay

Demethylation activity of 9 000 x g supernatant fractions of tissues was estimated by the modified method of Brookman and Kourounakis (1977) using aminopyrine as the substrate. 0.1 ml of 9 000 x g supernatant fraction sample representing 2.5 mg of protein was added to 1.4 ml of an incubation mixture containing (mmol/l): 0.86 NADP, 10.70 glucose-6-phosphate, 5.40 $MgCl_2$, 0.36 aminopyrine in 11 mmol/l TRIS-HCl buffer pH (7.4). The samples were incubated at 37 °C for 90 min, then the reaction was stopped by adding 1.0 ml of 0.6 mol/l trichloracetic acid. Reaction controls for each sample, to which trichloracetic acid had been added before incubation for arresting the enzymatic reaction, were run in an identical manner. After centrifugation at 3 000 x g for 10 min, 1.0 ml

of supernatant was used for the determination of formed formaldehyde by Nash reaction (Nash 1953). Samples were neutralized by adding 0.5 ml of 0.3 mol/l sodium hydroxide, then 1.5 ml of reagent was added consisting of 2.0 mol/l ammonium acetate, 0.02 mol/l acetylacetone, and 0.05 mol/l acetic acid. After incubation at 37 °C for 40 min, the absorbance of samples at 412 nm was measured with a reference sample prepared in the same manner with water instead of the incubation medium. The calibration curve for colorimetric assay was prepared with standard formaldehyde solutions in distilled water handled as the samples; it was linear in the range of concentration from 0.01 to 0.20 mmol/l.

Statistical evaluation

The data presented for each experiment were expressed as the mean ± standard deviation from the values of six-member groups of experimental animals. The statistical evaluation of data was performed by Student's t-test for unpaired data. The statistical significance of differences was set at the level $P<0.05$.

Results

Quantitative data on demethylation activity of 9 000 x g supernatant fractions of the kidney and liver tissue homogenates for the rat, rabbit and guinea-pig are presented in Fig.1. Demethylation activities of the renal tissue are in terms of an interspecies comparison on a similar level with a slight increase in the order guinea-pig, rabbit and rat.

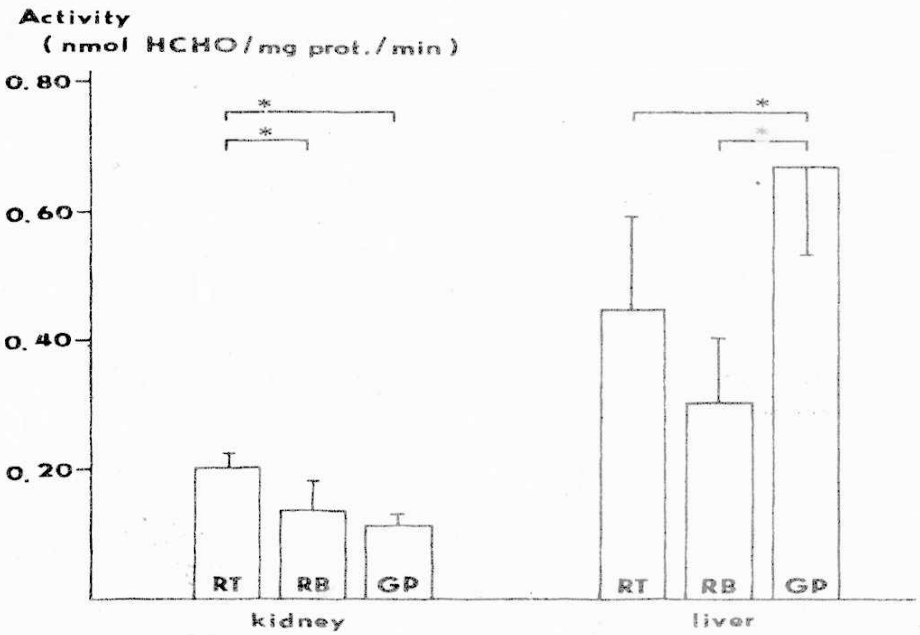

Fig.1.
Interspecies comparison of renal and hepatic demethylation activity (rat = RT; rabbit = RB; guinea-pig = GP; * = significantly different for $p<0.05$)

Relative demethylation activity of the renal tissue, i.e. demethylation activity expressed as a percentual part of the corresponding demethylation activity of the hepatic tissue in the same subject, was not statistically different in the rat and rabbit, but it was significantly lower in the guinea-pig reflecting higher hepatic demethylation activity in this species (Tab. 1).

Table 1

Demethylation activity of 9000 x g supernatant fraction of kidney and liver

Species	Demethylation activity (nmol HCHO/g prot./min)		Relative demethylation activity in the kidney (expressed in % of liver activity
	Kidney	Liver	Kidney
Rat	0.20 ± 0.02	0.45 ± 0.13	44.4 ± 12.4
Rabbit	0.14 ± 0.05	0.30 ± 0.10	46.7 ± 10.1
Guinea-pig	0.11 ± 0.02	0.68 ± 0.14	16.2 ± 5.9

The distribution of the MFO-system acting in aminopyrine oxidative demethylation in the kidney is presented in Table 2.

Table 2

Distribution of demethylation activity in the rabbit and guinea-pig kidney

Species	Demethylation activity (nmol HCHO/mg prot./min)		
	Total	Cortex	Medulla
Rabbit	0.14 ± 0.05	0.20 ± 0.06	0.00
Guinea-pig	0.11 ± 0.02	0.15 ± 0.03	0.02 ± 0.02

In the rabbit, the highest demethylation activity (142.9 % of total renal tissue activity) was found in the renal cortex. No demethylation activity was detected in the rabbit renal medulla. In the guinea-pig, the demethylation activity of the renal cortex (136.4 % of the total renal activity) was higher than that of the total renal tissue. The demethylation activity found in the renal medulla was at the limits of detection with a very high interindividual variability (Fig. 2). When interspecies differences are compared, the distribution of demethylation activity in the kidney was very similar in the rabbit and guinea-pig. Renal demethylation activity seemed to be concentrated in the renal cortex in both species.

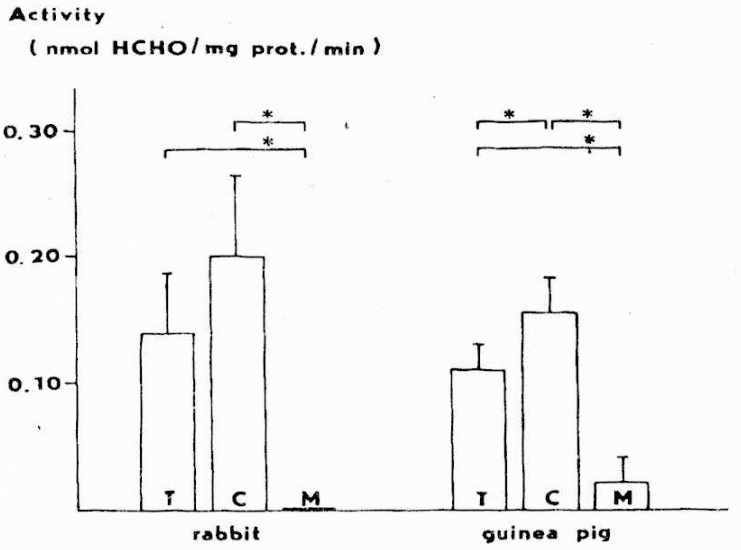

Fig.2.
Renal demethylation activity distribution in rabbit and guinea-pig (T = total kidney sample; C = cortex activity; M = medullary activity; * = significantly different for $p<0.05$)

Discussion

The investigation on the ability of renal and hepatic tissues to demethylate aminopyrine indicates the presence of MFO-system in these tissues in all three animal species examined. In order to facilitate a direct comparison with other papers, we have expressed the relative renal demethylation activity as a percentual part of the corresponding demethylation activity of the hepatic tissue in a given species. The values of relative activity were similar in the rat and rabbit, the one in the guinea-pig was significantly lower. Navran and Louis-Ferdinand (1975), using p-chloro-N-methylaniline as the substrate, presented the relative activity of the 9 000 x g supernatant fraction of rat renal tissue as 41 %, the demethylation activity is related to grams of tissue. Considering the protein concentration in 9 000 x g supernatant fractions, it may be supposed that when demethylation activity is related to milligrams of protein, the relative renal activity could reach about 80 %. Litterst *et al.* (1975) comparing the p-chloro-N-methylaniline demethylation activity of the liver and kidney microsome fractions gave a relative activity of 51 % for the rat.

With aminopyrine as the MFO-system substrate, Litterst *et al.* (1975) obtained relative demethylation activity values of renal microsome preparations of the rat, rabbit, and guinea-pig about 5 % for all the species tested. With respect to the investigation of Navran and Louis-Ferdinand (1975), who reported a considerable demethylation activity not only in the microsome fraction, but also in the cytosol fraction of the rat renal tissue, it may be considered that the lower demethylation activity in the microscope preparation could be due to a lack of cytosol demethylation activity present in the 9 000 x g supernatant fraction.

Results of the study of demethylation activity distribution in the rabbit kidney agree with the observation of Zenser *et al.* (1978) who, when testing demethylation activity in the rabbit renal cortex, outer medulla and the inner medulla, found the activity in the cortex preparation only. Also Davis *et al.* (1981) in their study on

cytochrome P-450 distribution reported the occurrence of this marker of the MFO-system in the rabbit renal cortex, not in the renal medulla. On the other hand, Mohandas *et al.* (1981) detected cytochrome P-450 in the rabbit renal outer medulla, its content being 14 % of cytochrome P-450 value in the cortex.

In the guinea-pig, demethylation activity has been found in both the renal cortex and medulla. Demethylation activity of the guinea-pig renal medulla was low, its values were at the limits of detection with a very high interindividual variability. Respecting these non-homogeneous assay results and the distribution of demethylation activity found in the rabbit, we cannot consider the presence of MFO-system activity in renal medulla to be sufficiently proved. The finding of demethylation activity in the guinea-pig renal medulla may be explained by contamination of the medulla preparation by the cortex tissue that has abundant high demethylation activity. The problem of contamination of the medullary preparation by the cortex tissue arose in the rat, where the small size of the kidney did not make it possible to separate the medullary and cortical tissue sufficiently accurately by the employed method. Though some quantitative differences in medullary and cortex demethylation activity were found in both the species examined, it may be concluded that the MFO-system responsible for renal demethylation activity is concentrated in the renal cortex and its distribution coincides in the rabbit and guinea-pig kidney.

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Reprint requests:
Dr. F. Trejtnar, Institute of Experimental Biopharmaceutics, Czechoslovak Academy of Sciences, 500 05 Hradec Králové, Heyrovského 1203.