*Biochemical Pharmacology*, Vol. 34, No. 3, pp. 439–440, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00


# KINETICS OF SPARTEINE METABOLISM IN RAT AND RABBIT LIVER MICROSOMES

E.E. Ohnhaus, M.E. McManus, D.M. Schwarz, S.S. Thorgeirsson

Department of Medicine, University of Essen, F.R.G. and
National Cancer Institute, Bethesda, Maryland, USA

Sparteine, an antiarrhythmic and oxytocic drug is oxidised polymorphically in man. Two distinct phenotypes have been observed, extensive metabolizers and poor metabolizers, with approximately 5% of the European population in the latter category (1). Sparteine has been shown to be metabolized to both 2- and 5-dehydrosparteine and these are thought to arise via a cytochrome P-450 mediated oxidation on the tertiary nitrogen (1). However, the involvement of the flavin-containing monooxygenase in this reaction can not be excluded (2). In an attempt to develop an animal model to study this polymorphic defect we have investigated the kinetics of sparteine metabolism in rat and rabbit liver microsomes and rat hepatocytes as so far not many reports have been published on animal experiments. In addition, we have assessed the ability of the purified hog flavin-containing monooxygenase and four purified forms of rabbit liver cytochrome P-450 to metabolise sparteine.

## Method and Material

Rat and rabbit liver microsomes of controls and following induction by phenobarbitone (60 or 30 mg/kg/4 days) or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD 25 or 20 µg/kg/1 day) were prepared. Microsomal protein (1 mg) was incubated with different sparteine concentration ranging from 5 to 4000 µmol in phosphate buffer solution pH 7.35 for 30 minutes in a 37°C shaking water bath. In addition, four purified forms of rabbit liver microsomal cytochrome P-450 (form 1, 3c, 4 and 6; provided by Dr. E.F. Johnson, Scripps, CA, USA) and hog flavin-containing monooxygenase were incubated together with 200 µMol sparteine, NADPH 1.8 mM, reductase, phospholipids and phosphate buffer solution. Reactions were stopped by 1 ml dichloromethane and extraction was performed by adding 0.2 ml 10 N NaOH mixing the sample for 1 minute on a rotary mixer. The estimation of sparteine and its metabolites was performed by gas chromatography (Hewlett Packard 5830 A) according to the method of Eichelbaum et al. (3) with some modification. 2-4 µl of the organic phase was injected onto the column. The apparatus conditions were as follows: injection temperature 230°C, column temperature 280°C, FID 240°; gas flows of nitrogen 35 ml/min, $H_2$ 25 ml/min and pressured air 250 ml/min. 17-Ethylsparteine was used as an internal standard, 2- and 5-dehydrosparteine as reference substances for the two major metabolites (provided by Prof. Dr. M. Eichelbaum, University of Bonn, FRG). Data were fitted according to Michaelis Menten kinetics using for the estimation of the model parameters and their respective variances a weighted non-linear least-squares regression analysis using a Levenberg-Marquardt algorithm (MLAB) (4).

## Results and Discussion

2-dehydrosparteine was the major metabolite measured, whereas 5-dehydrosparteine was only detectable at high substrate concentration (> 200 µMol). In control rat liver microsomes biphasic kinetics was observed and the data were best described by a two enzyme system. Eadie-Scatchard plots revealed a high affinity ($K_{m1}$= 6.3 ± 1.1 µM) low capacity enzyme ($V_{max1}$ = 3.1 ± 0.1 nmol/mg protein/30 min) and a low affinity ($K_{m2}$ > 2000 µM) high capacity enzyme ($V_{max2}$ = 25 nmol/mg protein/30 min.) The microsomes from rats exhibited similar $K_{m1}$ and $V_{max1}$ values after pretreatment with phenobarbital ($K_{m1}$ = 2.0 ± 0.5 uM, $V_{max1}$ = 2.5 ± 0.9 nmol/mg protein/30 min) and TCDD ($K_{m1}$ = 5.2 ± 1.6 uM, $V_{max1}$ = 2.1 ± 0.2 nmol/mg protein/30 min.) as control microsomes. However, in induced microsomes a more precise definition of the second enzyme component was achieved. The mean $K_{m2}$ values were 4.2 ± 0.7 mM and 9.1 ± 6.7 mM and the $V_{max2}$ values 43 ± 5 or 43 ± 25 nmol/mg protein/30 min. for phenobarbital and TCDD induced microsomes respectively.

Biphasic kinetics was also observed in control rat hepatocytes with similar $K_m$ and $V_{max}$ values ($K_{m1}$ = 3.8 ± 1.5 μM, $V_{max1}$ = 4.6 ± 0.5 nmol/mg protein/30 min.; $K_{m2}$ = 2.2 ± 1.2 mM, $V_{max2}$ = 26 ± 7.2 nmol/mg protein/30 min.) In contrast rabbit liver microsomes showed only a single metabolic pathway of sparteine to 2-dehydrosparteine best described by a single enzyme system. A $K_m$ of 36 ± 1.8 μM and a $V_{max}$ of 70 ± 10 nmol/mg protein/30 min. was obtained for control rabbit liver microsomes. Induction did not significantly alter these parameters. The 4 purified forms of rabbit liver cytochrome P-450 and the purified hog liver flavin-containing monooxygenase enzyme tested did not metabolize sparteine in all experiments performed.

The data of the present study show that in rat and rabbit liver microsomes a specific population of cytochrome P-450 metabolizes sparteine at low concentrations. At high substrate concentrations of sparteine in liver microsomes and hepatocytes a second population of cytochrome P-450 of low affinity can metabolize this substrate. The latter group would appear to be of limited significance at therapeutic concentrations of sparteine. The 4 purified forms of rabbit liver cytochrome P-450 and the flavin-containing monooxygenase tested did not metabolize sparteine. Therefore a specific form of cytochrome P-450 may to be involved in the metabolism of sparteine. As the flavin-containing monooxygenase showed no effect on the metabolism of sparteine this enzyme might be not connected with the N-oxidation of sparteine.

References:

1. M. Eichelbaum, Clin. Pharmacokinetics 7, 1 (1982)

2. D.M. Ziegler, Enzymatic basis of detoxication, 201, Academic Press, New York, 1982

3. M. Eichelbaum, N. Spannbrucker and H.J. Dengler, Eur. J. Clin. Pharmacol. 16, 109 (1979)

4. G.D. Knott, Comp. Prog. Biomed. 10, 271 (1979)