Biochemical Pharmacology, Vol. 37, No. 16, pp. 3155–3160, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00
Pergamon Press plc

IN-VIVO AND *IN-VITRO* DEXTROMETHORPHAN METABOLISM IN SD AND DA RAT

AN ANIMAL MODEL OF THE DEBRISOQUINE-TYPE POLYMORPHIC OXIDATION IN MAN

THOMAS ZYSSET,* TANJA ZEUGIN and ADRIAN KÜPFER
Department of Clinical Pharmacology, Murtenstr. 35, CH-3010 Berne, Switzerland

(*Received* 24 *September* 1987; *accepted* 22 *February* 1988)

Abstract—The female dark Agouti (DA) rat is well established as an animal model for the debrisoquine poor metabolizer phenotype (PM), whereas the SD rat represents the extensive metabolizer (EM). It is not known, however, if the DA rat also is representative for the dextromethorphan (DEM) PM, a compound recently demonstrated to be subjected to the debrisoquine phenotype in man. Studies were performed, therefore, to evaluate *in-vivo* and *in-vitro* metabolism of DEM in DA and SD rats. After oral administration of 50 mg/kg of DEM, the DA rat excreted 25 ± 6% of the dose in 72-hr urine as *O*-demethylated product (dextrorphan), whereas the SD excreted 40 ± 9% ($P < 0.002$). Metabolic ratio of *O*-demethylation was 0.46 ± 0.11 in DA and 0.02 ± 0.01 in SD ($P < 0.001$). As a compensatory mechanism, *N*-demethylation was ninefold increased in DA compared to SD (8.0 ± 3% of the dose excreted in urine of DA as methoxymorphinan vs 0.9 ± 0.7% in SD) ($P < 0.001$). Total plasma clearance of DEM was 95 ± 20 ml/min/kg in SD and 45 ± 13 ml/min/kg in DA ($P < 0.001$). *In vitro*, microsomal affinity for DEM *O*-demethylation was >50 times higher in SD than in DA rats ($P < 0.004$), whereas V_{max} did not differ statistically. V_{max} for *N*-demethylation was 80% increased in DA ($P < 0.01$), whereas corresponding K_m values did not differ. It appears that the differences in DEM metabolism between DA and SD rats are qualitatively similar to human EM and PM phenotypes, respectively. Whether this is also true for the underlying mechanism(s) however, remains to be resolved.

Dextromethorphan (DEM) is a widely used antitussive agent with low toxicity and low potential for drug dependency [1–3]. Recently, it has been demonstrated that in man, *O*-demethylation of this compound is genetically determined [4] and co-segregates with debrisoquine hydroxylation [5]. Due to its low toxicity and world-wide availability, DEM, therefore, appears to be an ideal test compound to evaluate the debrisoquine-type hydroxylator phenotype.

DEM metabolism undergoes two major metabolic pathways, namely *O*-demethylation to dextrorphan (DOR) and *N*-demethylation to methoxymorphinan (MEM). These metabolites may be further demethylated to hydroxymorphinan (HOM). The metabolic pathways of DEM are depicted in Fig. 1.

In order to perform studies on DEM metabolism at the level of hepatic microsomes, a suitable animal model would provide distinct advantages over studies in man. For the debrisoquine poor metabolizer phenotype, the dark Agouti rat (DA rat) has been demonstrated to represent a valid model [6]. It is not known, however, whether this rat strain would also provide a useful model for the poor DEM metabolizer phenotype. The aim of this study was, therefore, to evaluate DEM metabolism *in vivo* and *in vitro* in the DA rat and to compare it with the Sprague–Dawley rat.

* Author to whom correspondence should be addressed.

MATERIALS AND METHODS

Chemicals. Dextromethorphan hydrobromide (DEM), dextrorphan tartrate (DOR), 3-methoxymorphinan hydrochloride (MEM), 3-hydroxymorphinan (HOM), levallorphan tartrate, debrisoquine hemisulfate and 4-hydroxy-debrisoquine were kindly supplied by Hoffmann-La Roche, Basel, Switzerland. Glucose-6-phosphate-dehydrogenase (grade II), NADP, β-glucuronidase/arylsulfatase from *Helix pomatia* were from Boehringer (Mannheim, F.R.G.). Methanol and acetonitrile for HPLC were from Rathburn (Walkerburn, GB). All other chemicals were of analytical grade.

Animals. Female dark Agouti (DA) rats (mean body weight 211 ± 11 g) and female Sprague–Dawley (SD) rats (267 ± 25 g) were from Deutsche Versuchstierfarm Tuttlingen, F.R.G. They were kept in a 12 hr light–dark cycle in temperature- and humidity-controlled quarters. They had free access to water and were fed a maintenance diet for rats (Kliba, Basel, Switzerland).

In-vivo *studies*. Prior to the studies with DEM, the rats were phenotyped with debrisoquine (5 mg/kg body wt) as previously described [6]. To measure urinary DEM kinetics, 129.8 mg of DEM–HBr was dissolved in 0.5 ml propylenglycol, 200 μl of ethanol and 1.3 ml water. This solution was administered in a dose of 50 mg/kg body wt (calculated as free base) by gastric intubation. The rats were put into metabolic cages and urine was collected quantitatively in

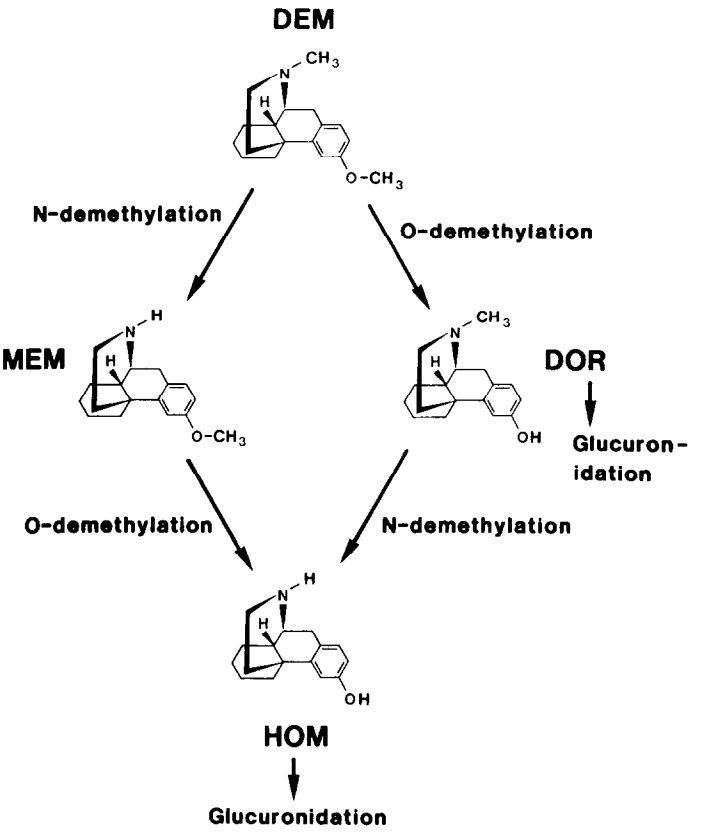

Fig. 1. Metabolic pathways of dextromethorphan. DEM, dextromethorphan; DOR, dextrorphan; MEM, methoxymorphinan; HOM, hydroxymorphinan.

12-hr intervals for 72 hr. Thereafter, the rats were kept in their usual quarters. One week later, PE-50 catheters were inserted under light ether anesthesia into the femoral vein and artery, respectively, and the rats were put into restraining cages. After a recovery period of about 30 min, DEM in a dose of 2 mg/kg body wt (free base) was injected into the jugular vein. Blood samples (250 μl) were taken at 0, 5, 10, 15, 20, 30, 50, 75 and 90 min and immediately replaced by an equal volume of heparinized blood previously taken from a donor rat by exsanguination. Urine was collected simultaneously. After collecting the last blood sample, the respective blood vessels were ligated, the catheters withdrawn and the skin incision was closed by sutures. The animals were kept in metabolic cages for three days as described above. This procedure was well tolerated, there was no weight loss during this time period. On the fourth day, the *in-vitro* assays were performed.

In-vitro *studies*. The rats were anesthetized with ether and killed by exsanguination. The livers were perfused through the portal vein with ice-cold physiological saline. Microsomes were prepared by differential centrifugation according to Mackinnon *et al.* [7]. Cytochrome P-450 and protein contents were analyzed by standard procedures [8, 9]. Microsomes were incubated for 30 min at 37°. 300 μl of incubation mixture contained 100 μl of microsomal suspension (corresponding to approx. 30 μg of microsomal protein), 3 μmol of glucose-6-phosphate, 1 μmol of NADP, 3 μmol of $MgCl_2$, 0.7 U of G6P-DH and DEM in concentrations ranging from 2.5 to 980 μM. The reaction was stopped with 20 μl of 60% perchloric acid.

Analytical methods. Debrisoquine and 4-OH-debrisoquine were determined after derivatization with hexafluoroacetylacetone as previously described [10]. A Carlo Erba gas chromatograph was used, equipped with an electron-capture detector. (Carlo Erba Strumentazione, Italy). The compounds were separated on a 6 ft 2.0 i.d. glass column filled with 3% XE-60 on Chromosorb W-HP (Supelco, Bellefonte, PA, U.S.A.). Peaks were integrated on a Hewlett Packard model 3390 integrator (Hewlett-Packard, Avondale, PA). DEM and its three major metabolites were analysed by HPLC: The HPLC system consisted of a Waters M-45 pump and a WISP 712 autoinjector (Waters, Milford, MA). A fluorescence detector (Merck-Hitachi, Merck Darmstadt, F.R.G.) was used. Wavelength settings were 270 nm (excitation) and 312 nm (emission). Peaks were integrated on a Spectraphysics SP 4290 integrator (Spectraphysics, San Jose, CA). A Nucleosil RP-phenyl column (ET 250/8/4, 7 μm) from Macherey-Nagel (Oensingen, Switzerland) was used. The solvent consisted of a mixture of 70% buffer (KH_2PO_4 10 mM, 1-octanesulfonic acid sodium salt monohydrate 2.5 mM, adjusted to pH

2.5 with phosphoric acid) and 30% of organic phase (acetonitrile/methanol 2:1). Flow rate was 1.3 ml/min, resulting in a back pressure of approx. 2200 psi.

DEM in blood was extracted as follows: to 100–200 μl of whole blood, 500 μl of 1 M glycine buffer pH 11.3, internal standard (60 ng/sample of levallorphan) and 5 ml of hexane containing 0.1% triethylamine were added. After shaking on a horizontal shaker for 15 min (Gerhardt, Bonn, F.R.G.) the upper organic layer was transferred to another tube and evaporated under a gentle stream of nitrogen in a water bath at 40°. 200 μl of HPLC solvent were added and 100 μl was injected onto the HPLC column. The assay was linear from 10 to 300 ng of DEM per tube. Overall recovery was >90%, day to day coefficient of variation of a spiked sample of 50 ng was <6% (N = 10).

DEM and its metabolites in urine were assayed as follows: To 250 μl of urine 20 μl of glusulase dissolved in 250 μl of 0.1 M acetate buffer pH 5.0 was added. Hydrolysis was performed for 12 hr at 37° in capped 10 ml Sovirel tubes in a shaking water bath. After addition of 1.5 ml of glycine buffer (pH 11.3) and 5 μg of internal standard (levallorphan), the samples were extracted with 5 ml of hexane containing 10% *n*-butyl alcohol. The organic phase was processed as described above. To the dried residues, 400 μl of the mobile phase was added and 10 μl was injected into the HPLC system. Calibration (using spiked blank urine) was linear up to 20 μg of DEM and metabolites. Extraction recoveries were the following: DEM 89 ± 6%, MEM 91 ± 3%, DOR 87 ± 8%, HOM 73 ± 3% (N = 4). Corresponding day-to-day coefficients of variation for these substances were 11.4, 5.7, 2.0 and 3.1%, respectively, for a control of 12 μg/ml (N = 6).

Assay in microsomes: After stopping the microsomal reaction with 20 μl of 60% perchloric acid, 1 μg of internal standard was added, the sample was briefly vortexed and centrifuged in an Eppendorf centrifuge at 10,000 rpm for 3 min. 15 μl of the supernatant was injected into the HPLC system without prior extraction. The assay was calibrated from 0 to 10 μg of DOR and MEM and to 1.0 μg of HOM per ml of microsomal incubation mixture. For calibration, ice-cold microsomal incubation mixture was spiked with a solution of DEM and metabolites, immediately followed by protein precipitation with perchloric acid.

Calculations. Metabolic ratio of debrisoquine and 4-OH-debrisoquine (MR_d) was calculated as previously described [11]:

MR_d = μmol debrisoquine/μmol 4-OH-debrisoquine excreted during 24 hr in urine.

Metabolic ratio of DEM-*O*-demethylation (MR_{DEM}) was estimated by calculating the ratio of the non-*O*-demethylated substrates (MEM and DEM) and the product of *O*-demethylation (DOR):

MR_{DEM} = (μmol DEM + μmol MEM)/μmol DOR excreted in 24 hr in urine.

Pharmacokinetics of DEM in blood were calculated using the R-strip fitting program (Micromath Scientific Software, Salt Lake City, U.S.A.). In all cases, the program selected a two-compartment model as the best fit of the data. AUC extrapolated to infinity (AUC_∞) was calculated by the R-strip program as:

$$AUC_\infty = \int_0^\infty C(t)\,dt.$$

Clearance was calculated as dose/AUC_∞, volume of distribution as

$$VD_{ss} = Dose_{iv} \cdot AUMC_\infty/(AUC)^2_\infty,$$

where AUMC (area under the moment curve) is defined as

$$AUMC_\infty = \int_0^\infty tC(t) \cdot dt.$$

[12]. K_m and V_{max} were estimated by nonlinear regression analysis [13].

All values are expressed as mean ± 1 standard deviation. Group means were compared by analysis of variance, followed by Student's *t*-test if the former showed significance [14].

RESULTS

(1) In-vivo *pharmacokinetics of dextromethorphan*

Metabolic ratio of debrisoquine was 0.334 ± 0.217 in SD (N = 6) and 1.474 ± 0.429 in DA rats (N = 9) ($P < 0.001$), whereas the ratio of DEM was 0.023 ± 0.012 in SD and 0.456 ± 0.106 in DA rats ($P < 0.001$). As shown in Fig. 2, there was no overlap of the metabolic ratios between SD and DA rats.

Between 56 and 61% of administered DEM was recovered during 72 hr in urine of SD and DA rats, mainly as metabolites. Total recovery after 72 hr, as well as at each 12-hr sampling interval, did not differ between the two strains. Identical values were obtained after intravenous and oral administration of DEM, suggesting complete intestinal absorption of the parent compound (Table 1). After oral administration, DEM *N*-demethylation to MEM as well as formation of the *N*- and *O*-demethylated product HOM was significantly increased in DA rats compared to SD. Interestingly, after intravenous administration, recovery of these two metabolites did not differ between SD and DA rats. After both i.v. and p.o. dosage, the DA rat excreted more unchanged DEM than the SD strain.

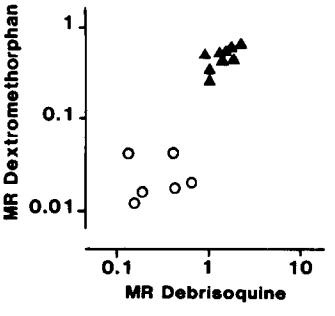

Fig. 2. Metabolic ratios of debrisoquine and DEM in DA and SD rats. ▲, DA (N = 9); ○, SD (N = 6).

Table 1. Cumulative urinary excretion of DEM and its three main metabolites in 72 hr in SD and DA rats

	DEM	DOR	MEM	HOM	Total
SD p.o. (N = 7)	1.3 ± 2.0	39.8 ± 9.1	0.9 ± 0.7	13.6 ± 6.0	55.7 ± 11.8
DA p.o. (N = 8)	4.1 ± 1.4	25.0 ± 5.6	8.0 ± 2.9	23.9 ± 4.1	61.1 ± 11.7
p SD vs DA	0.006	0.002	0.00002	0.0017	n.s.
SD i.v. (N = 6)	5.5 ± 2.1	31.5 ± 8.4	4.0 ± 1.3	18.3 ± 6.5	59.2 ± 12.6
DA i.v. (N = 5)	9.2 ± 2.0	20.4 ± 4.6	6.5 ± 4.9	19.6 ± 6.7	55.6 ± 8.9
p SD vs DA	0.016	0.027	n.s.	n.s.	n.s.

The doses were 50 mg/kg body weight p.o. and 2 mg/kg i.v. of free DEM base, respectively. Results are expressed as % of administered dose.

Total clearance of DEM in whole blood after i.v.-administration was 95 ± 20 ml/min/kg in SD (N = 7) and 45 ± 13 ml/min/kg in DA rats, respectively (N = 6, $P < 0.0003$). Terminal half life was significantly longer in DA (93 ± 21 min) than in SD rats (60 ± 24 min, $P < 0.03$). Apparent volumes of distribution (Vdss) were 22.4 ± 9.5 and 17.6 ± 5.4 l/kg body weight in SD and DA, respectively, and did not differ statistically. Mean DEM blood concentrations are depicted in Fig. 3.

(2) In vitro *dextromethorphan kinetics*

V_{max} of microsomal *O*-demethylation (DOR-formation) was 523 ± 174 pmol/min/mg microsomal protein in DA and 606 ± 95 pmol/min/mg protein in SD rats (P n.s.). There was a more than 50-fold difference, however, in K_m values: In DA, K_m was 210 ± 133 μM, whereas in SD, it was 4.1 ± 0.9 μM ($P < 0.004$). For *N*-demethylation (MEM-formation), K_m values did not differ (162 ± 90 vs 95 ± 47 μM in DA and SD, respectively), whereas V_{max} in DA was 80% higher (708 ± 237) than in SD rats (399 ± 80 pmol/min/mg protein, $P < 0.01$). The respective enzyme kinetics are depicted in Figs 4 and 5.

DISCUSSION

Our study demonstrated that the DA rat is a valid model for the poor DEM *O*-demethylation phenotype: the difference in metabolic ratios between DA and SD was 20-fold, whereas for debrisoquine, the respective difference was 4.4-fold. There was no overlap between the two strains. The DA rat apparently compensated the decreased DOR formation in part by an increase in *N*-demethylation.

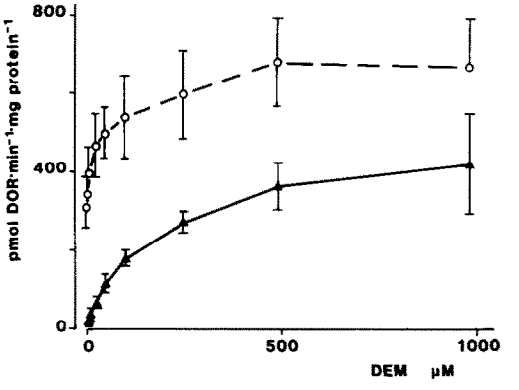

Fig. 4. Michaelis–Menten kinetics of DOR formation in microsomal incubations of DA and SD rats. Protein concentration in the incubation mixture was 0.1 mg/ml. ▲, DA (N = 6); ○, SD (N = 6).

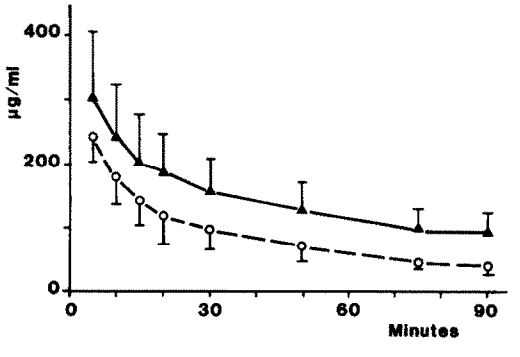

Fig. 3. Blood disappearance of DEM in SD (N = 7) and DA (N = 6) rats after intravenous administration of DEM hydrobromide (2 mg/kg body weight calculated as free base). The values represent the means ± 1 SD. ▲, DA (N = 6); ○, SD (N = 7).

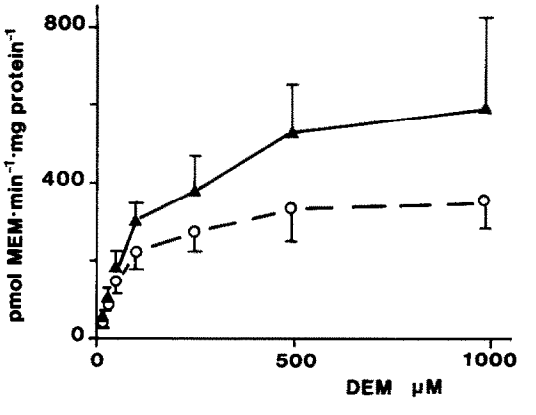

Fig. 5. Michaelis–Menten kinetics of MEM formation in microsomal incubations of DA and SD rats. Protein concentration in the incubation mixture was 0.1 mg/ml. ▲, DA (N = 6); ○, SD (N = 6).

These *in-vivo* findings qualitatively agree with results obtained in microsomal preparations: the increased urinary DOR output in the SD rat can be explained by the much higher enzymatic affinity for DEM (low K_m), whereas the increased MEM output in the DA strain is reflected *in vitro* by a higher V_{max} for *N*-demethylation.

Whereas the *in-vitro* metabolic pattern of DOR and MEM formation qualitatively agreed with urinary recovery of these metabolites after oral DEM administration, there was a discrepancy between *in-vivo* and *in-vitro* HOM formation. *In vivo*, HOM was a major urinary metabolite, whereas only trace amounts of HOM could be detected in the microsomal incubations. The reason for this discrepancy is not clear yet, preliminary *in-vitro* data using 10-fold higher microsomal protein concentrations suggested a mechanism compatible with product inhibition of HOM formation.

Interestingly, in contrast to oral administration, after intravenous DEM application, urinary recoveries of MEM and HOM did not differ between SD and DA rats (Table 1). A possible explanation for this finding may be the dose and route of administration: due to a considerable first pass effect of DEM, the oral dose was chosen 25 times higher than the intravenous one. Under these circumstances, therefore, the microsomal enzyme system may work near saturation conditions, which may be the reason for the discrepancies in relative recovery of metabolites between p.o. and i.v. administration.

The *in-vitro* findings suggest that the genetic differences between SD and DA rats are primarily located in the hepatic microsomal fraction. This does not exclude, however, the presence of extrahepatic DEM metabolism. This possibility is suggested by the high *in-vivo* clearance rate of DEM measured after intravenous DEM administration which in the SD rat was in the order of hepatic blood flow. Extensive tissue distribution [15] and/or extrahepatic metabolism may have been contributing factors to this high clearance rate.

Relatively few studies exist on DEM metabolism. An early study in male SD rats using tritiated DEM reported much lower urinary metabolite excretion than our study [16]. During four days following DEM administration, only about 20% of the dose was recovered in urine, whereas about 35% was found in feces. The reason for the lower urinary recovery in this study is not clear. Methodological and/or sex, age and strain differences may have been contributing factors. Major sex differences for DOR pharmakocinetics in the rat have been reported [17].

Comparison of DEM metabolism between man and the DA–SD rat model showed profound quantitative differences, whereas the qualitative pattern was similar: in a group of human volunteers corresponding most likely to extensive metabolizer phenotypes, Pfaff *et al.* [18] recovered 86% of the DEM dose in urine during 48 hr. The main metabolites were DOR and HOM, whereas no MEM could be detected. In a second group presumably corresponding to poor metabolizers, only 19% of the dose was recovered in urine, mainly as unmetabolized DEM. In contrast to the extensive metabolizers, this group also excreted *N*-demethylated metabolite (MEM). Total recovery in man, therefore, is in contrast to our rat model, where similar percentages of a DEM dose could be recovered in urine of both DA and SD rats. Qualitatively, however, there were some similarities between human poor and extensive metabolizers and the SD and DA rat: both human poor metabolizers and the DA rat showed decreased DOR and increased MEM and DEM output compared to the extensive metabolizer and the SD rat, respectively.

The exact mechanism for the metabolic differences between SD and DA rats are not clear yet. Total cytochrome P-450 content in hepatic microsomes did not differ between SD and DA (0.81 ± 0.09 vs 0.79 ± 0.09 nmol cytochrome P-450/mg protein, P n.s.). It may be speculated, however, that the two strains differ in the composition or amount of cytochrome P-450 isozymes: Investigations by the group of Guengerich suggested that one isozyme ($P\text{-}450_{UT\text{-}H}$) is responsible for debrisoquine hydroxylation. Studies in hepatic microsomes revealed that the female DA rat is deficient in this isozyme, showing levels amounting to only about 5% of those in male or female SD rats [19]. The same group demonstrated that the amount and catalytic activity of this isozyme is regulated at the level of the mRNA [20]. Studies on debrisoquine 4-hydroxylase and bufuralol 1′-hydroxylation in the rat, on the other hand, suggested that at least three isozymes might be involved in the hydroxylation of these substrates [21, 22]. The differences in V_{max} and K_m between SD and DA rats for *N*- and *O*-demethylation, respectively, indicate that these pathways may be under different genetic control. These hypotheses are supported by a preliminary finding indicating that linear transformation of DOR formation in hepatic microsomes (V vs V/S) suggested in some, but not in all of the SD rats two enzymatic binding sites for DEM (plots not shown). Increased assay sensitivity by several orders of magnitude would be required to elucidate the exact mechanisms involved. Appropriate studies using tritium-labelled DEM are in progress in our institution. Regardless of the exact mechanisms, our study demonstrated that DEM metabolism in the female DA rat follows a qualitatively similar pattern to the human debrisoquine poor metabolizer phenotype. The quantitative metabolic pattern, however, markedly differed from the one observed in man. Further studies on the level of the genes coding for P-450 isozymes are needed to elucidate the mechanisms and significance of our results with regard to the human PM.

Acknowledgements—This work was supported by the Swiss National Science Foundation, Grants Nr. 3.825.0.84 and 3.823.084, and the "Sandoz Stiftung zur Förderung der Medizinisch-Biologischen Wissenschaften". The expert analytical assistance of Mrs C. Talos is greatly appreciated.

REFERENCES

1. Benson WM, Stefko PL and Randall LO, Comparative pharmacology of levorphan, racemorphan and dextrorphan and related methyl ethers. *J Pharmacol Exp Ther* **109**: 189–200, 1953.
2. Cass LJ and Frederik WS, Evaluation of a new antitussive agent. *N Engl J Med* **249**: 132–136, 1953.

3. Matthys H, Bleicher B and Bleicher U, Dextromethorphan and codeine: objective assessment of antitussive activity in patients with chronic cough. *J Int Med Res* **11**: 92–100, 1983.
4. Küpfer A, Schmid B and Pfaff G, Pharmacogenetics of dextromethorphan *O*-demethylation in man. *Xenobiotica* **16**: 421–433, 1986.
5. Schmid B, Bircher J, Preisig R and Küpfer A, Polymorphic dextromethorphan metabolism: co-segregation of oxidative *O*-demethylation with debrisoquin hydroxylation. *Clin Pharm Ther* **38**: 618–624, 1985.
6. Al-Dabbagh SG, Idle JR and Smith RL, Animal modelling of human polymorphic drug oxidation—the metabolism of debrisoquine and phenacetin in rat inbred strains. *J Pharm Pharmacol* **33**: 161–164, 1981.
7. Mackinnon M, Sutherland E and Simon FR, Effects of ethinyl estradiol on hepatic microsomal proteins and the turnover of cytochrome P-450. *J Lab Clin Med* **90**: 1096–1106, 1977.
8. Omura T and Sato R, The carbon monoxide-binding pigment of liver microsomes, *J Biol Chem* **239**: 2370–2378, 1964.
9. Lowry OH, Rosenbrough NJ, Farr LA and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
10. Erdtmansky P and Goehl TJ, Gas–liquid chromatographic electron capture determination of some monosubstituted guanido-containing drugs. *Analyt Chem* **47**: 750–752, 1975.
11. Dick B, Küpfer A, Molnàr J, Braunschweig S and Preisig R, Hydroxylierungsdefekt für Medikamente (Typus Debrisoquin) in einer Stichprobe der Schweizer Bevölkerung. *Schweiz Med Wochenschr* **112**: 1061–1067, 1982.
12. Gibaldi M and Perrier D, *Pharmacokinetics* (2nd Edn). Marcel Dekker, New York, 1982.
13. Wilkinson GN, Statistical estimations in enzyme kinetics. *Biochem J* **80**: 324–332, 1961.
14. Snedecor GW and Cochran WG, *Statistical Methods*. Iowa State University Press, Ames, Iowa, 1967.
15. Roos F, Zysset T and Reichen J, Determinants of hepatic dextromethorphan (DM) extraction (E) in the rat. *Experientia* **43**: 708, 1987.
16. Kamm JJ, Taddea AB and van Loon EJ, Metabolism and excretion of tritiated dextromethorphan by the rat. *J Pharmacol Exp Ther* **158**: 437–444, 1967.
17. Ramachander G, Bapatla KR and Emele JF, Sex differences in plasma half-life of dextrorphan in rats administered dextromethorphan. *J Pharm Sci* **67**: 1326–1327, 1978.
18. Pfaff G, Briegel P and Lamprecht I, Inter-individual variation in the metabolism of dextromethorphan. *Int J Pharm* **14**: 173–189, 1983.
19. Larrey D, Distlerath LM, Dannan GA, Wilkinson GR and Guengerich FP, Purification and characterization of the rat liver microsomal cytochrome P-450 involved in the 4-hydroxylation of debrisoquine, a prototype of genetic variation in oxidative drug metabolism. *Biochemistry* **23**: 2787–2795, 1984.
20. Churchill PF, Churchill SA, Martin MV and Guengerich FP, Characterization of a rat liver cytochrome $P\text{-}450_{UT\text{-}H}$ cDNA clone and comparison of mRNA levels with catalytic activity. *Mol Pharmacol* **31**: 152–158, 1987.
21. Kahn GC, Rubenfield M, Davies DS, Murray S and Boobis AR, Sex and strain differences in hepatic debrisoquine 4-hydroxylase activity of the rat. *Drug Metab Dispos* **13**: 510–516, 1985.
22. Boobis AR, Seddon CE and Davies DS, Bufuralol 1′-hydroxylase activity of the rat. *Biochem Pharmacol* **35**: 2961–2965, 1986.