Pergamon

Biochemical Pharmacology, Vol. 49, No. 4, pp. 453–460, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(94)00481-1

ETHYLMORPHINE O-DEETHYLATION IN ISOLATED RAT HEPATOCYTES

INVOLVEMENT OF CODEINE O-DEMETHYLATION ENZYME SYSTEMS*

BANG QIAN XU,† TOR A. AASMUNDSTAD, ANDERS BJØRNEBOE, ASBJØRG S. CHRISTOPHERSEN and JØRG MØRLAND

National Institute of Forensic Toxicology, P.O. Box 16 Gaustad, N-0320 Oslo 3, Norway

(*Received* 31 *May* 1994; *accepted* 17 *October* 1994)

Abstract—The O-dealkylation of ethylmorphine (EM) and codeine (CD) to morphine (M) co-segregates with debrisoquine/sparteine genetic polymorphism in man. CD O-demethylation is catalysed by cytochrome P450 2D1 (CYP2D1) in rats. In the present study, the O-deethylation of EM was examined and compared with that of CD in suspensions of freshly-isolated hepatocytes prepared by a collagenase method from Wistar rats with and without CYP2D1 inhibitors. Isolated hepatocytes were also prepared from Dark Agouti (DA) rats deficient in CYP2D1, and were incubated with EM or CD. EM, CD and their metabolites were quantified by HPLC with UV detection. EM had a similar pattern of metabolism to that of CD in suspensions of hepatocytes from Wistar rats. Both EM and CD were O-dealkylated to form M plus morphine-3-glucuronide (M3G) and N-demethylated to form norethylmorphine (NEM) or norcodeine (NCD), respectively, which were further metabolized to normorphine (NM) and finally glucuronidated to normorphine-3-glucuronide (NM3G). As compared to hepatocytes from Wistar rats, DA rats were characterized by a markedly decreased formation (70~75% reduction) of M plus M3G from both EM and CD. Quinine, quinidine, propafenone and sparteine all inhibited EM O-deethylation as well as CD O-demethylation. Quinine was the most potent inhibitor of both these O-dealkylations ($K_i = 0.2$ μM for both EM and CD, respectively). Quinine as well as the other inhibitors inhibited both EM and CD O-dealkylation competitively and with small differences in K_i versus EM and CD, respectively. The metabolism of EM to M plus M3G and that of CD to M plus M3G was highly correlated when results from the various separate cell suspensions were plotted. In conclusion all findings indicated that the enzyme responsible for O-demethylation of CD, CYP2D1 was also responsible for the O-deethylation of EM to M.

Key words: ethylmorphine; O-deethylation; metabolism; morphine; CYP2D1, rat hepatocytes

EM‡ is a commonly used opioid antitussive drug with potential for abuse [1]. This might be a consequence of its O-deethylation to M [2, 3]. The other major biotransformation pathways in humans are N-demethylation to norethylmorphine (NEM) [4, 5], and EM glucuronidation to form ethylmorphine-6-glucuronide [6, §], similar to what has been shown for CD [7] (Fig. 1). In rats EM and CD are not glucuronidated [8, 9]. The human O-demethylation of CD to M is mediated by CYP2D6, [10–13] which also metabolizes, for example, debrisoquine/sparteine. This particular P450 is subject to genetic polymorphism, with 5–10% of a Caucasian population being deficient in this enzyme and designated poor metabolizers [14].

Recently, Rane *et al.* [6] demonstrated that the *in vivo* O-deethylation of EM to M co-segregates with debrisoquine/sparteine genetic metabolic polymorphism in man. Previously, it has been shown that CD along with debrisoquine, dextromethorphan and bufuralol are subjected to impaired oxidation in DA rats [15–19]. The absence of CYP2D1, the rat counterpart of human CYP2D6 [20, 21], gene expression in DA rats leads to a reduced metabolism of these drugs [21]. CD is closely related structurally to EM. The aim of the present study was to investigate if the O-dealkylation of EM to M was mediated by the same enzyme, CYP2D1, that O-demethylates CD to M. This was done by comparing the metabolism of EM with CD as a reference in experiments with suspensions of hepatocytes isolated from DA rats and Wistar rats.

MATERIALS AND METHODS

Animals. Male Wistar rats (250–320 g), male DA rats (200–230 g), and female DA rats (160–180 g)

* Part of this work was presented at the 9th International Symposium on Microsomes and Drug Oxidations, Jerusalem, July 1992.

† Corresponding author. Tel. (47) 22142190; FAX (47) 22143334.

‡ Abbreviations: CD, codeine; CYP2D1, cytochrome P450 2D1; CYP2D6, cytochrome P450 2D6; DA, Dark Agouti; EM, ethylmorphine; K_{el}, the elimination rate constant; K_i, inhibition constant; M, morphine; M3G, morphine-3-glucuronide; NCD, norcodeine; NEM, norethylmorphine; NM, normorphine; NM3G, normorphine-3-glucuronide.

§ Bugge A, Aasmundstad TA, Christophersen AS, Morgenlie S, Aasen AJ and Mørland J, Synthesis and detection of ethylmorphine-6-β-D-glucuronide, a metabolite of ethylmorphine, *Acta Chem Scand*, in press.

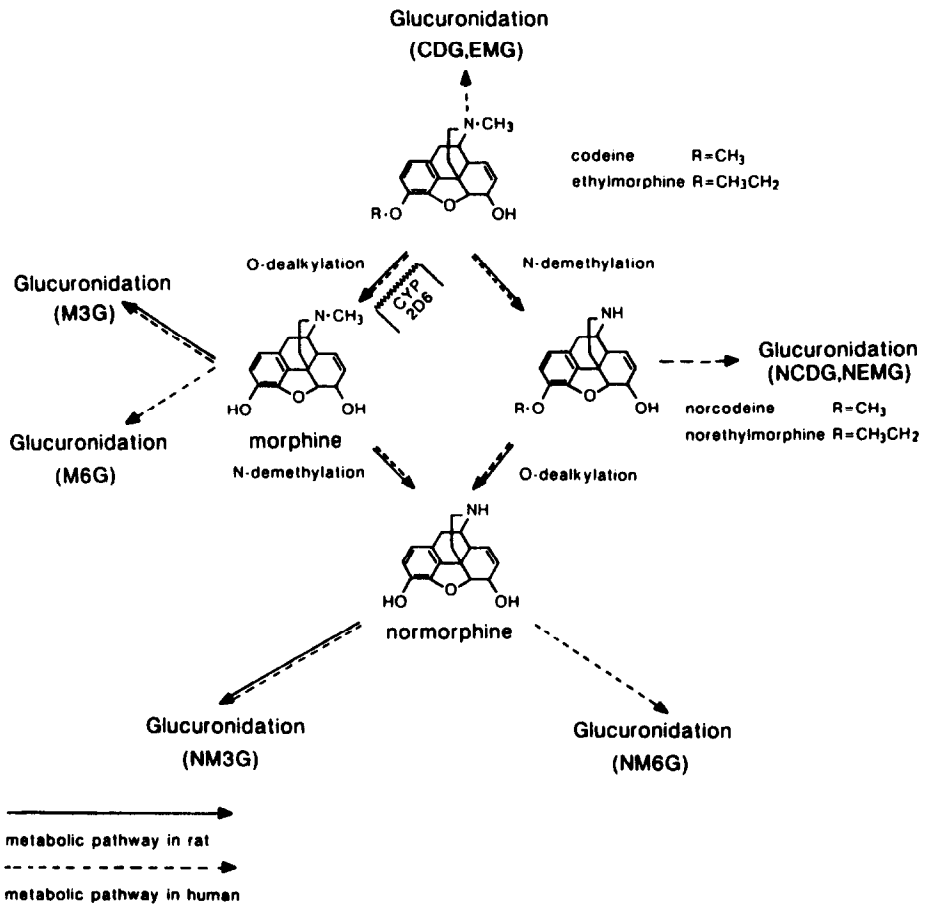

Fig. 1. Major metabolic pathways of CD and EM. Abbreviations: CDG, codeine-6-glucuronide; EMG, ethylmorphine-6-glucuronide; NCDG, norcodeine-6-glucuronide; NEMG, norethylmorphine-6-glucuronide; M6G, morphine-6-glucuronide; NM6G, normorphine-6-glucuronide.

(Møllegaard Hansens Avlslaboratorier A/S Ejby, Denmark) were maintained on a 12 hr light/dark cycle, at 23 ± 2° and allowed free access to standard pelleted food (B & K Universal A/S Nedre Holter, Norway) and water.

Chemicals and reagents. Collagenase (type IV, from Clostridium histolyticum), HEPES, β-glucuronidase (type L-II), BSA (fraction V defatted), M3G, NCD hydrochloride trihydrate, quinine hydrochloride, quinidine hydrochloride monohydrate, (-)-sparteine sulfate and propafenone hydrochloride were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). NM hydrochloride was obtained from Supelco, Inc. (Bellefonte, PA, U.S.A.). M hydrochloride, CD phosphate and naloxone hydrochloride (internal standard) were purchased from Norsk Medisinaldepot (Oslo, Norway). EM hydrochloride was obtained from Weiders Farmasøytiske A/S (Oslo, Norway). NEM was synthesized from EM [22]. Acetonitrile (FSA Laboratory Supplies, Loughborough U.K.) was of HPLC grade. All other reagents were of analytical grade and used as received.

Preparation of hepatocytes. Isolated hepatocytes were prepared according to a previously described method [23]. The viability of hepatocytes was checked by Trypan blue exclusion before and after incubation. The viabilities were 95–97% at the beginning of incubation, and 90–95% after 2 hr incubation. The protein concentration was determined according to Lowry *et al.* [24] with BSA used as standard.

Incubation conditions. Cell suspensions (1.6×10^6 cells/mL) of freshly-isolated hepatocytes from DA or Wistar rats were incubated at 37° in a final volume of 40 mL [8]. The cells were preincubated for 10 min before addition of EM or CD to final concentrations ranging from 5 to 100 μmol/L. For the inhibition experiments the inhibitors were dissolved in deionized water (Milli-Q^{UF}_{PLUS} Ultra-pure water system, Millipore Corporation, Bedford, MA, U.S.A.) and freshly prepared on the day of use. Various concentrations of inhibitors were added simultaneously with the substrate. Samples of suspension were drawn at 5, 10, 15, 20, 30, 40, 60 and 120 min, unless otherwise specified, and immediately frozen in liquid N_2, and stored at −80° until sonication and analysis [8].

Sample purification and analysis. EM, CD and their metabolites were quantitated by use of an

HPLC (Shimadzu LC-6A pump and SIL-9A auto injector, Kyoto, Japan) assay with UV detection (Shimadzu SPD-6A UV spectrophometric detector, Kyoto, Japan) after purification of the samples using a solid phase extraction (Bond Elut C_{18}) procedure [8].

Normorphine-3-glucuronide (NM3G) was determined indirectly as NM after hydrolysis with β-glucuronidase [8], due to lack of the reference standard. Recovery of glucuronide hydrolysis was checked by the incubation of different concentrations (5, 10, 20, and 50 μM) of M3G standards. The recovery of enzymatic hydrolysis of M3G was approximately 80%. However, due to lack of reference standard, the exact recovery of enzymatic hydrolysis of NM3G was impossible to determine.

Data analysis. The inhibitory constants (K_i values) were obtained graphically using Dixon plots [25]. Rats have almost no capacity for glucuronidation at the 6-position of morphine and its analogues [9, 26–28]. Furthermore 6-glucuronides were not detected in preliminary studies, and accordingly we did not seek these conjugates in the cell suspensions. The O-dealkylation of EM and CD was quantified by the formation of M plus M3G. Comparisons between results obtained from DA and Wistar rats were made using the Mann–Whitney test (Minitab® statistical software) and a value of $P < 0.05$ was considered significant. The elimination rate constants (K_{el}) were calculated by a log-linear regression analysis (Microsoft Excel®) computer program.

RESULTS

EM and CD metabolism in Wistar rat hepatocytes

The time profiles for the metabolism of EM and CD (20 μM) in suspensions of Wistar rat hepatocytes are outlined in Fig. 2. The similarities in biotransformation of the two drugs are clearly illustrated. Both EM and CD were metabolized to the intermediates M and NEM/NCD. M was then glucuronidated to M3G and N-demethylated to NM. NEM and NCD were also further metabolized to NM, which was subsequently glucuronidated to NM3G. After 2 hr incubation, approx. 51 ± 8% and 18 ± 4% (mean ± SD, N = 4) of the given dose of EM was recovered as NM plus NM3G and M3G, respectively, while M and NEM were no longer detected. In comparison, approx. 40 ± 9% and 26 ± 6% of the given dose of CD was recovered as NM plus NM3G and M3G, respectively. These results indicated that M (+ M3G) is one of the major metabolites of both EM and CD in rat hepatocytes. The elimination constants demonstrated that CD (20 μM) was metabolized at a slower rate than EM (20 μM) ($K_{el\,(CD)}$ 0.14 ± 0.03 min^{-1}, $K_{el\,(EM)}$ 0.36 ± 0.04 min^{-1}, mean ± SD, N = 4). The K_{el} depended on the substrate concentration indicating saturation of CD and EM metabolism. The K_{el} of 100 μM CD was 0.07 ± 0.01 min^{-1}, and of 100 μM EM 0.13 ± 0.03 min^{-1}.

EM and CD metabolism in DA rat hepatocytes

The metabolism of EM and CD (20 μM) in suspensions of isolated hepatocytes from male DA rats is depicted in Fig. 3. The same metabolites, as formed in hepatocytes of Wistar rats, were formed from both EM and CD, but there were quantitative differences in their metabolic pattern compared with the Wistar rats. The formation of M3G was reduced by more than 50% from both EM and CD (20 μM) after 2 hr incubation. On the other hand the formation of NEM and NCD was somewhat increased. The K_{el} (20 μM) was 0.05 ± 0.02 min^{-1} and 0.16 ± 0.04 min^{-1} for CD and EM, respectively, while the K_{el} of 100 μM of CD and EM was 0.02 ± 0.01 min^{-1} and 0.06 ± 0.02 min^{-1} (mean ± SD, N = 4), demonstrating that DA rat hepatocytes metabolized both CD and EM at a rate somewhat lower than 50% of the rate observed in Wistar hepatocytes.

The O-dealkylation of EM exhibited marked differences between Wistar and DA rat hepatocytes. The formation of M plus M3G was 70–75% lower ($P = 0.002$) in male DA than in Wistar rats in incubations with 100 μM of EM (Fig. 4a), demonstrating that the O-deethylation of EM to M was reduced in DA rats. Even less M plus M3G were produced from EM in suspensions of hepatocytes from female DA rats compared to male DA rats (Fig. 4A). There was a very similar pattern of impaired O-demethylation of CD in both male and female DA rats (Fig. 4B).

Inhibition of O-dealkylation of EM and CD in Wistar rat hepatocytes.

Co-incubation of EM with quinine resulted in the inhibition of O-deethylation of EM to such an extent that Wistar rat hepatocytes mimiced DA rats with regard to their O-dealkylation of EM. The formation of M plus M3G from EM (100 μM) was reduced by 65–70% ($P = 0.002$) as compared to the control after 10 min incubation (Fig. 4) in the presence of 5 μM quinine. The O-demethylation of CD was inhibited by quinine to the same extent as the O-deethylation of EM. Quinine was a more potent inhibitor of both EM and CD metabolism than quinidine, sparteine and propafenone (Table 1) which are all inhibitors of the O-dealkylation of CD.

Table 1 shows the inhibition of O-dealkylation of 20 μM of EM and CD with different concentrations of inhibitors used. Dose-dependent effects were observed for all the inhibitors. Other substrate concentrations of EM and CD were then incubated with different inhibitor concentrations to determine the inhibitor constants from Dixon plots. All inhibition constants (K_i) determined are presented in Table 2.

Correlation of EM O-deethylation and CD O-demethylation

The results for each single cell suspension with respect to per cent of initial dose of EM or CD metabolized to M plus M3G after 10 min were plotted in Fig. 5. A high correlation ($r = 0.97$) was observed between the corresponding values for the formation of M plus M3G from EM and CD for all hepatocytes studied (Wistar and DA strains). This correlation demonstrated a very high degree of co-variation of O-deethylation and the O-demethylation reactions.

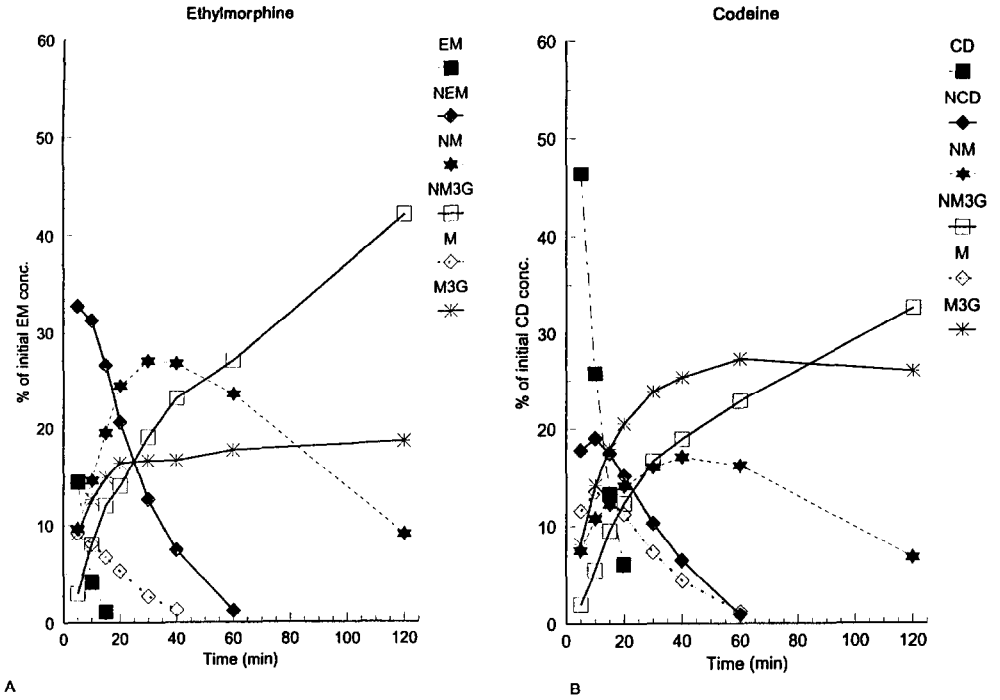

Fig. 2. Metabolism of EM and CD in suspensions of isolated hepatocytes from Wistar rats. The final concentration of EM or CD was 20 μM in this set of experiments. The results are mean data from four experiments.

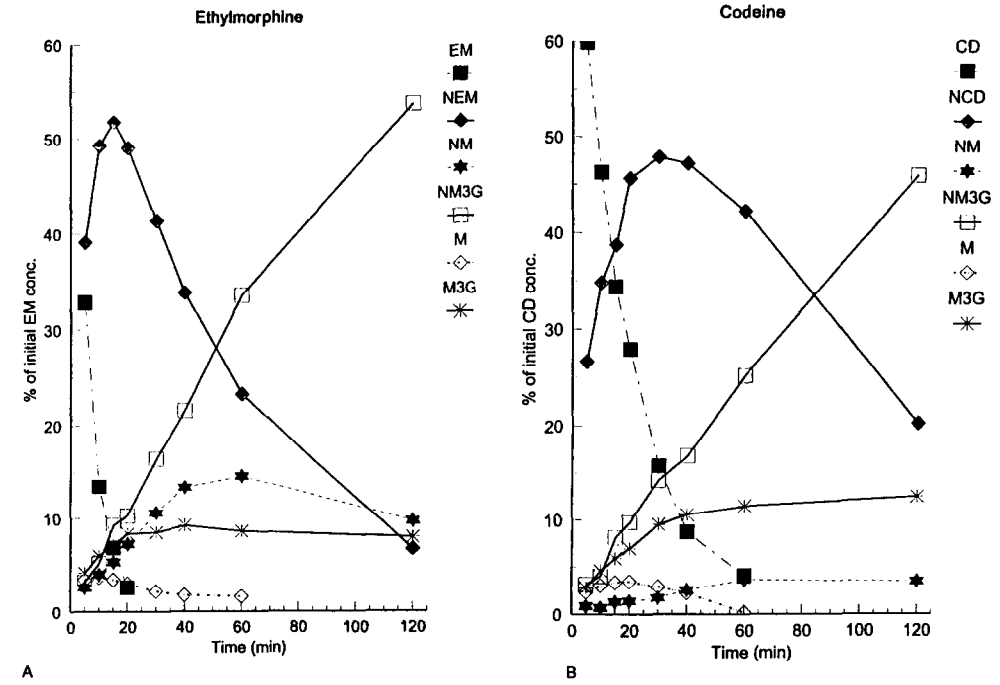

Fig. 3. Metabolism of EM and CD in suspensions of isolated hepatocytes from male DA rats. The final concentration of EM or CD was 20 μM in this set of experiments. The results are mean data from four experiments.

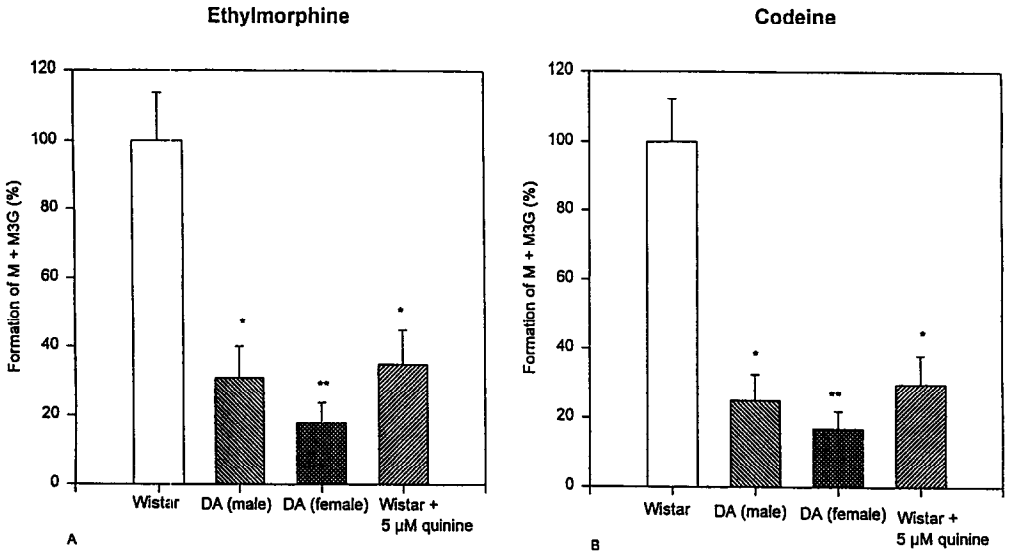

Fig. 4. Formation of M plus M3G in suspensions of hepatocytes isolated from Wistar and DA rats. Starting concentrations of EM and CD were 100 μM. Each bar represents mean values obtained from four experiments after 10 min incubation. The results are expressed as a percentage of the activity measured in suspensions of Wistar rat hepatocytes. Difference between male DA, female DA or Wistar in the presence of 5 μM quinine and Wistar control *(P = 0.002); **(P = 0.001).

Table 1. The inhibitory effect of quinidine, quinine, sparteine and propafenone on EM and CD O-dealkylation (formation of M plus M3G) after incubation for 30 min in suspensions of hepatocytes isolated from Wistar rats. Starting concentration of EM and CD 20 μM

Inhibitors	Ethylmorphine	Codeine
Quinidine		
0.5 μM	82†	62†
1 μM	71†	56†
5 μM	45‡	38‡
Quinine		
0.5 μM	32‡	22‡
1 μM	29‡	17§
5 μM	22§	10§
Sparteine		
5 μM	59*	55*
25 μM	30*	26*
Propafenone		
1 μM	85†	79†
5 μM	49‡	40‡
20 μM	15§	8§
50 μM	8§	4§

Data are mean values of four incubations; *data are mean values of two incubations.

The results are expressed as percentages of control activity (in the absence of inhibitor).

Significant difference between incubation with and without inhibitor. † (P = 0.01); ‡ (P = 0.002); § (P = 0.001).

Table 2. Inhibition constants (K_i) of inhibitors in isolated rat hepatocytes

Inhibitors	K_i (μM)	
	Ethylmorphine	Codeine
Quinine	0.2	0.2
Quinidine	1.7	1.1
Propafenone	1.8	1.3
Sparteine	5.6	4.7

All results are the means of two experiments.

The inhibition constants (K_i) were obtained graphically from Dixon plots.

DISCUSSION

Our experiments demonstrated that EM had a very similar pattern of metabolism to that of CD in suspensions of isolated hepatocytes from Wistar rats. Wistar and DA (male and female) rats showed a distinct strain difference for both EM and CD O-dealkylation. Competitive inhibitors of CD O-demethylation demonstrated similar K_i values for CD and EM and similar inhibition types for both substrates. Finally O-demethylation and O-deethylation activity were highly correlated for each single hepatocyte suspension. Taken together this indicated that the O-dealkylation of CD and EM was mediated by the same enzyme.

This study examined the O-dealkylation pathways of EM and CD in Wistar and DA rat strains. The latter exhibits a deficiency only in CYP2D1, which is mainly responsible for the metabolism of substrates

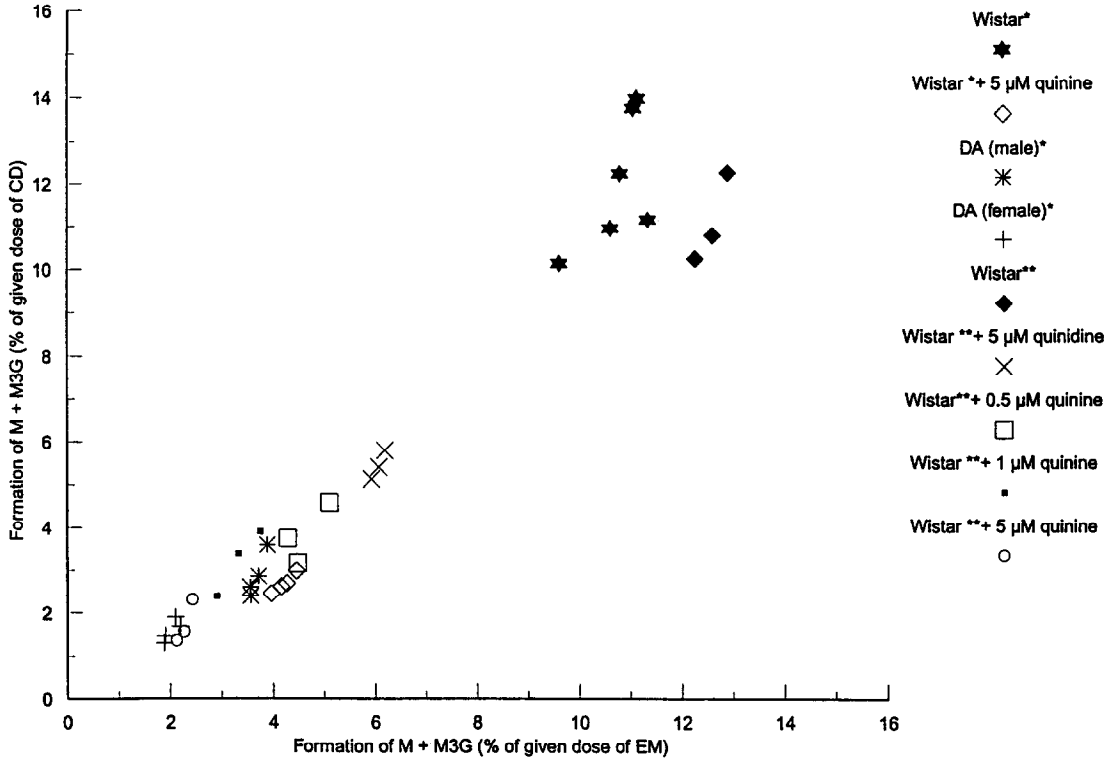

Fig. 5. Correlation between formation of M plus M3G from CD and formation of M plus M3G from EM in hepatocytes of individual rats of both rat strains after 10 min incubation. Substrate concentration: * = 100 μM; ** = 50 μM.

of the debrisoquine/sparteine type in Wistar rats. Therefore, DA rats could be a useful animal model to study the EM O-deethylation pathway. In this study O-dealkylation of EM and CD also showed this strain difference. The results obtained with DA rats agreed with the previously reported differences in CD metabolism in rats [18]. The difference between male and female DA rats may be due to the participation of more than one enzyme in CD and EM O-dealkylation. It has been shown that CYP2C11, a male-specific P450 isozyme, contributes to the metabolism of debrisoquine/sparteine oxidation [19]. On the other hand, other investigators [29] have failed to demonstrate inhibition of EM O-dealkylation by a monoclonal antibody against rat liver CYP2C11. This antibody was, however, a strong inhibitor of EM N-demethylation. Our observations strongly suggest that CYP2D1 are involved in the metabolism of CD and EM, since the CYP2D1 deficiency diminished O-dealkylation and prolonged the half-life of both CD and EM. Taken together our results and previous reports [6, 10–12, 15–19, 30, 31] strongly suggest that DA rats are a suitable animal model for the investigation of the CYP2D6/CYP2D1 dependent metabolism.

The activity of both CYP2D1 and CYP2D6 is inhibited by the specific inhibitor, quinidine, and its stereoisomer, quinine *in vitro* [32] and *in vivo* [33]. This inhibition can transform the extensive metabolizer phenotype into a pseudo-poor metabolizer phenotype [34]. Sparteine and propafenone are also substrates for CYP2D1 and CYP2D6 [18, 35], and act as competitive inhibitors for the O-demethylation of CD. Our observations are in good agreement with previously published results on quinidine and quinine [36] and the inhibition of CD O-demethylation [18]. The concordance between EM and CD in the presence of inhibitors suggested that the O-dealkylations of EM and CD were catalysed by the same enzyme.

In the present study, we used suspensions of freshly-isolated rat hepatocytes. As opposed to the use of microsomes or other subcellular fractions for metabolic studies, isolated hepatocytes represent a more *in vivo*-like model of hepatic metabolism capable of performing a variety of aspects of drug biotransformation under physiological conditions. Furthermore, we did not separate cell pellets and supernatants after incubation, but sonicated the total cell suspension before HPLC analysis. This should have reduced difficulties of the type previously reported [37] in which drugs actively transported into cells caused lower K_m values to be observed.

REFERENCES

1. Gjerde H and Mørland J, A case of high opiate tolerance: implications for drug analyses and interpretations. *Int J Leg Med* **104**: 239–240, 1991.

2. Ripel Å, Christophersen AS, Bjørneboe A and Mørland J, Morphine formation after intake of ethylmorphine. *Pharmacol Toxicol* **70**: 228–229, 1992.
3. Jarvi E, Stolznbach JC and Larson RE, Simultaneous quantification of ethylmorphine *O*-deethylase and *N*-demethylase activity by high-performance liquid chromatography. *J Chromatogr* **377**: 261–268, 1986.
4. Nerland DE and Mannering GJ, Species, sex and developmental difference in the O- and N-dealkylation of ethylmorphine by hepatic microsomes. *Drug Metab Dispos* **6**: 150–153, 1978.
5. Ladona MG, Spalding DJM, Ekman L, Lindström B and Rane A, Human fetal and adult liver metabolism of ethylmorphine: relation to immunodetected cytochrome P-450 PCN and interactions with important foetal corticosteroids. *Biochem Pharmacol* **38**: 3147–3155, 1989.
6. Rane A, Modiri AR and Gerdin E, Ethylmorphine O-deethylation cosegregates with the debrisoquine genetic metabolic polymorphism. *Clin Pharmacol Ther* **52**: 257–264, 1992.
7. Adler TK, Fujimoto JM, Way EL and Baker EM, The metabolic fate of codeine in man. *J Pharmacol Exp Ther* **114**: 251–262, 1955.
8. Xu BQ, Bjørneboe A, Ripel Å, Aasmundstad TA, Christophersen AS and Mørland J, Ethylmorphine metabolism in isolated rat hepatocytes. *Pharmacol Toxicol* **73**: 35–40, 1993.
9. Bodd E, Drevon CA, Kveseth N, Olsen H and Mørland J, Ethanol inhibition of codeine and morphine metabolism in isolated rat hepatocytes. *J Pharmacol Exp Ther* **237**: 260–264, 1986.
10. Dayer P, Desmeules J, Leemann T and Striberni R, Bioactivation of the narcotic drug codeine in human liver is mediated by the polymorphic monooxygenase catalyzing debrisoquine 4-hydroxylation (cytochrome P-450 dbl/buff). *Biochem Biophys Res Commun* **152**: 411–416, 1988.
11. Yue QY, Svensson JO, Alm C, Sjöqvist F and Säwe J, Codeine O-demethylation co-segregates with polymorphic debrisoquine hydroxylation. *Br J Clin Pharmacol* **28**: 639–645, 1989.
12. Mortimer Ö, Persson K, Ladona MG, Spalding D, Zanger UM, Meyer UA and Rane A, Polymorphic formation of morphine from codeine in poor and extensive metabolizers of dextromethorphan: relationship to the presence of immunoidentified cytochrome P-450IID1. *Clin Pharmacol Ther* **47**: 27–35, 1990.
13. Nebert DW, Nelson DR, Coon MJ, Estabrook RW, Feyereisen R, Fujii-Kuriyama Y, Gonzalez FJ, Guengerich FP, Gunsalus IC, Johnson EF, Loper JC, Sato R, Waterman MR and Waxman DJ, The p450 superfamily: update on new sequences, gene mapping, and recommended nomenclature. *DNA Cell Biol* **10**: 1–14, 1991.
14. Alván G, Bechtel P, Iselius L and Gundert-Remy U, Hydroxylation polymorphisms of debrisoquine and mephenytoin in European populations. *Eur J Clin Pharmacol* **39**: 533–537, 1990.
15. Al-Dabbagh SG, Idle JR and Smith RL, Animal modelling of human polymorphic oxidation the metabolism of debrisoquine and phenacetin in rat inbred strains. *J Pharm Pharmacol* **33**: 161–164, 1981.
16. Boobis AR, Seddon CE and Davies DS, Bufuralol 1′-hydroxylase activity of the rat strain differences and the effects of inhibitors. *Biochem Pharmacol* **35**: 2961–2965, 1986.
17. Zysset T, Zeugin T and Küpfer A, *In-vivo* and *in-vitro* dextromethorphan metabolism in SD and DA rat: an animal model of the debrisoquine-type polymorphic oxidation in man. *Biochem Pharmacol* **37**: 3155–3160, 1988.
18. Mikus G, Somogyi AA, Bochner F and Eichelbaum M, Codeine O-demethylation: rat strain differences and the effects of inhibitors. *Biochem Pharmacol* **41**: 757–762, 1991.
19. Larrey D, Distlerath LM, Dannan GA, Wilkinson GR and Guengerich FP, Purification and characterization of rat liver microsomal cytochrome P-450 involved in the 4-hydroxylation of debrisoquine, a prototype for genetic variation in oxidative drug metabolism. *Biochemistry* **23**: 2787–2795, 1984.
20. Nebert DW, Nelson DR, Adsenik M, Coon MJ, Estabrook RW, Gonzalez FJ, Guengerich FP, Gunsalus IC, Johnson EF, Kemper B, Levin W, Phillips JR, Sato R and Waterman MR, The P450 superfamily: update listing of all genes and recommended nomenclature for the chromosomal loci. *DNA* **8**: 1–13, 1989.
21. Matsunaga E, Zanger UM, Hardwick JP, Gelboin HV, Meyer UA and Gonzalez FJ, The CYP2D gene subfamily: analysis of the molecular basis of the debrisoquine 4-hydroxylase deficiency in DA rats. *Biochemistry* **28**: 7349–7355, 1989.
22. Xu BQ, Bugge A, Gunderson H, Malterud KE and Christophersen AS, Synthesis and spectroscopic studies of norethylmorphine. *J Pharm Biomed Anal* **10**: 303–307, 1992.
23. Mørland J, Bessesen A and Svendsen L, Incorporation of labelled amino acids into proteins of isolated parenchymal and nonparenchymal rat liver cells in the absence and presence of ethanol. *Biochim Biophys Acta* **561**: 464–473, 1979.
24. Lowry OH, Rosebrough AL, Farr AL and Randall RJ, Protein measurement with Folin phenol reagent. *J Biol Chem* **93**: 265–275, 1951.
25. Dixon M, The determination of enzyme inhibitor constants. *Biochem J* **55**: 170–171, 1953.
26. Hanioka N, Hoshikawa Y, Mitsui T, Oguri K and Yoshimura H, Species difference in codeine uridine diphosphate-glucuronyl-transferase activity of liver microsomes. *J Pharmacobio-Dyn* **13**: 712–717, 1990.
27. Oguri K, Hanioka N and Yoshimura H, Species difference in metabolism of codeine urinary excretion of codeine glucuronide, morphine-3-glucuronide and morphine-6-glucuronide in mice, rats, guinea pigs and rabbits. *Xenobiotica* **20**: 683–688, 1990.
28. Aasmundstad TA, Ripel Å, Bodd E, Bjørneboe A and Mørland J, Different biotransformation of morphine in isolated liver cells from guinea pig and rat. *Biochem Pharmacol* **46**: 961–968, 1993.
29. Rane A and Ask B, A conspicuous down-regulating effect of morphine on essential steroid hydroxylation reactions and certain drug N-demethylations. *J Steroid Biochem Mol Biol* **41**: 91–98, 1992.
30. Mikus G, Somogyi AA, Bochner F and Eichelbaum M, Thebaine O-demethylation to oripavine: genetic differences between two rat strains. *Xenobiotica* **21**: 1501–1509, 1991.
31. Kerry N, Somogyi AA, Mikus G, and Bochner F, Primary and secondary oxidative metabolism of dextromethorphan: In vitro studies with female Sprague-Dawley and Dark Agouti rat liver microsomes. *Biochem Pharmacol* **45**: 833–839, 1993.
32. Dayer P, Kronbach T and Eichelbaum M, Enzymatic basis of the debrisoquine/sparteine type genetic polymorphism of drug oxidation-characterization of bufuralol 1′-hydroxylation in liver microsomes of *in vivo* phenotyped carriers of the genetic deficiency. *Biochem Pharmacol* **36**: 4145–4152, 1987.
33. Leemann T, Dayer P and Meyer UA, Single-dose quinidine treatment inhibits metoprolol oxidation in extensive metabolizers. *Eur J Clin Pharmacol* **29**: 739–741, 1986.
34. Funck-Brentano C, Kroemer HK, Pavlon H, Woosley RL and Roden DM, Genetically-determined interaction between propafenone and low dose quinidine.

Role of active metabolites in modulating net drug effect. *Br J Clin Pharmacol* **27**: 435–444, 1989.
35. Siddoway LA, Thompson KA, McAllister CB, Wang T, Wilkinson GR, Roden DM and Woosley RL, Polymorphism of propafenone metabolism and disposition in man: clinical and pharmacokinetic consequences. *Circulation* **75**: 785–791, 1987.
36. Kobayashi S, Murray S, Watson D, Sesardic D, Davies DS and Boobis AR, The specificity of inhibition of debrisoquine 4-hydroxylase activity by quinidine and quinine in the rat is the inverse of that in man. *Biochem Pharmacol* **38**: 2795–2799, 1989.
37. Erickson RR, Yu-Drent P and Holtzman JL, The kinetics of ethylmorphine N-demethylation in isolated hepatocytes: The effect of drug transport and oxygen concentration. *J Pharmacol Exp Ther* **220**: 35–38, 1982.