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Presence of flavin-containing monooxygenase in rat brain

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Recent interest has centered on the capability of the brain to metabolize xenobiotics and the presence of cytochrome P450 (P450*) and associated monooxygenase activity have indicated the importance of drug metabolism in the brain [1–4]. Besides P450, flavin-containing monooxygenase (FMO, EC 1.14.13.8) is also known to catalyse the oxidation of a wide variety of nitrogen-, sulphur- and phosphorus-containing drugs, pesticides and industrial chemicals [5, 6]. FMO which is localized in microsomes has been detected in a wide variety of species [5]. In addition to liver, FMO activity has been detected in other organs, namely, lung, adrenal, kidney and thymus [5].

Some of the best known substrates for hepatic and pulmonary FMO are the psychoactive drugs, namely, antipsychotics (e.g. chloropromazine, trifluopromazine) and tricyclic antidepressants (e.g. imipramine, amitryptaline). Since the major site of action of these drugs is the central nervous system, cerebral FMO could play an important role in pharmacological modulation of these drugs in the brain. In view of this, the presence of FMO in the brain was investigated using model substrates like *N,N*,dimethylaniline (DMA), methimazole (MEM) and thiobenzamide (TB). Metabolism of DMA, MEM and TB by FMO leads to the formation of *N,N*,dimethylaniline-*N*-oxide, methimazole-*S*-oxide and thiobenzamide-*S*-oxide, respectively.

Materials and Methods

Male Wistar rats (6 months old) from the Central Animal Research Facility of the institute, were anaesthesized with ether and perfused transcardially (in order to minimize haemoglobin contamination in the microsomal preparation) with ice-cold 0.1 M Tris–HCl (pH 7.4). The animals were decapitated and the brains were quickly removed and microsomes prepared by a calcium precipitation method [7] with slight modification. Microsomes were washed twice to ensure complete removal of calcium [8]. The homogenization buffer consisted of 0.1 M Tris–HCl (pH 7.4) containing 1 mM EDTA, 0.1 mM PMSF, 1.15% KCl (w/v), 10% glycerol (v/v) and 0.1% BSA (w/v). Microsomes were prepared from pooled brains of 10 rats for each experiment and stored in liquid nitrogen until use. Protein was determined by a dye-binding method [9].

The metabolism of DMA and MEM was determined by measuring the change in absorbance at 340 nm due to oxidation of NADPH [8], in the presence of *n*-octylamine (an inhibitor of cytochrome P450) [10]. The assay buffer consisted of 0.2 M Tris–HCl (pH 8.4) containing 1 mM EDTA, 3 mM *n*-octylamine and 0.1% (v/v) Triton N-101 and was oxygenated before use. Microsomes (30–100 μg) were incubated with 0.1 mM NADPH in the above buffer and endogenous rates were recorded. FMO activity was measured following the addition of substrates (3 mM DMA or 1 mM MEM) and absorbance changes at 340 nm were recorded for 1–5 min. Reaction rates were determined using molar absorptivity of 6220 $M^{-1}cm^{-1}$ for NADPH, after subtracting the endogenous rates. The linearity of substrate-stimulated oxidation of NADPH with increasing microsomal protein concentration was maintained in the FMO assays with the three different substrates.

Thiobenzamide-S-oxidation (TBSO) was determined as described [11]. Triton N-101 (0.1%) was added to assay buffer.

Antisera against purified rat liver NADPH cytochrome P450 reductase (reductase) was raised in rabbits [2] and added to microsomes prior to assay of FMO activity. The amount of antisera necessary to completely inhibit rat brain reductase was as described earlier [2]. FMO activity was measured, as described above, in the microsomes treated with the above antisera to reductase.

Results and Discussion

FMO activity could be demonstrated in brain microsomes, using the model substrates DMA, MEM and TB (Table 1). The FMO activity in the brain was significantly higher than that observed in the liver. The rate of TBSO formation in rat brain was 147% of the corresponding hepatic rate. In a similar manner the rates of DMA- and MEM-stimulated NADPH oxidation were 160 and 133%, respectively, of the corresponding activity in the liver microsomes. The enzyme activity was determined in the presence of *n*-octylamine, a potent inhibitor of P450 [10], in order to prevent any contribution through P450-mediated metabolism. Microsomes were preincubated at 37° for 5 min with NADPH (to determine endogenous rates).

* Abbreviations: P450, cytochrome P450; FMO, flavin-containing monooxygenase; DMA, *N,N*,dimethylaniline; TB, thiobenzamide; TBSO, thiobenzamide-*S*-oxide; MEM, methimazole; reductase, NADPH cytochrome P450 reductase.

Table 1. Flavin-containing monooxygenase activity in rat brain and liver

	Brain	Liver
Thiobenzamide*	550.27 ± 47.46§	374.55 ± 23.84
N,N,Dimethylaniline†	176.00 ± 3.62§	110.70 ± 5.70
Methimazole†	19.00 ± 1.46‡	14.30 ± 1.12

Values are mean ± SEM (N = 4–7 individual experiments).

* Enzyme activity is expressed as nmoles of thiobenzamide-*S*-oxide formed/min/mg protein.

† Enzyme activity is expressed as nmoles of NADPH oxidized/min/mg protein.

Enzyme activities significantly higher than hepatic activities are indicated by: ‡$P < 0.01$; §$P < 0.001$.

Table 2. Effect of detergent, *n*-octylamine and reductase antibody in rat brain FMO activity

	DMA (nmoles of NADPH oxidized/min/mg protein)	MEM (nmoles of NADPH oxidized/min/mg protein)
Whole system	176.20 ± 3.62	19.0 ± 1.46
Whole system + antisera to reductase (4°)	135.10 ± 27.30	16.3 ± 1.59
+ Triton N-101 − *n*-Octylamine	161.70 ± 8.22	18.1 ± 2.30
− Triton N-101 − *n*-Octylamine	ND	ND
− Triton N-101 + *n*-Octylamine	ND	ND

Whole system incubations consisted of microsomal protein (30–200 μg) in 0.2 M Tris–HCl (pH 8.4) containing 1 mM EDTA, 3 mM *n*-octylamine, 0.1 mM NADPH, 0.1% Triton N-101 and appropriate substrates

Values are expressed as mean ± SEM (N = 4–7).

ND, not detected.

Antiserum to reductase was added at a concentration 10 μL of antisera per 160 μg microsomal protein and incubated for 30 min at 4° prior to determination of enzyme activity.

Endogenous rates of NADPH oxidation were negligible after this period of preincubation. This preincubation did not result in the loss of FMO activity.

NADPH cytochrome P450 reductase is an essential component of the P450 monooxygenase system. Antisera raised to rat liver reductase has been shown to effectively inhibit rat brain reductase activity [2]. In order to determine if there was any contribution of P450-mediated metabolism in NADPH-dependent oxidation of DMA and MEM, microsomes were preincubated with antisera to reductase, prior to assay of FMO activity. Preincubation with the above antisera had no effect on the oxidation of DMA or MEM (Table 2). This indicates that the metabolism of DMA and MEM as measured in the present study was mediated by FMO alone. The effect of detergent on FMO activity was also examined. FMO activity measured in the absence of detergent, namely, Triton N-101 (a non-ionic detergent) was very low compared with the activity detectable in the presence of the detergent. Solubilization of microsomes with detergents is known to enhance certain microsomal enzyme activities, namely hexose-6-phosphate dehydrogenase and glucuronyl transferase [12]. This enhancement could be attributed to increased cofactor or substrate accessibility to the enzyme [12]. FMO activity was determined in the absence of *n*-octylamine, in order to determine whether *n*-octylamine was also a positive effector of brain FMO. There was no significant difference in the FMO-mediated oxidation of DMA and MEM in the presence or absence of *n*-octylamine, indicating that the brain FMO activity was unaffected by the presence of *n*-octylamine (Table 2).

The present study demonstrates the presence of FMO in the brain. There has been one earlier report of FMO activity in the corpus striatum of rat brain [13], wherein FMO-mediated metabolism of TB to thiobenzamide-*S*-oxide was examined. In the present study we have demonstrated the presence of FMO in whole brain microsomes using three model substrates and significantly higher rates of oxidation of TB to TBSO has been detected in the brain microsomes as compared to the earlier study [13]. This may be due to the conditions used for the preparation of microsomes [7]. Similar results have been obtained in the measurement of cerebral P450 [1–4]. However, when higher concentrations of microsomal protein, namely, 1–2 mg were added to unoxygenated assay buffer which did not contain any detergent, the rate of oxidation of TB to TBSO was 10.03 ± 1.05 and 7.61 ± 3.15 nmoles of TBSO formed/min/mg protein in brain and liver, respectively. This is comparable to the activity reported in the earlier study [13].

The demonstration of FMO activity in the brain has wide ranging pharmacological implications. Several psychoactive drugs are substrates for hepatic and pulmonary FMO [6] and the metabolites formed (namely, the *N*- and *S*-oxides) are pharmacologically active. Thus, FMO-mediated metabolism of these drugs, within the CNS would be important, with particular reference to combination drug therapy. Since the brain exhibits considerable regional and cellular heterogeneity, the regional variation of FMO and its cellular localization within brain needs to be determined.

In summary, the present study demonstrates the presence of FMO activity in brain using three model substrates namely, DMA, MEM and TB. The brain FMO activity determined using the above three substrates is significantly higher than that in the liver.

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Differential expression and ciprofibrate induction of hepatic UDP-glucuronyltransferases for thyroxine and triiodothyronine in Fischer rats

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The possible implication of thyroid hormone in the hypolipidaemic action of phenoxyisobutyrate (fibrate) drugs has been suggested by the finding that clofibrate stimulates liver mitochondrial α-glycerophosphate dehydrogenase activity, a classical thyromimetic response [1]. Observations of a drug-induced increase in hepatic uptake of thyroxine (T_4*) have been interpreted in support of this view [1]. Although clofibrate treatment raises the plasma level of T_4-binding proteins, competitive displacement of T_4 from these sites by the drug augments the plasma free T_4 fraction with a resultant shift into the tissues [1–3]. However, it is questionable if this results in a thyrotoxic state of the tissues, since the plasma free T_4 concentration during chronic clofibrate treatment is normal [2, 3].

Enzymatic deiodination determines the bioactivity of T_4 through conversion to the active hormone 3,3′,5-triiodothyronine (T_3) or to the inactive isomer 3,3′,5′-triiodothyronine (reverse T_3) [4]. Both metabolites are also further metabolized by deiodination. Other important pathways in the metabolism of thyroid hormone involve the conjugation of the phenolic hydroxyl group with glucuronic acid or sulfate [4]. The sulfate conjugates are rapidly degraded in the liver by the type I iodothyronine deiodinase, and T_4 and T_3 are excreted in rat bile largely as glucuronides [4]. In contrast to an early report [5], clofibrate has been shown to stimulate the biliary clearance of T_4 [6]. Not only clofibrate [6, 7] but also fenofibrate [7], the fibrate derivative nafenopin [8] and ciprofibrate [9] accelerate the metabolic clearance of T_4 in rats. It has been demonstrated that nafenopin and ciprofibrate strongly increase the faecal clearance of T_4, whereas the urinary (deiodinative) clearance of T_4 is not affected [8] or even inhibited [9]. Little effect of these drugs was noted on the metabolic clearance of T_3 [8, 9]. Fibrates are well-known inducers of bilirubin UDP-glucuronyltransferase (UDPGT) in the liver [10, 11]. In this study we examined the effects of ciprofibrate administration to rats on hepatic T_4 and T_3 UDPGT activities. In order to gain more insight into the isozymes responsible for the glucuronidation of T_4 and T_3 we investigated, in parallel, the effects of ciprofibrate on *p*-nitrophenol (PNP) and androsterone UDPGT activities.

Materials and Methods

L-T_4, L-T_3, PNP and Brij 56 were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.), [^{125}I]T_4 (1500 μCi/μg) and [^{125}I]T_3 (2800 μCi/μg) from Amersham (Amersham, U.K.), androsterone from Steraloids (Wilton, NH, U.S.A.), [^{3}H]androsterone (116 μCi/μg) from New England Nuclear (Boston, MA, U.S.A.) and UDP-glucuronic acid (UDPGA) from Boehringer (Mannheim, F.R.G.). Fischer 344 rats were obtained from Charles River (Margate, U.K.) and Wistar rats from Harlan

* Abbreviations: DTT, dithiothreitol; HA, high activity; LA, low activity; MC, 3-methylcholanthrene; PNP, *p*-nitrophenyl; PCB, polychlorobiphenyl; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; T_3, 3,3′,5-triiodothyronine; T_4, thyroxine; TBG, T_4-binding globulin; UDPGA, UDP-glucuronic acid; UDPGT, UDP-glucuronyltransferase.