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Short communication

Flavin-containing monooxygenase mediated metabolism of psychoactive drugs by human brain microsomes

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Abstract

Flavin-containing monooxygenases (FMO) catalyze the oxidation of certain xenobiotics and drugs which contain a nucleophilic heteroatom. Here we report the first assessment of human brain flavin-containing monooxygenase from tissues obtained at autopsy from seven traffic accident victims. Human brain microsomes catalyzed the *S*-oxidation or *N*-oxidation of model substrates methimazole and *N,N*-dimethylaniline, respectively. The psychoactive drugs chlorpromazine, imipramine and fluoxetine, were also metabolized by human brain FMO. 'Western' immunoblot analyses revealed immunological cross-reactivity of the human brain FMO with rabbit pulmonary FMO. Immunocytochemistry further revealed the localization of the FMO predominantly in the neuronal cell bodies in the magnocellular reticular nuclei, colliculi and substantia nigra. Human brain clearly contains an active FMO system, and it is conceivable that such enzyme(s) are significantly involved in the local metabolism and modulation of pharmacological effects of psychoactive drugs.

Keywords: Brain; Flavin-containing monooxygenase; Metabolism; Neuroleptic; Antidepressant; Monooxygenase; Human brain

Microsomal flavin-containing monooxygenases (FMOs) catalyze the NADPH-dependent oxidation of a variety of xenobiotics which contain nucleophilic heteroatoms. Typically, nitrogen-, sulfur- or phosphorus-containing functional groups are sites for oxidation by the enzyme [18]. Many psychoactive drugs fall into this category and are substrates for hepatic and pulmonary flavin-containing monooxygenase (FMO). The presence of FMO has been documented in several organs including liver [13], lung [17] and kidney [4].

Hepatic and pulmonary FMOs have been shown to be distinct proteins [16,17,19]. FMO-mediated oxidation of model substrate *N,N*-dimethylaniline has been demonstrated in adult and fetal human liver [8]. More recently, FMO-mediated *N*-oxidation of imipramine in human kidney has been reported [10]. Human hepatic FMO has been cloned and sequenced [7,11]. While the existence of multiple forms of FMO has been known, recent interest has centered on the polymorphism seen in the *N*-oxygenation of trimethylamine [6] and nicotine [1] in humans probably due to a genetic defect in one or more forms of FMO.

Studies from our laboratory have demonstrated the presence of FMO in the rat brain and the enzyme's ability to metabolize model substrates [2] and antidepressants such as imipramine and fluoxetine [3]. To our knowledge the presence of FMO in human brain has not heretofore been demonstrated.

Human brain tissues were obtained at autopsy from seven, male traffic accident victims. The average age of the subjects was 45.4 ± 18.2 years, and the interval between death and autopsy was 6.2 ± 3.3 h. None of the subjects had any known neurological disorders. Following autopsy, the meninges and blood vessels were removed and the brain regions (cortex, cerebellum, thalamus, striatum, hippocampus, pons, midbrain

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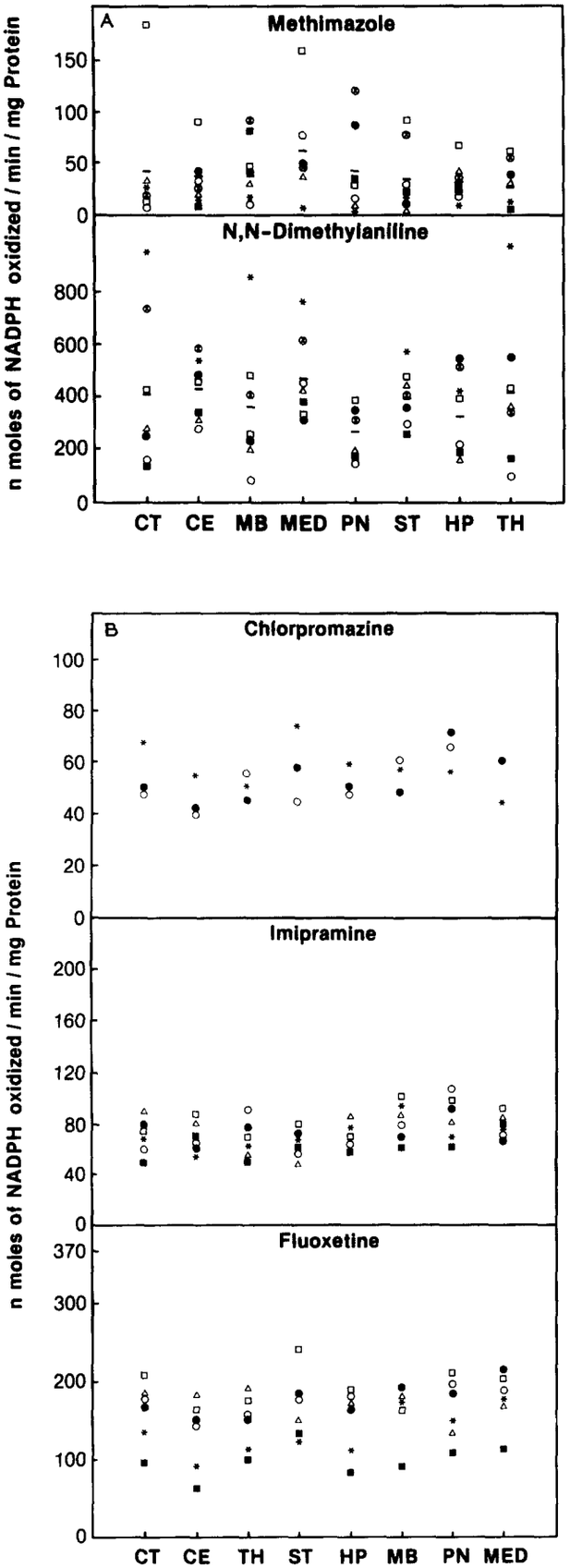

Table 1
Apparent kinetic constants, K_m and V_{max}, for flavin-containing monooxygenase mediated oxidation of various substrates by human brain microsomes

Substrates	K_m	V_{max}
Chlorpromazine	19.5 μM	231.0
Imipramine	22.0 μM	271.0
Fluoxetine	16.0 μM	650.0
Methimazole	17.1 μM	29.8
N,N-Dimethylaniline ('low')	2.3 mM	565.0
N,N-Dimethylaniline ('high')	1.2 μM	5.4

The K_m and V_{max} values were determined in cortical microsomes obtained at autopsy (case VI, see legend to Fig. 1). V_{max} is expressed as nmol of NADPH oxidized/min/mg protein.

and medulla) were dissected out using appropriate anatomical landmarks. The tissues were stored at −70°C prior to analysis.

Microsomes were prepared by calcium aggregation method as described [3]. Microsomal protein concentrations were estimated by a dye-binding method [5] and by the method of Lowry [12]. FMO activity was measured using a variety of substrates, namely methimazole, *N,N*-dimethylaniline, imipramine, fluoxetine and chlorpromazine. The substrate-stimulated rate of NADPH oxidation was measured essentially as described [3].

Immunoblot analyses were carried out following sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [9], and transfer of protein to nitrocellulose membrane [15], using antibody to pig hepatic FMO [19] or rabbit pulmonary FMO [17].

Different regions from human brain were fixed in formalin; frozen sections (10–20 μm) were cut on a cryostat. The immunocytochemical localization was carried out essentially as described [3] using the antibody to rabbit pulmonary FMO. Control sections were incubated with non-immune immunoglobulin.

The FMO-mediated oxidation of model substrates methimazole and *N,N*-dimethylaniline by microsomes from human brain regions is depicted in Fig. 1A. Significant activity was detectable in all the brain regions examined. FMO-mediated oxidation of the psychoactive drugs chlorpromazine, imipramine and fluoxetine was also observed in human brain microsomes (Fig. 1B). The distribution of FMO among the various

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Fig. 1. FMO-mediated oxidation of (A) methimazole and *N,N*-dimethylaniline and (B) chlorpromazine, imipramine and fluoxetine by human brain microsomes. The deceased individuals and corresponding ages were: case I, 50 years (○); case II, 65 years (△); case III, 16 years (□); case IV, 60 years (⊗); case V, 32 years (●); case VI, 60 years (*) and case VII, 35 years (■). The horizontal bar indicates the mean enzyme activity. The human brain regions are shown as cortex (CT), cerebellum (CE), midbrain (MB), medulla (MED), pons (PN), striatum (ST), hippocampus (HP) and thalamus (TH).

regions of brain did not reveal any significant differences (Fig. 1A and B).

The apparent kinetic constants K_m and V_{max} for FMO-mediated oxidation of various substrates by human brain cortical microsomes (Case VI, see legend to Fig. 1A) are given in Table 1. All the psychoactive drugs examined namely, chlorpromazine, imipramine and fluoxetine, exhibited high affinity (19.5, 22, 16 μM, respectively) and high activity for the enzyme. The model substrate *N,N*-dimethylaniline exhibited biphasic kinetics namely, a high-affinity (1.2 μM), low activity component and a low-affinity (2.3 mM), high activity component. Although the K_m for methimazole was 17.1 μM, the V_{max} was significantly lower than that observed for the psychoactive drugs (Table 1).

'Western' immunoblot analyses of the microsomes from brain regions of two subjects (case VI and VII; see legend to Fig. 1A) using the antibody to purified rabbit pulmonary FMO revealed the presence of a single immunoreactive protein in all the regions of the brain that were examined (Fig. 2). Immunoblot experiments with antiserum to pig hepatic FMO did not reveal any cross-reactivity with the human brain FMO (data not shown).

Immunocytochemical localization was carried out likewise using the above antibody. Among the brain regions (namely, cortex, cerebellum, midbrain and medulla) that were investigated for localization of FMO, significant immunolabelling was observed in the large reticular neurons of medulla oblongata (Fig. 3B), the substantia nigra (Fig. 3C) and the colliculi (Fig. 3D). In the pons and medulla oblongata, the neurons of the V, VI, VII, IX and X cranial nerve nuclei and the pontine nuclei were stained. There was a variable degree of labelling of the neurons of the inferior olivary nucleus; the immunostaining extended further to the distant dendritic arborization (Figs. 3B and D). In the cerebellum, the large Purkinje cells and the small granule cells were stained. In the cerebral cortex, the staining was essentially restricted to the large pyramidal neurons of layer 5. Neuropil staining was also observed. The glia and the vascular elements were not stained and the fibre tracts showed variable intensity of staining (data not shown).

The present study demonstrates the FMO-mediated metabolism of model substrates methimazole and N,N-dimethylaniline, and the psychoactive drugs imipramine, fluoxetine and chlorpromazine by human brain microsomes. Although the presence of FMO had been demonstrated in rat brain microsomes [2,3], to our knowledge the presence of this enzyme system had not been known in human brain.

The distribution of FMO activity among the various regions of the brain that were examined did not reveal any striking differences (Fig. 1A and B). This is unlike that observed in the distribution of cytochrome P450 and associated monooxygenase activities in the human brain wherein higher concentrations of that enzyme system were detected in the pons, medulla and midbrain regions [14]. However, considerable inter individual variations were detectable in the FMO activity from various human brain samples. The FMO-mediated oxidation of *N,N*-dimethylaniline exhibited biphasic kinetics (Table 1), in a manner similar to that seen previously in rat brain [3]. A low-affinity, high-activity component of *N,N*-dimethylaniline oxidase activity was detectable when the substrate concentration was in the millimolar range, and a high-affinity, low-activity component was detectable when the substrate concentration was in the micromolar range. The oxidation of psychoactive drugs by human brain FMO exhibited both high affinity and high activity as compared with the model substrates (Table 1).

FMO activity has been determined in human liver biopsy sample using *N,N*-dimethylaniline as a substrate [8]. The FMO activity in liver *homogenate* varied from 1.06–1.96 nmol of *N*-oxide formed/min/mg protein. This is comparable to the high affinity component of FMO activity seen in brain *microsomes* (V_{max} = 5.4 nmol of NADPH utilized/min/mg protein) using *N,N*-dimethylaniline as substrate. Imipramine is metabolized poorly by human liver FMO, while human kidney exhibits comparatively higher activity [10].

We observed that the human brain FMO mediated

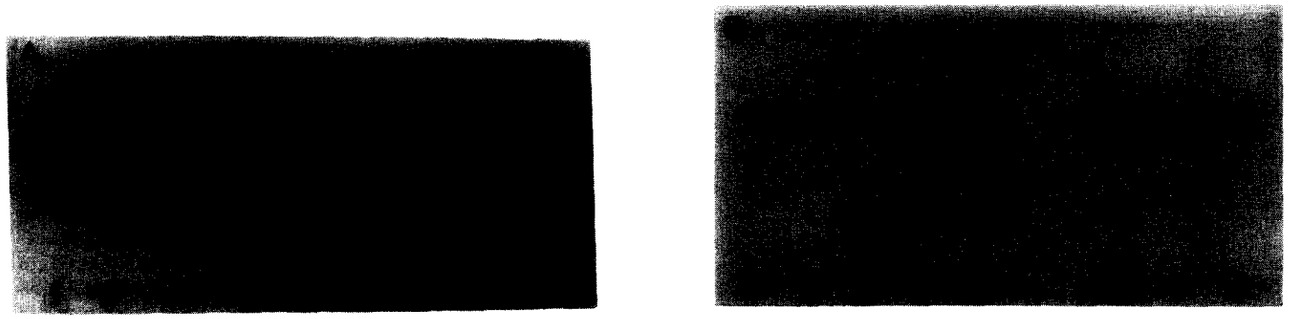

Fig. 2. 'Western' immunoblot of microsomes from human brain regions from two autopsy samples (A) case VI and (B) case VII, stained with antibody to purified rabbit pulmonary FMO. Each lane was loaded with 50 μg of microsomal protein. The lanes were loaded as 1, cortex; 2, cerebellum; 3, midbrain; 4, medulla; 5, pons; 6, striatum; 7, hippocampus; and 8, thalamus.

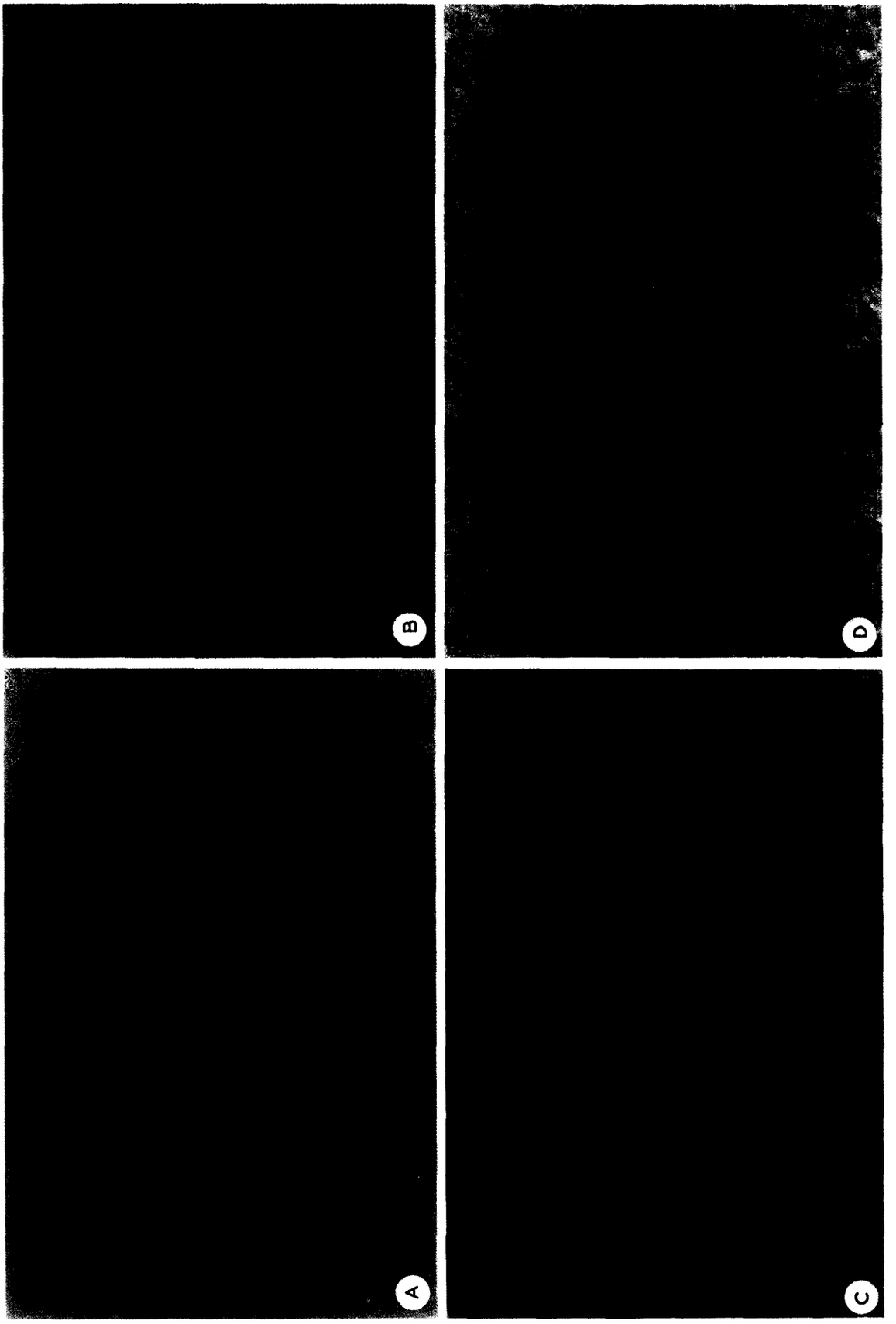

Fig. 3. Immunolabelling of human brain sections by the antibody to the pulmonary FMO. A: control section from medulla oblongata incubated with non-immune goat IgG and processed identical to the test. Arrows point out unlabelled neurons having light yellow stain. ×240. B: immunolabelling of the neuronal perikaryon and the extension of the immune reaction to the dendrites of the reticular neurons in medulla oblongata. ×360. C: pigmented neurons of substantia nigra revealing particulate immunolabelling with antibody to FMO. ×360. D: a large neuron in the superior coliculus immunostained by antibody to FMO. ×360.

imipramine *N*-oxidase activity varied depending on the concentration of microsomal protein present in the incubation medium. Thus, when the microsomal protein concentration was 5–15 μg/ml incubation, the activity was 96.5 nmol/min/mg protein (Fig. 1). When the microsomal protein concentration was increased to 50 and 100 μg of protein/ml incubation, the activity was observed to be 16.1 and 12.9 nmol of NADPH oxidized/min/mg protein respectively (data not shown). In an earlier study using human kidney microsomes, the rate of imipramine N-oxidation was observed to be 1.22 nmol of imipramine *N*-oxide formed/min/mg protein, using 700 μg of microsomal protein/ml incubation. Thus, a direct comparison of the activities determined in the present study with those described in the earlier study [10] is difficult.

The human brain FMO exhibited immunological similarity with rabbit pulmonary FMO as examined by 'Western' immunoblot. Immunochemical studies using the above antibody revealed the localization of human brain FMO predominantly in the neuronal cell bodies. Earlier immunocytochemical studies on the localization of cytochrome P450 in human brain had also revealed the preferential localization of P450 in the neuronal cell soma [14]. It thus appears that both P450 and FMO are co-localized in the brain. Thus, the in situ FMO-mediated oxidative metabolism of psychoactive drugs in brain probably takes place in neuronal cells, some of which presumably are the site(s) of action of these agents. Since the *N*-oxides of these drugs may have different pharmacological activities than the parent compounds, the neuronal metabolism of these agents conceivably could modulate significantly their local pharmacological effects.

In conclusion, the present study demonstrates the presence of FMO activity in diverse human brain regions and points to its potential role in the local pharmacological modulation of pharmacological activity and/or toxicity of psychotropic drugs, and possibly other foreign compounds, that are subject to FMO-mediated oxidative metabolism. The considerable interindividual variations seen in the distribution of FMO activity in human brain might help to explain the variable psychotropic drug responses seen typically in a diverse patient population.

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