Accumulation of the 1-Methyl-4-phenylpyridinium Ion in Suncus (*Suncus murinus*) Brain: Implication for Flavin-Containing Monooxygenase Activity in Brain Microvessels

Taisei Mushiroda,[†] Noritaka Ariyoshi,[†] Tsuyoshi Yokoi,[†] Eiji Takahara,[‡] Osamu Nagata,[‡] Hideo Kato,[‡] and Tetsuya Kamataki*,[†]

Laboratory of Drug Metabolism, Division of Pharmacobio-dynamics, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Hokkaido 060-0812, Japan, and Research & Development Division, Hokuriku Seiyaku Company, Katsuyama, Fukui 911-8555, Japan

Received June 9, 2000

The metabolism of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was examined in an effort to evaluate the role of flavin-containing monooxygenase (FMO) expressed in the brain of suncus (*Suncus murinus*) and rats. MPTP was metabolized to generate both 1-methyl-4-phenylpyridinium ion (MPP^+) and MPTP *N*-oxide by brain homogenates from rats. Although the level of MPP^+-producing activity was similar in suncus and rats, a remarkable difference was found between the animal species in MPTP *N*-oxygenase activity, which was not detectable in brain homogenates from suncus. The concentrations of MPP^+ in suncus brain after a single ip administration of MPTP were markedly higher than that in rats, probably because of the lack of FMO activity in the suncus brain. The MPTP *N*-oxygenase activity of microvessel homogenates of rat brain was 21-fold greater than that of whole brain homogenates. These results suggest that FMO(s) plays a significant role in the detoxification of MPTP in cerebral endothelial cells.

Introduction

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)[1] is known to be a neurotoxic substance that induces the degeneration of dopaminergic neurons and causes a parkinsonism-like syndrome in humans (*1*) and monkeys (*2*). This compound is metabolized by mitochondrial monoamine oxidase type B (MAO-B) in the brain to yield 1-methyl-4-phenyl-2,3-dihydropyridinium ion ($MPDP^+$), which is further oxidized to the ultimate toxic compound, 1-methyl-4-phenylpyridinium ion (MPP^+) (Figure 1) (*3*). MPTP is also metabolized by the microsomal enzymes flavin-containing monooxygenase (FMO) and cytochrome P450 (P450) to generate MPTP *N*-oxide and 4-phenyl-1,2,3,6-tetrahydropyridine (PTP), respectively, in the liver (*4*, *5*). Although both of these two metabolic processes are considered the detoxification pathways of MPTP, the N-oxygenation has been proposed to be primarily responsible for the detoxification on the basis of the evidence that the K_m value (i.e., 1.8 μM) for the N-oxygenation with rat FMO1 was much smaller than that with P450 2D6 expressed in yeast cells (i.e., 39 μM) (*6*, *7*). The role of FMO in the N-oxygenation of MPTP has been studied with mouse, rat, pig, and human liver microsomes and purified pig liver FMO (*4*, *8*, *9*). A recent study has shown that the FMO activity in human brain microsomes measured as imipramine *N*-oxygenase was 2-fold higher than that in rat brain microsomes (*10*). However, no detailed studies demonstrating the participation of the brain FMO in MPTP detoxification have been reported so far.

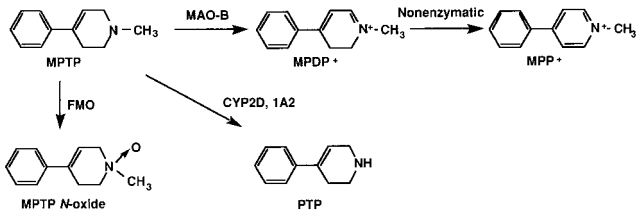

Figure 1. Metabolic pathways of MPTP.

In this report, we intended to evaluate the contribution of brain FMO in the detoxification of MPTP by comparing *N*-oxygenase activity in the suncus (*Suncus murinus*) and rat brain. The suncus is an animal species which belongs to the family Soricidae of the order Insectivora and is distributed throughout Southeast Asia, including the southern part of Japan. The suncus is classified as a species phylogenetically closer to primates than rodents (*11*). In previous studies, we found that the level of FMO mRNA and the activity of trimethylamine *N*-oxygenase were negligible in the suncus liver (*12*, *13*). Thus, we intended to examine the effects of FMO-mediated N-oxygenation of MPTP in both suncus and rat brain in vitro and in vivo. We report herein that the level of MPP^+ becomes higher in suncus after administration of MPTP than in rats.

* To whom correspondence should be addressed: Laboratory of Drug Metabolism, Division of Pharmacobio-dynamics, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Hokkaido 060-0812, Japan. Telephone: 81-11-706-3233. Fax: 81-11-706-4978. E-mail: kamataki@pharm.hokudai.ac.jp.

[†] Hokkaido University.

[‡] Hokuriku Seiyaku Co.

[1] Abbreviations: BBB, blood−brain barrier; P450, cytochrome P450; FMO, flavine-containing monooxygenase; MAO, monoamine oxidase; $MPDP^+$, 1-methyl-4-phenyl-2,3-dihydropyridinium ion; MPP^+, 1-methyl-4-phenylpyridinium ion; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PTP, 4-phenyl-1,2,3,6-tetrahydropyridine.

10.1021/tx0001225 CCC: $20.00
Published on Web 01/30/2001

Experimental Procedures

Materials. MPTP hydrochloride, PTP hydrochloride, and MPP^+ iodide were obtained from Funakoshi (Tokyo, Japan). Pargyline hydrochloride was purchased from Wako Pure Chemicals (Tokyo, Japan). All other chemicals were of the highest grade commercially available.

Animals. Male suncus (6 weeks old) were obtained from Clea Japan (Tokyo, Japan). Male Sprague-Dawley rats (7 weeks old) were obtained from Japan SLC (Shizuoka, Japan). Animals were maintained at a constant temperature of 23 ± 1 °C under controlled lighting conditions (lights on from 7 a.m. to 7 p.m.) and given a commercial chow (Clea Japan) and tap water ad libitum.

Preparations of Whole Brain and Brain Microvessel Homogenates. After the animals had been sacrificed by decapitation, brains were removed immediately and homogenized with 4 volumes of 0.25 M sucrose. The homogenates were stored at −80 °C until they were used. A microvessel preparation was isolated according to the procedure of Ghersi-Egea et al. (*14*). Briefly, after the brains were removed, the cortices and cerebella were excised. The tissue was homogenized with 10 volumes of 0.32 M sucrose. The homogenates were centrifuged at 1000*g* for 15 min, and the pellet was resuspended in 20 mL of a Krebs-Ringel buffer followed by centrifugation at 1500*g* for 10 min. The pellet was suspended in 10 mL of 0.25 M sucrose, layered over a 1.0 to 1.5 M sucrose gradient (each 12 mL), and then centrifuged at 58000*g* for 30 min. The microvessels were resuspended in the Krebs-Ringel buffer and stored at −80 °C until they were used. The protein concentration was determined by the method of Lowry et al. (*15*) using bovine serum albumin as a standard.

Analytical Procedures in Vitro. A typical reaction mixture consisted of 100 mM potassium phosphate buffer (pH 7.4), 0.05 mM EDTA, an NADPH-generating system (5 mM $MgCl_2$, 5 mM glucose 6-phosphate, 0.5 mM $NADP^+$, and 1 unit/mL glucose-6-phosphate dehydrogenase), and 1.5 mg (whole brain) or 90 μg (microvessel) of homogenate protein in a final volume of 0.25 mL. The reaction mixture was preincubated at 37 °C for 3 min. The reaction was initiated by the addition of MPTP at concentrations ranging from 0.02 to 1 mM. After incubation for 20 min, ice-cold acetonitrile (250 μL) was added to stop the reaction. The mixture was centrifuged at 1300*g* for 10 min. An aliquot of the clear supernatant sample (50 μL) was subjected to the HPLC system (HITACHI model L-7000 series, Hitachi, Tokyo, Japan) equipped with an Inertsil ODS-3 reversed-phase column (5 μm, 4.6 mm i.d. × 150 mm, GL Science, Tokyo, Japan). Separation was carried out with a mobile phase [consisting of 50 mM potassium phosphate buffer (pH 7.4) and acetonitrile, 85:15, v/v] at a flow rate of 1 mL/min at 40 °C. Elutions of MPP^+ and MPTP *N*-oxide were monitored at 244 nm.

Determination of the MPTP Level in Plasma. MPTP hydrochloride was dissolved in saline and administered to suncus and rats by a single ip injection at a dose of 5 mg/kg of body weight. The dose of the compound was calculated as its free base. Blood samples were collected from the vein 15 min, 30 min, and 1 h after the administration and centrifuged to obtain plasma. Nine volumes of 0.5 M perchloic acid containing 2 mM sodium sulfite was added to an aliquot of plasma (10 μL). After centrifugation (7000*g* for 5 min), the supernatant was analyzed for MPP^+ with the HPLC system as described above. Separation was carried out with a mobile phase [consisting of 50 mM potassium phosphate buffer (pH 7.4) and acetonitrile, 70:30, v/v] at a flow rate of 1 mL/min at 40 °C. Elution of MPTP was monitored at 244 nm.

Determination of the MPP^+ Level in the Brain. MPTP hydrochloride was dissolved in saline and administered to suncus and rats by a single ip injection at a dose of 5 mg/kg of body weight. Suncus and rats were decapitated 15 min, 30 min, and 1, 2, 4, 6, and 8 h after the administration. The brain was removed and then homogenized with 9 volumes of 0.5 M perchloic acid containing 2 mM sodium sulfite. After centrifugation (7000*g* for 5 min), the supernatant was analyzed for MPP^+ by the HPLC system as described above.

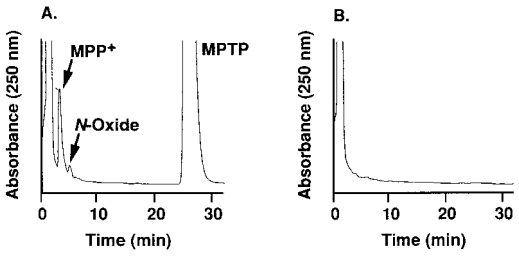

Figure 2. Representative HPLC chromatograms for the formation of MPP^+ and MPTP *N*-oxide in rat brain homogenates. The reaction mixture containing 1.5 mg protein of homogenates was incubated with 100 μM MPTP (A) or without MPTP (B).

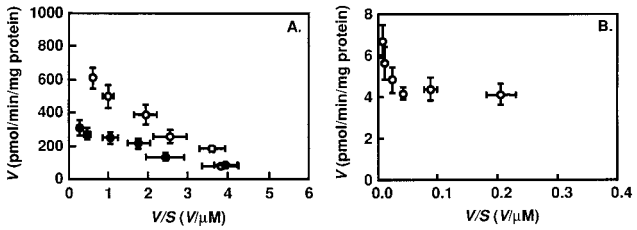

Figure 3. Eadie−Hofstee plots for the metabolism of MPTP by brain homogenates from suncus and rats: (A) MPP^+ formation and (B) MPTP N-oxygenation. Each point represents the mean ± SEM of three animals. *V* is the velocity of the MPTP metabolism (picomoles per minute per milligram of protein), and *S* is the substrate concentration (micromolar): (●) suncus and (○) rat. The incubation mixture consisted of 100 mM potassium phosphate buffer (pH 7.4), the NADPH-generating system, and 1.5 mg of protein of the brain homogenates as described in detail in Experimental Procedures.

Results

Metabolism of MPTP by Homogenates of Whole Brain and the Brain Microvessel from Suncus and Rats. MPTP, MPTP *N*-oxide, and MPP^+ were well separated under the HPLC conditions (Figure 2). Brain homogenates from suncus and rats produced MPP^+. Eadie−Hofstee plots for MPP^+ formation by brain homogenates of both suncus and rats exhibited a monophasic profile (Figure 3A). The K_m and V_{max} values for MPP^+ formation by brain homogenates are summarized in Table 1. Suncus exhibited 2.4- and 2.8-fold lower V_{max} and K_m values, respectively, than the rat. Therefore, CL_i (V_{max}/K_m) values of both animals were comparable.

Eadie−Hofstee plots for MPTP N-oxygenation by brain homogenates of rats exhibited a biphasic pattern (Figure 3B). These results suggest that at least two enzymes may be involved in the MPTP N-oxygenation in the rat. In contrast to the results with rats, MPTP *N*-oxide could not be detected over the substrate concentration range of 0.02−1 mM in the suncus.

It is noteworthy that the *N*-oxygenase activity in the microvessel homogenates was 21-fold higher than in the whole brain homogenates in the rat (Figure 4).

Effect of the MAO Inhibitor and Heat Treatment of Brain Homogenates on MPTP Metabolism. The MAO inhibitor, pargyline, was used to examine the contribution of MAO in MPP^+ formation by brain homogenates of rats. The formation of MPP^+ was inhibited to 2 and 7% of the control level by 1 and 10 mM pargyline, respectively, while the N-oxygenation was unaffected by the treatment with pargyline (Figure 5A).

Table 1. Michaelis−Menten Kinetic Parameters for the MPTP Metabolism in Brain Homogenates from Suncus and Rats[a]

	MPP^+ formation			N-oxygenation		
	K_m (μM)	V_{max} [nmol min^{-1} (mg of protein)$^{-1}$]	CL_i [μL min^{-1} (mg of protein)$^{-1}$]	K_m (μM)	V_{max} [pmol min^{-1} (mg of protein)$^{-1}$]	CL_i [μL min^{-1} (mg of protein)$^{-1}$]
suncus	50 ± 9	0.27 ± 0.05	5.3 ± 0.3	−	−	−
rat	140 ± 10	0.66 ± 0.10	4.7 ± 0.4	2.0 ± 0.2	4.5 ± 0.6	2.3 ± 0.3
				96 ± 3	2.5 ± 0.6	0.026 ± 0.006

[a] Each value represents the mean ± SEM of three animals. K_m is the Michaelis−Menten constant, V_{max} the maximum velocity of MPTP metabolism, and CL_i the intrinsic clearance calculated as V_{max}/K_m. −, not calculated.

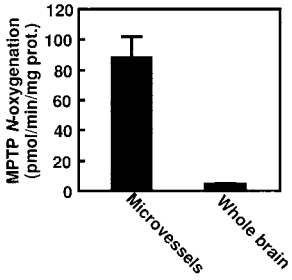

Figure 4. MPTP *N*-oxygenase activity in brain microvessel homogenates prepared from rats. Each column represents the mean ± SEM of three animals. The incubation mixture consisted of 100 mM potassium phosphate buffer (pH 7.4), the NADPH-generating system, 75 μg of protein of the brain microvessel homogenates, and 100 μM MPTP as described in detail in Experimental Procedures.

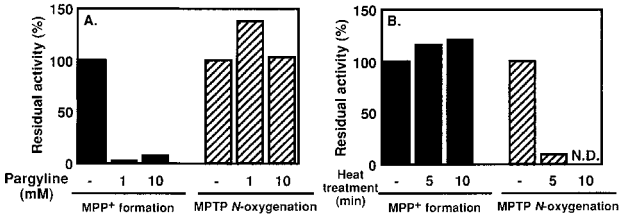

Figure 5. Effects of pargyline, MAO inhibitor, and heat treatment on the metabolism of MPTP by the rat brain homogenates. (A) Effect of pargyline on the formation of MPP^+ (black bars) and the N-oxygenation of MPTP (striped bars). (B) Effect of treatment at 45 °C on the formation of MPP^+ (black bars) and the N-oxygenation of MPTP (striped bars). Each column represents the mean of two determinations. ND means not detectable. The rates of MPP^+ formation in the absence of pargyline and without heat treatment were 0.24 (A) and 0.26 nmol min^{-1} (ng of protein)$^{-1}$ (B), respectively. The rates of N-oxygenation in the absence of pargyline and without heat treatment were 4.76 (A) and 5.70 pmol min^{-1} (mg of protein)$^{-1}$ (B), respectively.

FMO is unstable toward heat treatment. Figure 5B shows the effect of heat treatment of rat brain homogenates on the activity of MPTP N-oxygenation. The *N*-oxygenase activity decreased time-dependently by heat treatment at 45 °C, and the activity was completely lost after treatment for 10 min, supporting the idea that N-oxygenation of MPTP in the brain was catalyzed by FMO. On the contrary, MPP^+ formation was unchanged by the treatment of homogenates at 45 °C for 10 min.

MPTP Concentration in Plasma after a Single Administration of MPTP. After an ip injection of MPTP at a dose of 5 mg/kg, the concentrations of MPTP in plasma were 410 ng/mL in suncus and 253 ng/mL in rats at the first sampling point, 15 min pd (Figure 6). The concentrations declined rapidly with $T_{1/2}$ values of 0.30 and 0.52 h in suncus and rats, respectively (Table 2).

MPP^+ Concentration in Brain after a Single Administration of MPTP. Levels of MPP^+ in brain increased with a T_{max} value of 0.74 h, reached the C_{max} of 2.4 μg/g, and then declined with a $T_{1/2}$ of 2.1 h after administration of MPTP to the rats. On the other hand, the concentration of MPP^+ reached the maximum level of 4.2 μg/g at 0.15 h after a single administration and declined with a $T_{1/2}$ of 27.7 h in the suncus brain. Consequently, the $AUC_{0-\infty}$ of suncus was calculated to be 18-fold higher than that of the rat (Figure 7 and Table 3).

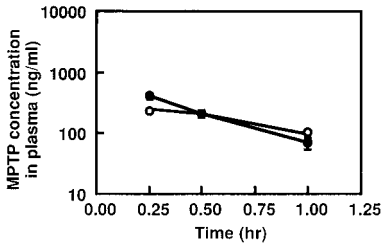

Figure 6. MPTP concentrations in the plasma after a single ip injection of MPTP into suncus and rats at a dose of 5 mg/kg. Each point represents the mean ± SEM of two to four animals: (●) suncus and (○) rat.

Table 2. Pharmacokinetic Parameters for MPTP in Plasma after a Single ip Administration to Suncus and Rats at a Dose of 5 mg/kg[a]

	$AUC_{0-\infty}$ (μg h mL^{-1})	$T_{1/2}$ (h)
suncus	300	0.30
rat	240	0.52

[a] Each value represents the mean of two to four animals.

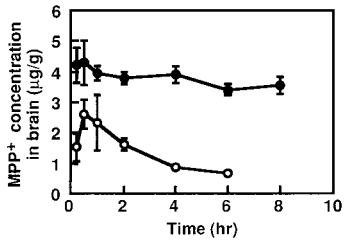

Figure 7. MPP^+ concentrations in the brain after a single ip injection of MPTP into suncus and rats at a dose of 5 mg/kg. Each point represents the mean ± SEM of three animals: (●) suncus and (○) rat.

Table 3. Pharmacokinetic Parameters for MPP^+ Formation from MPTP in Brain after a Single ip Administration of MPTP to Suncus and Rats at a Dose of 5 mg/kg[a]

	C_{max} (μg/g)	T_{max} (h)	$AUC_{0-\infty}$ (μg h g^{-1})	$T_{1/2}$ (h)
suncus	4.2	0.15	170	28
rat	2.4	0.74	9.4	2.1

[a] Each value represents the mean of three animals.

Discussion

The brain is partially protected from exposure to xenobiotics by the tight junctions that link adjacent cerebral endocelial cells and form the structural basis of

the BBB. In addition to the BBB, a recent report suggests that several drug-metabolizing enzymes in the small microvessels of the brain may act as an enzymatic barrier to the influx of xenobiotics (*16*). The drug-metabolizing enzymes known to be present in the brain are P450 and FMO, which are detected in the rodent and human brain (*10*, *17*, *18*). It is of interest to characterize the FMO in the brain with respect to the pharmacological significance of this enzyme for several psychoactive drugs, including imipramine and clozapine, since these drugs are mainly metabolized by both P450s and FMO (*19*, *20*). Recently, cDNAs encoding FMOs were isolated from pigs, mice, rats, rabbits, guinea pigs, and humans (*21*). On the basis of the similarity of the deduced amino acid sequences, FMOs have been classified into five gene subfamilies (*21*). In our previous study, we found that the suncus was a unique animal which exhibited an extremely low level of expression of mRNAs for FMOs (*12*). Although the level is very low, FMO1 was assumed to be the predominant isoform of FMO. In fact, the level of FMO4 mRNA was below the detection limit. At present, the mechanisms of the extremely low level of expression of mRNAs for FMOs are not understood. The study presented here revealed that MPTP *N*-oxygenase activity was at a level below the detection limit in the suncus brain. When the detection limit is taken into account, the MPTP *N*-oxygenase activity in the suncus brain was estimated to be less than $^1/_{15}$ of that in rat brain at an MPTP concentration of 1 mM. This result strongly suggests that suncus expresses no FMO or an extremely low level of FMO in the brain.

Furthermore, little expression of FMO in the suncus brain was strengthened by pharmacokinetic data in vivo after a single administration of MPTP. Although we intended to determine the MPTP *N*-oxide concentration in rat brain after an ip injection, the concentrations of MPTP *N*-oxide in rat brain were below the detection limit. Therefore, we determined that MPP^+ is an ultimate metabolite responsible for causing parkinsonism-like neurotoxicity. The C_{max} and $AUC_{0-\infty}$ in suncus were 1.8- and 18-fold greater than those in the rat, respectively. In the suncus brain, the concentration of MPP^+ declined with a $T_{1/2}$ of 27.2 h, which was approximately 19- and 14-fold larger than those reported for the cortex and striatum of mice, respectively (*22*). Since the plasma concentration of the unchanged MPTP after a single ip injection in suncus at a dose of 5 mg/kg was similar to that of rats, it is unlikely that the high level of accumulation of MPP^+ in the suncus brain is due to the low level of the hepatic first-pass effect in MPTP metabolism. Thus, it seems that the influx of MPTP into suncus brain was much greater than that into rat brain. Only a smaller portion of MPTP given to rats is expected to enter into rat brain, because MPTP might be converted to MPTP *N*-oxide by FMO localizing in cerebral endothelial cells prior to penetrating the BBB. Additional evidence for the localization of FMO in cerebral endothelial cells comes from the observation that the MPTP *N*-oxygenase activity by microvessel homogenates of rat brain was 21-fold greater than that by whole brain homogenates.

Generally, mice have been experimentally used for investigation of parkinsonism-like behavior. To measure the degree of MPTP-induced bradykinesia, mice were treated by repeated ip injection of MPTP at a higher dose (30 mg/kg/day, 4 days) (*23*). Chiba et al. (*22*) found that pretreatment of mice with an alternate substrate of FMO, *N*-methylmercaptoimidazole, enhanced the MPTP-induced reduction of striatal dopamine and its metabolites. Furthermore, pretreatment of mice with *N*-methylmercaptoimidazole caused a significant increase in the level of MPP^+ in both the striatum and cortex (*8*). These results suggest that an increased level of MPP^+ appears to enhance the neurotoxicity of MPTP. In the study presented here, an AUC value for MPP^+ in the suncus brain was approximately 400-fold greater than that reported for the striatum of mice (*22*). Therefore, the suncus might be a useful animal species for studying a symptom of parkinsonism when the animal is treated with MPTP repeatedly because of a high level of accumulation of MPP^+ in the brain even after a single administration of MPTP at a small dose.

In conclusion, this study revealed that FMO activity in the suncus brain was extremely low. The low FMO activity in suncus may be responsible for a higher permeability of MPTP into the brain than into the rat brain, presumably leading to an accumulation of MPP^+ in the suncus brain. These findings strongly favor the idea that FMO(s) in the small microvessels of the brain may act as an enzymatic barrier to the influx of xenobiotics (*16*). Gilham et al. (*24*) reported that P450 2D6 was localized in the pigmented neurons of the substantia nigra. The data presented here demonstrated that FMO(s) in brain microvessels might play a role in the detoxification of MPTP in cooperation with P450 2D6 expressed in the target cells of the brain.

Acknowledgment. This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan.

References

(1) Langston, J. W., and Irwin, I. (1986) MPTP: Current concepts and controversies. *Clin. Neurol.* **9**, 487−507.

(2) Tipton, K. F., and Singer, T. P. (1993) Advances in our understanding of the mechanism of the neurotoxicity of MPTP and related compounds. *J. Neurochem.* **61**, 1191.

(3) Chiba, K., Trevor, A., and Castagnoli, N., Jr. (1984) Metabolism of the neurotoxic tertiary amine, MPTP, by brain monoamine oxidase. *Biochem. Biophys. Res. Commun.* **120**, 574−578.

(4) Cashman, J. R., and Zieglar, D. M. (1986) Contribution of *N*-oxygenation of the metabolism of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) by various liver preparations. *Mol. Pharmacol.* **29**, 163−169.

(5) Weisman, J., Trevor, A., Chiba, K., Peterson, L. A., Caldera, P., Castagnoli, N., Jr., and Baillie, T. (1985) Metabolism of the nigrostriatal toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine by liver homogenate fractions. *J. Med. Chem.* **28**, 997−1001.

(6) Chiba, K., Kobayashi, K., Itoh, K., Itoh, S., Chiba, T., Ishizaki, T., and Kamataki, T. (1995) *N*-Oxygenation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine by the rat liver flavine-containing monooxygenase expressed in yeast cells. *Eur. J. Pharmacol.* **293**, 97−100.

(7) Coleman, T., Ellis, S. W., Martin, I. J., Lennard, M. S., and Tucker, G. T. (1996) 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is *N*-demethylated by Cytochrome P450 2D6, 1A2 and 3A4: Implications for susceptibility to Parkinson's disease. *J. Pharmacol. Exp. Ther.* **227**, 685−690.

(8) Chiba, K., Kubota, E., Miyakawa, T., Kato, Y., and Ishizaki, T. (1988) Characterization of hepatic microsomal metabolism as an *in vitro* detoxification pathways of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mice. *J. Pharmacol. Exp. Ther.* **246**, 1108−1115.

(9) Wu, R.-F., and Ichikawa, Y. (1994) Characteristic properties and kinetic analysis with neurotoxins of porcine FAD-containing monooxygenase. *Biochim. Biophys. Acta* **1208**, 204−210.

(10) Bharme, S., Bhagwat, S. V., Shanker, S. K., Boyd, M. R., and Ravindranath, V. (1995) Flavin-containing monooxygenase mediated metabolism of psychoactive drugs by human liver microsomes. *Brain Res.* **672**, 276−280.

(11) Ueno, S., Matsuki, N., and Saito, H. (1987) *Suncus murinus*: Experimental model in emesis research. *Life Sci.* **41**, 513−518.

(12) Mushiroda, T., Yokoi, T., Nunoya, K., Nakagawa, T., Kubota, M., Takahara, E., Nagata, O., Kato, H., and Kamataki, T. (2000) The house musk shrew (*Suncus murinus*): a unique animal with extremely low level of expression of mRNAs for CYP3A and flavin-containing monooxygenase. *Comp. Biochem. Physiol., Part C* **126**, 225−234.

(13) Mushiroda, T., Yokoi, T., Takahara, E., Nagata, O., Kato, H., and Kamataki, T. (2000) The suncus (*Suncus murinus*) shows poor metabolic phenotype for trimethylamine *N*-oxygenation. *Toxicol. Appl. Pharmacol.* **162**, 44−48.

(14) Ghersi-Egea, J.-F., Minn, A., and Siest, G. (1988) A new aspect of the protective functions of the blood-brain barrier: Activities of four drug-metabolizing enzymes in isolated rat brain microvessels. *Life Sci.* **42**, 2513−2523.

(15) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265−275.

(16) Ghersi-Egea, J. F., Leininger-Muller, B., Cecchelli, R., and Fenstermacher, J. D. (1995) Blood-brain interfaces: relevance to cerebral drug metabolism. *Toxicol. Lett.* **82/83**, 645−653.

(17) Ravindranath, V., Bhamre, S., Bhagwat, S. V., and Anandatheerthavarada, H. K. (1995) Xenobiotic metabolism in brain. *Toxicol. Lett.* **82/83**, 633−638.

(18) Bhamre, S., Bhagwat, S. V., Shanker, S. K., Williams, D. E., and Ravindranath, V. (1993) Cerebral flavin-containing monooxygenase-mediated metabolism of antidepressants in brain: immunochemical properties and immunocytochemical localization. *J. Pharmacol. Exp. Ther.* **267**, 555−559.

(19) Bhagwat, S. V., Bhamre, S., Boyd, M. R., and Ravindranath, V. (1996) Cerebral metabolism of imipramine and a purified flavin-containing monooxygenase from human brain. *Neuropsychopharmacology* **15**, 133−142.

(20) Tugnait, M., Hawes, E. M., Mckay, G., Rettie, A. E., Haining, R. L., and Midha, K. K. (1997) *N*-Oxygenation of clozapine by flavin-containing monooxygenase. *Drug Metab. Dispos.* **25**, 524−527.

(21) Lawton, M. P., Cashman, J. R., Cresteil, T., Dolphin, C. T., Elfarra, A. A., Hines, R. N., Hodgson, E., Kimura, T., Ozols, J., Phillips, I. R., Philpot, R. M., Poulsen, L. L., Rettie, A. E., Shephard, E. A., Williams, D. E., and Ziegler, D. M. (1994) A nomenclature for the mammalian flavin-containing monooxygenase gene family based on amino acid sequence identities. *Arch. Biochem. Biophys.* **308**, 254−257.

(22) Chiba, K., Horii, H., Kubota, E., Ishizaki, T., and Kato, Y. (1990) Effects of *N*-methylmercaptoimidazole on the disposition of MPTP and its metabolites in mice. *Eur. J. Pharmacol.* **180**, 59−67.

(23) Tasaki, Y., Makino, Y., Ohta, S., and Hirobe, M. (1991) 1-Methyl-1,2,3,4-tetrahydroisoquinoline, decreasing in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated mouse, prevents parkinsonism-like behavior abnormality. *J. Neurochem.* **57**, 1940−1943.

(24) Gilham, D. G., Cairns, W., Paine, M. J. I., Modi, S., Poulsom, R., Roberts, G. C. K., and Wolf, C. R. (1997) Metabolism of MPTP by cytochrome P4502D6 and the demonstration of 2D6 mRNA in human foetal and adult brain by *in situ* hybridization. *Xenobiotica* **27**, 111−125.

TX0001225