Effects of d-Methamphetamine on Monkey Brain Monoamine Oxidase, In Vivo and In Vitro

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Accepted May 30, 1987

Abstract—A and B form MAO activities in mitochondria and synaptosome were measured in the brain of monkeys administered d-methamphetamine (d-MP) 2 mg/kg, i.m., daily for 7 days. When mitochondria were used as an enzyme preparation, the $K_m$ and $V_{max}$ values decreased with 5-HT (serotonin for A-form MAO substrate) and $\beta$-phenylethylamine ($\beta$-PEA for B-form MAO substrate), while in the synaptosome, a significant increase of the $K_m$ and $V_{max}$ values was observed with 5-HT and dopamine as substrates. The mitochondrial MAO treated with d-MP was inhibited strongly by clorgyline and deprenyl with $\beta$-PEA as a substrate, while synaptosomal MAO was highly sensitive to these MAO inhibitors with 5-HT as a substrate. MP and amphetamine (AP) were found in brain mitochondrial and synaptosomal preparations of monkeys administered 2 mg/kg d-MP, i.m. daily for 7 days; MP and AP contents were 5.05±0.22 pg/mg protein and 37.3±3.8 ng/mg protein in mitochondria and 2.35±0.35 pg/mg protein and 46.4±1.5 ng/mg protein in synaptosomes, respectively. MAO was inhibited by MP and its metabolites, AP, p-hydroxymethamphetamine (OH-MP) and p-hydroxyamphetamine (OH-AP), with 5-HT, $\beta$-PEA and dopamine as substrates, in vitro. MP and its metabolites were more potent inhibitors of A-form MAO than B-form MAO.

The effects of amphetamine or methamphetamine are commonly considered to result from catecholamine release and reuptake blockade in vivo. However, the biochemical basis of the central stimulant action of these drugs is still uncertain. Recently, it has been reported that treatment with repeated large doses of methamphetamine produces a decrease of dopamine level and decreases in tyrosine hydroxylase and tryptophan hydroxylase activities (1–4). Similar depressive effects on tyrosine hydroxylase contents have also been reported after chronic treatment with large doses of amphetamine (5–7). However, the mechanism by which amphetamine or methamphetamine depresses these enzyme activities has not been elucidated.

Although it also has been known that amphetamine inhibits monoamine oxidase (MAO) in brain mitochondria, in vivo (8, 9) and in vitro (10–12), there remains some doubt about whether this effect is important with regards to the central stimulant action of amphetamine because a relatively high concentration of amphetamine is required to produce a significant inhibition of MAO (13). Recently, there has been the renewed interest in the MAO inhibitory properties of amphetamine since the multiplicity of MAO (14–16) and the selective inhibition of type A MAO by amphetamine (8, 12) have been reported. However, there have been many reports which associated MAO activity with amphetamine, but few studies on methamphetamine have been reported (17).

In the present investigation, we have undertaken further studies on the possible MAO inhibitory effect in monkey brains by the repeated administration of d-methampet-
amine.

Materials and Methods

1. Enzyme preparations: Crab-eating female monkeys (2.2–3.6 kg) were fed ad lib with ordinary feed and water. Monkeys were intramuscularly (i.m.) administered 2 mg/kg/day d-methamphetamine (d-MP) dissolved in saline solution for 7 days. Control monkeys were given the vehicle only. Following the repeated administration of MP to monkeys, the effects of MP on locomotor activity were slightly potentiated. They were anesthetized with ketalar (50 mg/kg, i.m.) 24 hr after the last dose as indicated, and their whole brains were quickly removed and homogenized in 5 volumes of 0.32 M sucrose (previously adjusted to pH 7.0 with 0.5 M NaHCO₃). Mitochondrial and synaptosomal fractions were prepared by sucrose density gradient centrifugation as described earlier (18). The separated mitochondria and synapotosome were used as the enzyme preparations in these experiments.

2. MAO activity: MAO activity was estimated with 14C-5-HT (200 nM), 14C-(3-phenylethylamine ((3-PEA, 50 µM) and 14C-dopamine (DA, 200 µM) as substrates, as described earlier (19). The incubation medium contained a suitable amount of the enzyme to give a linear reaction for at least 40 min in a total volume of 225 µl of 0.1 M phosphate buffer, pH 7.2. The reaction was started by adding 25 µl of labelled substrate, and incubation was carried out for 20 min at 37°C. Then the reaction was stopped by adding 200 µl of 2 N HCl. The products of the reaction were extracted with 2 ml of benzene-ethylacetate (1:1, v/v). To 1 ml samples of the extract, 1 ml of NaOH saturated with NaCl and 6 ml of n-pentane were added. After 15 min of shaking, 5 ml of the n-pentane phase was transferred into a 10 ml glass-stopped test tube containing 0.1 ml heptafluorobutyric anhydride (HFBA). After heating at 60°C for 30 min, the mixture was dried under nitrogen at 40°C. The residue was dissolved in 0.1 ml of acetone and analyzed by g.l.c.-mass spectrometry. Methamphetamine (MP), amphetamine (AP) and their p-hydroxylated metabolites, p-hydroxymethamphetamine (OH-MP) and p-hydroxyamphetamine (OH-AP) were quantified as HFBA derivatives by a selected ion monitoring (S.I.M.) technique using a g.l.c.-mass spectrometry instrument (JEOL, D-300) connected to a computer (JMA-2000). The determination conditions were as follows: column: 2% OV-17 on Uniport HP (80–100 mesh), glass column (2 mm int. diam., 2 m length); temperature: column oven [140–160°C (5°C/min)], sample injection port (200°C), separator (250°C), ionizing chamber (150°C); carrier gas: helium (inlet pressure 0.8 kg/cm²); ionizing voltage: 70 eV; ionizing current: 300 µA. To determine the amounts of AP and OH-AP and MP and OH-AP, the selective ions were monitored at m/z 240 and 254, respectively. The retention times of the HFBA derivatives of AP, MP, OH-AP and OH-MP were 2.5, 4.1, 4.6 and 6.5 min, respectively. The usual recoveries of MP and its metabolites after this extraction procedure were 75–85%.

3. Protein: Protein concentration of the preparation was measured by the method of Lowry et al. (20) with bovine serum albumin as a standard.

4. Methamphetamine and its metabolites assay: Mitochondria or synaptosome preparations were homogenized in 4 volumes of 0.4 N perchloric acid. To 1 ml of the homogenate, 1 ml of 5 N NaOH saturated with NaCl and 6 ml of n-pentane were added. After 15 min of shaking, 5 ml of the n-pentane phase was transferred into a 10 ml glass-stopped test tube containing 0.1 ml heptafluorobutyric anhydride (HFBA). After heating at 60°C for 30 min, the mixture was dried under nitrogen at 40°C. The residue was dissolved in 0.1 ml of acetone and analyzed by g.l.c.-mass spectrometry. Methamphetamine (MP), amphetamine (AP) and their p-hydroxylated metabolites, p-hydroxymethamphetamine (OH-MP) and p-hydroxyamphetamine (OH-AP) were quantified as HFBA derivatives by a selected ion monitoring (S.I.M.) technique using a g.l.c.-mass spectrometry instrument (JEOL, D-300) connected to a computer (JMA-2000). The determination conditions were as follows: column: 2% OV-17 on Uniport HP (80–100 mesh), glass column (2 mm int. diam., 2 m length); temperature: column oven [140–160°C (5°C/min)], sample injection port (200°C), separator (250°C), ionizing chamber (150°C); carrier gas: helium (inlet pressure 0.8 kg/cm²); ionizing voltage: 70 eV; ionizing current: 300 µA. To determine the amounts of AP and OH-AP and MP and OH-AP, the selective ions were monitored at m/z 240 and 254, respectively. The retention times of the HFBA derivatives of AP, MP, OH-AP and OH-MP were 2.5, 4.1, 4.6 and 6.5 min, respectively. The usual recoveries of MP and its metabolites after this extraction procedure were 75–85%.

5. Materials: Methamphetamine-HCl and ephedrine-HCl were purchased from Dai-nippon Pharmaceutical Co., Ltd. p-Hydroxymethamphetamine sulfate and p-hydroxyamphetamine oxalate were prepared from p-methoxyphenyl acetone by the method of Buzas and DuFour (21). Amphetamine sulfate was prepared by the method of Magidson et al. (22). The purities of the products were checked by g.l.c. and g.l.c.-mass spectrometry. Radiochemical substrates, hydroxytryptamine binoxalate, 5-[2-14C]-serotonin binoxalate, 5-HT (46 mCi/mmol), dihydroxyphenylethylamine hydrobromide, 3,4-[8-14C]-dopamine (DA) (46.8 mCi/mmol) and phenylethylamine hydrochloride, β-[ethyl-1-14C]-(3-PEA) (48 mCi/mmol).
were purchased from New England Nuclear, Boston, Mass.

Results

1. MAO activity in brain mitochondria and synaptosome of monkey after administration (i.m.) of 2 mg/kg of MP: MAO activities in monkey brain mitochondria and synaptosome were measured after administration of 2 mg/kg of MP once a day for 7 days with 5-HT and β-PEA as substrates. Slight inhibition of MAO activity in mitochondria was observed with 5-HT and β-PEA as substrates. However, MAO activity in synaptosome was not significantly changed (Table 1).

The $K_m$ and $V_{max}$ values for the mitochondrial and synaptosomal preparations of monkeys administered MP or saline for 7 days were determined from Lineweaver-Burk double reciprocal plots of values obtained from graphic representation of kinetic data. The results with 5-HT, β-PEA and DA as substrates are shown in Table 2. When 5-HT and β-PEA are used as substrates, the $K_m$ and $V_{max}$ values decreased slightly using the mitochondrial preparation from monkeys administered MP for 7 days. In contrast, when the synaptosomal preparation from monkeys administered MP for 7 days was used, the $K_m$ and $V_{max}$ values with 5-HT and DA increased compared with that of the control group.

2. Effects of clorgyline and deprenyl on MAO in brain of monkey administered MP: Using the mitochondrial and synaptosomal preparations from monkey brains administered MP, the inhibitions of MAO activity by various concentrations of clorgyline (inhibitor for A-form MAO) and deprenyl (inhibitor for B-form MAO) were studied (Fig. 1). Using the mitochondria and synaptosome from the brains of monkeys administered a saline solution, typical sigmoidal inhibition curves were obtained: MAO activity with 5-HT as a substrate was highly sensitive, the activity with β-PEA was less sensitive to the clorgyline, while β-PEA oxidation was the most sensitive, and 5-HT oxidation was the least sensitive to the deprenyl. In the mitochondrial preparations from the brains of monkeys administered MP, the inhibition by clorgyline and deprenyl increased with β-PEA as a substrate. When synaptosome was used as the enzyme preparation, the inhibition by deprenyl and

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**Table 1.** Effect of repeated administration of d-methamphetamine on MAO activity in brain mitochondria and synaptosome of monkeys

|                | Mitochondria | Synaptosome |
|---------------|--------------|-------------|
|               | 5-HT         | β-PEA       | 5-HT         | β-PEA       |
| Control       | 0.95±0.03    | 1.49±0.03   | 1.02±0.02    | 0.77±0.03   |
| Methamphetamine treated | 0.78±0.05*   | 1.38±0.02*  | 1.07±0.05    | 0.80±0.02   |

MAO activity was expressed as nmole/min/mg of protein. MAO activity is assayed radiochemically using 200 μM 5-HT and 50 μM β-PEA as substrates. d-Methamphetamine (2 mg/kg, i.m.) and saline were administered to three monkeys daily for 7 days, respectively. *P<0.1.

**Table 2.** $K_{in}$ and $V_{max}$ values of MAO of mitochondria and synaptosome of monkey brain

|       | Control |          | MP-treated |          |
|-------|---------|----------|------------|----------|
|       | $K_{in}$| $V_{max}$| $K_{in}$   | $V_{max}$|
| 5-HT  | M       | 191.0±8.5| 4.47±0.56  | 154.6±10.4* | 1.96±0.05*  |
|       | S       | 29.7±0.5 | 2.08±0.12  | 61.4±1.8*   | 3.87±0.37*  |
| β-PEA | M       | 49.7±6.0 | 7.13±0.07  | 35.9±4.2*   | 6.03±0.14*  |
|       | S       | 27.7±1.5 | 4.85±0.07  | 44.9±15.1   | 4.04±0.92   |
| DA    | M       | 48.6±2.1 | 6.45±0.24  | 48.7±3.4    | 6.41±0.45   |
|       | S       | 47.8±1.3 | 4.39±0.18  | 56.9±4.1*   | 5.49±0.04*  |

$K_{in}$: μM, $V_{max}$: nmole/min/mg of protein. *P<0.05. Each value represents the mean±S.E. of three monkeys. M: mitochondria, S: synaptosome.
Fig. 1. Effects of clorgyline and deprenyl on MAO in monkey brains administered methamphetamine. After incubation at 37°C for 20 min with the various concentration of clorgyline (left) and deprenyl (right), MAO activity was determined with 5-HT and β-PEA as substrates at 37°C for 20 min. The protein concentration of each of the mitochondrial (upper) and synaptosomal (bottom) preparations was adjusted to 2 mg/ml. The results are means of triplicate assays. Enzyme preparations from monkey brains by repeated administration of methamphetamine (▲ —▲ 5-HT, ●—● β-PEA) and saline (∧—∧ 5-HT, ○——○ β-PEA).

Table 3. Methamphetamine and its metabolite contents in mitochondria and synaptosome of monkey brain given methamphetamine

|                         | Mitochondria | Synaptosome |
|-------------------------|--------------|-------------|
| Methamphetamine        | 5.05±0.22    | 2.35±0.35   |
| Amphetamine             | 37.3±3.8     | 46.4±1.5    |
| p-Hydroxy methamphetamine | n.d.       | n.d.        |
| p-Hydroxy amphetamine   | n.d.         | n.d.        |

Monkeys were treated with d-methamphetamine once a day for 7 days (2 mg/kg, i.m.). The contents of MP and its metabolites in mitochondria and synaptosome were determined by the following S.I.M. technique using g.l.c.-mass spectrometry (see Method). Methamphetamine: pg/mg of protein, Amphetamine: ng/mg of protein, n.d.: not detectable. Each value represents the means±S.E. of three monkeys.

clorgyline increased with 5-HT as a substrate.

3. MP and its metabolite contents in mitochondria and synaptosome from monkey brains given MP: The contents of MP and its metabolites in mitochondrial and synaptosomal preparations of monkey brain administered MP for 7 days were determined by the following S.I.M. technique using g.l.c.-mass spectrometry (see Method). Using the mitochondrial preparation, the contents of MP and AP were about 5.05±0.22 pg/mg of protein and 37.3±3.8 ng/mg of protein, respectively. In the synaptosomal preparation, the contents of MP and AP were about 2.35±0.35 pg/mg of protein and 46.4±1.5 ng/mg of protein, respectively. However, OH-MP and OH-AP could not be detected in the mitochondrial and synaptosomal preparations from monkey brains (Table 3).

4. Effects of MP, AP, OH-MP and OH-AP on MAO activity, in vitro: To determine the mechanism of inhibition of MAO activity by
MP and its metabolites, AP, OH-MP and OH-AP, the effects of various concentrations of these reagents on MAO in monkey brain mitochondria and synaptosome, in vitro, were studied using 5-HT, β-PEA and DA as substrates. As can be seen in Fig. 2, MAO activities decreased with increase in the concentrations of these reagents for all substrates. Particularly, when 5-HT was used as a substrate, MAO was inhibited by these reagents more highly than that toward β-PEA and DA, and inhibition was in the following order: AP > OH-MP > MP > OH-AP. The activities toward β-PEA and DA were highly inhibited by these reagents, except for OH-AP. Similar results were obtained using synaptosomes as the enzyme preparation (data not shown).

5. Inhibition of monkey brain mitochondrial and synaptosomal MAO by various concentrations of MP, AP, OH-MP and OH-AP: Figures 3–5 show the inhibition of monkey brain mitochondrial and synaptosomal MAO by these reagents with 5-HT, DA and β-PEA as substrates. As shown in Figs. 3–5, several graphs of I/v against I/s were, straight lines in the presence and absence of these reagents and these lines all crossed at a single point on the ordinate; therefore, all of these reagents are competitive inhibitors of monkey brain mitochondrial and synaptosomal MAO with 5-HT, DA and β-PEA as substrates. The respective Kᵢ values are calculated from the apparent Michaelis constants, and the Kᵢ and Kᵢ values are presented in Table 4. When synaptosomes were used as the enzyme preparation, the Kᵢ values toward these reagents were similar to the Kᵢ values calculated from Lineweaver-Burk plots with 5-HT as the substrate. While, the Kᵢ values toward OH-MP and OH-AP were higher than the Kᵢ values with β-PEA and DA as substrates. The Kᵢ values toward AP were much lower than that of MP, OH-MP and OH-AP with 5-HT and β-PEA as substrates.

Discussion

It is known that MP and AP are competitive MAO inhibitors that are more potent for type A MAO than for type B MAO, in

Fig. 2. Effects of methamphetamine and its metabolites on MAO activity in monkey brain mitochondria. After incubation at 25°C for 20 min with the various concentrations of these reagents, MAO activity was determined with 200 µM 5-HT (Δ—Δ), 200 µM dopamine (●—●) and 50 µM β-PEA (○—○) as substrates at 37°C for 20 min. The mean control values for MAO activity in mitochondria were 0.92, 1.12 and 1.45 nmole/min/mg of protein with 5-HT, dopamine and β-PEA as substrates, respectively. The results are means of triplicate assays. MP: methamphetamine, AP: amphetamine, OH-MP: p-hydroxymethamphetamine, OH-AP: p-hydroxyamphetamine.
Fig. 3. Inhibition by MP, AP, OH-MP and OH-AP of 5-HT oxidation by monkey brain mitochondrial and synaptosomal MAO. Lineweaver-Burk plots of the reciprocal of the initial velocity of 5-HT oxidation against the reciprocal of the 5-HT concentration in the presence of these reagents. Abscissa: 1/substrate concentration of mM, Ordinate: 1/initial velocity in nmole/min/mg of protein. The results are means of triplicate assays. ■ 1 x 10^{-4} M OH-MP, △ 1 x 10^{-4} M AP, ▲ 1 x 10^{-4} M OH-AP, □ 1 x 10^{-4} M MP, ○ no additional drugs.

Fig. 4. Inhibition by MP, AP, OH-MP and OH-AP of dopamine oxidation by monkey brain mitochondrial and synaptosomal MAO. Experimental conditions and symbols are as in Fig. 3. The results are means of triplicate assays. ■ 1 x 10^{-4} M OH-MP, △ 1 x 10^{-4} M AP, ▲ 1 x 10^{-3} M OH-AP, □ 1 x 10^{-4} M MP, ○ no additional drugs.
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Fig. 5. Inhibition by MP, AP, OH-MP and OH-AP of β-PEA oxidation by monkey brain mitochondrial and synaptosomal MAO. Experimental conditions and symbols are as in Fig. 3. The results are means of triplicate assays. ■—■ 1×10⁻⁴ M OH-MP, △—△ 1×10⁻⁴ M AP, ▲—▲ 1×10⁻³ M OH-AP, □—□ 1×10⁻⁴ M MP, (--) no additional drugs.

Table 4. Substrate and inhibitor constants for monkey brain mitochondrial and synaptosomal MAO

| Substrate or Inhibitor | Kₘ (μM) | Kᵢ (μM) values toward: |
|------------------------|---------|------------------------|
|                         | MP      | AP                     | OH-MP                  | OH-AP                  |
| 5-HT                   |         | 191.0±8.5              | 150.6±0.8              | 44.0±1.0               | 72.3±6.3               | 225.3±35.0             |
|                        | M       | S                      |                        |                        |                        |                       |
| β-PEA                  |         | 29.7±0.5               | 68.0±2.6               | 34.6±0.8               | 26.6±0.6               | 51.6±1.2               |
|                        | S       | 49.7±6.0               | 108.0±4.7              | 84.6±4.9               | 590.0±82.2             | 2700.3±278.4           |
| DA                     |         | 27.7±1.5               | 93.0±4.1               | 52.6±2.0               | 415.6±12.2             | 1387.6±16.6            |
|                        | M       | S                      |                        |                        |                        |                       |
|                        | 49.6±2.1| 95.7±2.9               | 71.9±1.7               | 88.6±2.2               | 1391.3±81.0            |                       |
|                        | S       | 47.8±1.3               | 125.8±3.2              | 71.1±2.2               | 75.6±2.4               | 1329.1±41.2            |

All assays were performed in triplicate. Kᵢ values were calculated for the data by the method of Dixon and expressed as mean±S.E. for determinations in three monkey brain preparations. M: mitochondria, S: synaptosome

vitro (8, 12, 17). This effect has been supposed to produce some change of MAO in vivo, since selectively high concentrations of MP or its metabolites are required for significant inhibition following repeated MP administration. Bakhit et al. (3) reported that the Vₘₐₓ of the neostriatal tyrosine hydroxylase in rats markedly decreased after having been given large, repeated doses of MP, but no change in the Kₘ was detected. Similar results have been obtained in other enzymes with MP and AP (4, 23, 24). However, choline acetyltransferase and glutamate decarboxylase activities were not altered chronically by MP administration (4). On the other hand, results obtained recently by Wagner et al. (25) and Heffner et al. (26) suggest that repeated administration of MP produces long-term decreases in dopamine levels and in the number of DA uptake sites, and the depression of locomotor activity by amphetamine is mediated by increased serotonergic neurotransmission. In this present study, slight decreases of the Kₘ and Vₘₐₓ values in mitochondria were observed with 5-HT and β-PEA as substrates after administration of MP, 2 mg/kg, once a day for 7 days, while in the mitochondrial pre-
parations from the brains of monkeys administered MP, the inhibition by clorgyline (A-form MAO inhibitor) and deprenyl (B-form MAO inhibitor) increased with β-PEA (B-form MAO substrate) as a substrate. These results indicate that MP and its metabolites themselves did not decrease the $K_m$ and $V_{max}$ value of MAO directly by binding to the MAO enzyme, but that the content of B-form MAO decreased. However, in the case of the synaptosomes, significant increases of the $K_m$ and $V_{max}$ values and a decrease of the content of A-form MAO were observed with 5-HT and DA as substrates. The reason for the discrepancy with these data is not clear. It could be related to the difference in localization of the MAO enzyme, e.g., MAO in the synapse and the mitochondrial MAO in the cell body (18) or to the finding that multiple doses of MP might be toxic to both central serotonergic and dopaminergic neurons (27). Moreover, these findings also indicate that the inhibitory effects of MP on MAO activity cannot be attributed to a destruction of all neurons but are localized only at selected biogenic amine nerve terminals.

Since mitochondrial MAO is believed to exist in many tissues in two functional forms called A- and B-form MAO (28, 29), many investigators have studied the inhibition of A-form and B-form MAO by various reagents (30–32). A-form MAO which is mainly responsible for the deamination of 5-HT and norepinephrine is selectively inhibited by clorgyline. The other enzyme form (B-form MAO) shows greatest affinity for β-PEA as a substrate and is selectively inhibited by deprenyl. In vitro, d-AP and d-MP are competitive, reversible A-form MAO inhibitors (8, 12, 17). l-AP is five times less active than the d-form on A-form MAO (9). Green and El Hait (33) demonstrated that p-methoxyamphetamine is over 20 times more potent than d-AP as an A-form MAO inhibitor, in vivo and in vitro. In this experiment, when 5-HT was used a substrate, monkey brain MAO also was inhibited by MP and its metabolites more highly than that toward β-PEA and dopamine, and was in the order: AP>OH-MP>MP>OH-AP. In addition, these reagents were competitive inhibitors of monkey brain mitochondrial and synaptosomal MAO with 5-HT, DA and β-PEA as substrates. When synaptosomes were used as the enzyme preparation, the $K_m$ values toward these reagents were similar to that of the corresponding $K_m$ values with 5-HT as a substrate. These results show that these reagents are competitive MAO inhibitors that are more potent for A-form MAO than for B-form MAO.

Many studies on the metabolic pathways of AP have been reported in various species (34–36). However, there are few reports on the metabolism of MP in vivo (37). These reports revealed that marked species differences in the metabolism of MP and AP are observed: and the main reaction in the rat is aromatic hydroxylation and in the guinea pig, demethylation and deamination, whereas in monkeys and humans, much of the drug, possibly one-half, is excreted in an unchanged form. In our experiment, MP and AP were found in brain mitochondrial and synaptosomal preparations from monkeys administered d-MP, 2 mg/kg, i.m., daily for 7 days, although the monkey was sacrificed 24 hr after the last dose. However, its metabolites, OH-MP and OH-AP, could not be detected in the brains. It may be considered that the production of OH-MP or OH-AP is low in the monkey and is excreted immediately. These results indicate that MP and AP might be accumulated slowly in central nerve terminals by repeated administration of MP, although no trace of MP and its metabolites could be detected in the brain after 24 hr by a single administration of MP. It is considered that this accumulation of MP and AP might be one of the causes of the alteration or toxicity to nerve terminals produced by the inhibition of enzyme activity.

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