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# PREFERENTIAL INHIBITION OF THE B-FORM OF MONOAMINE OXIDASE IN THE LIVER OF RATS GIVEN 3′-METHYL-4-DIMETHYLAMINOAZOBENZENE IN THE DIET

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**Abstract**—A and B-form monoamine oxidase (MAO) activities were measured in the liver of rats maintained with a diet containing 0.06% 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB). A-form MAO activity was similar to the control value throughout the feeding periods with serotonin as substrate. In contrast, B-form MAO activity decreased rapidly and the level of MAO activity was maintained at about 30% with β-phenylethylamine (β-PEA) as substrate. 3′-Me-DAB feeding did not cause any changes in MAO activity in the brain of rats. A single administration of 3′-Me-DAB (100 mg/kg p.o.) failed to alter A and B-form MAO activities for up to 4 days after its administration. The mechanism of inhibition of B-form MAO activity in rat liver mitochondria by 3′-Me-DAB was investigated. The inhibition of 3′-Me-DAB of B-form MAO activity, *in vitro*, was competitive and reversible. There was no difference in the apparent Michaelis constant toward β-PEA between control and 3′-Me-DAB fed rats. B-form MAO in rat liver mitochondria was titrated with (−)deprenyl; this compound is selective to and an irreversible inhibitor of B-form MAO. The content of B-form MAO in liver mitochondria of rats fed 3′-Me-DAB for 3 weeks was decreased to about 60% of the control level.

Since Johnston's theory [1] proposing that monoamine oxidase (MAO) [monoamine:$O_2$ oxidoreductase (deaminating) EC 1.4.3.4] has A and B-forms (based on the selective inhibitory action of clorgyline) many workers have investigated the existence of two forms of MAO. A-form MAO has been shown to be active with serotonin and sensitive to inhibition by a low concentration of clorgyline [2]. B-form MAO has been shown to be active with β-phenylethylamine (β-PEA) and sensitive to inhibition by a low concentration of (−)deprenyl [3]. When tyramine, dopamine and kynuramine, common substrates both forms of the enzyme, were used as substrate, a double-sigmoid curve was obtained for the inhibition of MAO activity by clorgyline or (−)deprenyl [4].

However, studies of this type show that the relative activities of the two forms vary widely between the various organs of rats [5] and between different animal species [6]. In addition, it has also been shown that the relative activities vary with the conditions of assay of MAO activity, i.e., the period of preincubation time with clorgyline and (−)deprenyl [7] and the concentration of substrate used, *in vitro* [8, 9].

In a preliminary study we found [10] that the MAO activity in liver mitochondria of rats maintained on a diet containing 0.06% 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB) decreased proportionated with the period of 3′-Me-DAB ingestion; the level of MAO activity was maintained at about 30% from the 3rd to the 9th weeek with kynuramine as substrate. We also acquired evidence for changes in the ratio of MAO-A/MAO-B in rat liver mitochondria while the animals were fed a diet containing 3′-Me-DAB.

In the present paper, we have investigated further details of the mechanism of the inhibition of MAO activity in rat liver mitochondria by 3′-Me-DAB ingestion. In addition we acquired evidence that this phenomenon depends on a preferential decrease in B-form MAO in the rat liver mitochondrial preparation.

## MATERIALS AND METHODS

*Preparation of enzyme.* Male Donryu rats weighing 80–100 g were fed on a diet containing 0.06% 3′-Me-DAB for several definite periods. Matched controls were fed a diet only. The rats were anesthetized with 10 mg/kg of sodium pentobarbital given i.p. and their livers were quickly removed and homogenized in 10 vol. 0.25 M sucrose (previously adjusted to pH 7.0 with 0.5 M $NaHCO_3$). The mitochondrial fractions were prepared by the differential centrifugation method described earlier [11]. The mitochondria were washed once by resuspension in 0.25 M sucrose solution and used as the enzyme preparation. Rat brains were homogenized in 10 vol. 0.32 M sucrose, pH 7.0, and the mitochondrial fractions were prepared by differential centrifugation. The mitochondria were washed once by resuspension in 0.32 M sucrose solution and used as the enzyme preparation. All operations were carried out at 4°.

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In studies of short term administration of 3'-Me-DAB, a dose of 100 mg/kg of 3'-Me-DAB in 1.0 ml corn oil was given orally to rats. The rats were anesthetized with 10 mg/kg pentobarbital given i.p. and their livers were quickly removed at the times indicated.

In studies utilizing inhibitors of protein synthesis, rats were treated with cycloheximide, 1 mg/kg (i.p.) twice every 12 hr and next day treated by injection with 1 mg/kg cycloheximide plus 20 mg/kg of pargyline. The animals fed on a diet containing 0.06% 3'-Me-DAB were killed at various times after administration as indicated in the figure.

*MAO activity.* MAO activity was measured by using an ammonia selective electrode as modified by the method of Mayerson *et al.* [12]. The incubation medium consisted of 0.1 M potassium phosphate buffers of pH 7.2, 1 mg of protein, various concentration of substrates and deionized water to a final volume of 2.0 ml. Incubation was carried out for 20 min at 37° and the reaction was stopped by the addition of 4.0 ml of potassium–sodium hydroxide buffer of pH 12.0. Substrates were added to control tubes after the termination of the incubation. The ammonia electrode was immersed in the agitated reaction mixture and its millivolt equilibrium potential was read. Control and test ammonia concentrations were calculated from the standard curve, and the difference between the samples was taken as the amount of ammonia formed enzymatically. MAO activity was expressed as nmole of ammonia formed/min/mg of protein. The ammonia production was linear between 0.5 to 3.0 mg of protein from rat liver mitochondria as the enzyme source; it was also linear with respect to time for the first 30 min of the incubation. Substrate concentrations used were 1 mM tyramine and dopamine, 200 $\mu$M serotonin and 100 $\mu$M $\beta$-PEA as the final concentration.

*Determination of Michaelis constants.* Studies to determine the $K_m$ values for serotonin and $\beta$-PEA were performed utilizing control rat liver mitochondria and 3'-Me-DAB rat liver mitochondria as enzyme preparations. Six concentrations of substrates, each run in duplicate, were utilized for each determination of $K_m$. Appropriate control tubes were maintained at each of the various substrate concentrations. The other details of assay conditions were described above. Kinetic constants were determined from graphic treatment of data as described by Lineweaver and Burk.

In investigating the effect of 3'-Me-DAB on MAO activity, *in vitro,* the enzyme was preincubated for 30 min at 37° with 3'-Me-DAB at the concentrations stated in the individual figures before the addition of the substrates.

*Estimation of protein.* Protein concentrations of the preparations were measured by the method of Lowry *et al.* [13] with bovine albumin as the standard. The protein concentration of the enzyme preparation was adjusted to 10 mg/ml.

*Titration with inhibitors* [7, 14]. Rat liver mitochondria (containing 0.5 mg of protein) were incubated for 2 hr at 37° in small test tubes containing 0.1 M potassium phosphate buffer at pH 7.2 and the desired concentration of inhibitor in 1.0 ml final volume. After the addition of substrates the remaining MAO activity was measured using the ammonia electrode. Inhibitors used were (−)deprenyl for B-form MAO and clorgyline for A-form MAO, respectively.

*Other enzyme activity.* Kynurenine hydroxylase [15]: This was assayed in a medium containing 0.1 M Tris buffer pH 8.1, 30 mM KCl, 0.14 mM NADPH, mitochondrial preparation (1.0 mg of protein) and deionized water to a final volume of 3.0 ml. The reaction was started by adding 10 $\mu$l kynurenine to give a final concentration of 0.3 mM. The decrease in $A_{340}$ was measured over 10 min periods. One unit of activity was defined as the amount of enzyme necessary to produce a change in $A_{340}$ of 0.001/10 min. Specific activity was expressed as units/min/mg of protein.

Malate dehydrogenase [16]: The assay mixture contained 0.1 M phosphate buffer pH 7.5, 10 mg/ml NADH, mitochondrial preparation (25 $\mu$g of protein) and deionized water to a final volume of 3.1 ml. The reaction was started by adding 0.1 ml oxalo acetic acid. The decrease in $A_{340}$ was measured over 2 min periods. One unit of activity was defined as the amount of enzyme necessary to produce a change in $A_{340}$ of 0.001/min. Specific activity was expressed as units/min/mg of protein.

## RESULTS

*Changes of MAO activity in liver mitochondria of rats ingesting 0.06% 3'-Me-DAB in the diet for several definite periods.* The MAO activity in liver mitochondria of rats maintained on a diet containing 0.06% 3'-Me-DAB during a 5 week period was determined. When tyramine was used as substrate (substrate for both forms of MAO), MAO activity decreased and was about 60% of the control value in the first week. After that, the level of MAO activity was maintained at about 50% from the 2nd to the 5th week. The MAO activity decreased more

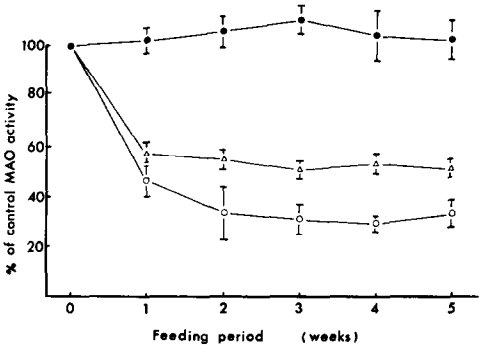


Fig. 1. MAO activity in liver mitochondria of rats ingesting 3'-Me-DAB in the diet (0.06%) for several definite periods. MAO activity is assayed using an ammonia electrode at 37° for 20 min. Specific activities are expressed as nmoles of $NH_3$ formed/min/mg of protein. The mean ± S.E. control values for MAO activity were 15.54 ± 1.64 nmoles/min/mg protein for tyramine, 9.51 ± 0.62 nmole/min/mg protein for serotonin and 8.35 ± 0.55 nmole/min/mg protein for $\beta$-PEA, respectively. Each point represents the mean percentages (± S.E.) of the activity of the enzyme in triplicate experiments. △——△, tyramine; ●——●, serotonin; ○——○, $\beta$-PEA.

Table 1. Mitochondrial enzyme activities in liver of rats ingesting 3′-Me-DAB in the diet for several definite periods

| | monoamine oxidase ( nmole/min/mg of protein ) | | kynurenine hydroxylase ( units/mg of protein ) | malate dehydrogenase ( units/mg of protein) |
|---|---|---|---|---|
| | serotonin | β-PEA | | |
| control | 9.51 ± 0.62 | 8.35 ± 0.55 | 46.06 ± 2.03 | 1045.8 ± 26.2 |
| 1 week | 9.75 ± 0.28 | 3.85 ± 0.42* | 13.63 ± 0.95* | 749.6 ± 33.7* |
| 3 week | 10.40 ± 0.60 | 2.50 ± 0.25* | 8.57 ± 1.02* | 597.2 ± 14.9* |
| 5 week | 9.65 ± 0.66 | 2.80 ± 0.43* | 9.33 ± 0.29* | 708.8 ± 19.5* |

Enzyme activities are defined in Materials and Methods.
Values are means ± S.E. for three separate preparations.
* $P < 0.05$.

rapidly and finally levelled off at about 30% with β-PEA as substrate (preferential substrate for B-form MAO). On the contrary, when serotonin was used as a substrate (preferential substrate for A-form MAO), change of MAO activity was not observed during the 5th week periods (Fig. 1).

For estimation of the effect of other enzymes in mitochondria by 3′-Me-DAB feeding, the activities of kynurenine hydroxylase, an outer membrane marker, and that of malate dehydrogenase, an inner membrane marker, were measured. Both the activity of kynurenine hydroxylase and that of malate dehydrogenase were also significantly decreased proportionally to the periods of 3′-Me-DAB ingestion (Table 1).

MAO activities in brain mitochondria of rats were not changed with every substrate used during the 5th week periods (results not shown).

*MAO activity in liver mitochondria of rats after administration (p.o.) of 100 mg/kg 3′-Me-DAB.* MAO activities in rat liver mitochondria were measured after administration of 100 mg/kg of 3′-Me-DAB with tyramine, dopamine, serotonin and β-PEA as substrates. The significant changes of MAO activity by single administration of 3′-Me-DAB were not observed during 108 hr with every substrate used (results not shown). However, when the rats were administered 100 mg/kg (p.o.) 3′-Me-DAB once a day for 3 days, MAO activity was decreased about 20% with tyramine and dopamine as substrates and about 30% with β-PEA. However, MAO activity was not changed with serotonin as substrate (Table 2).

*Inhibition of rat liver mitochondrial MAO by various concentrations of 3′-Me-DAB.* Figure 2 shows the inhibition of rat liver mitochondrial MAO by 3′-Me-DAB with β-PEA and serotonin as substrates. The reaction rates were estimated by measuring the ammonia produced. As shown in Fig. 2, values of $1/v$ vs $1/s$ gave straight lines in the presence and absence of 3′-Me-DAB and these lines crossed at a single point on the ordinate; therefore 3′-Me-DAB is a competitive inhibitor of rat liver mitochondrial MAO with β-PEA and serotonin as substrates. The $K_i$ values were calculated from apparent Michaelis constants and were $9.9 \times 10^{-7}$ M and $2.5 \times 10^{-7}$ M at the concentration of $4 \times 10^{-7}$ M 3′-Me-DAB with serotonin and β-PEA as substrates, respectively.

*Reversibility of the effect of 3′-Me-DAB.* A sample of a mitochondrial preparation was mixed with an equal volume of $4 \times 10^{-6}$ M 3′-Me-DAB and, as a control, another sample of the suspension was mixed with an equal volume of 0.1 M phosphate buffer. Both mixtures were dialyzed over night against 0.001 M phosphate buffer at pH 7.2 at 4° before their activities were compared. As shown in Table 3,

Table 2. Effect of multiple administration of 3′-Me-DAB on MAO activity in liver mitochondria of rats

| | tyramine | serotonin | β-PEA | dopamine |
|---|---|---|---|---|
| control | 14.58 ± 1.34 | 9.83 ± 0.60 | 8.55 ± 0.54 | 13.11 ± 1.05 |
| 3′-Me-DAB treatment | 11.80 ± 1.01* | 9.66 ± 0.53 | 5.98 ± 0.48* | 10.75 ± 0.92* |

* $P < 0.05$ MAO activity is assayed using an ammonia electrode at 37° for 20 min.
Specific activity is expressed as nmoles of $NH_3$ formed/min/mg of protein.
3′-Me-DAB (100 mg/kg, p.o.) was administered to rats daily for 3 days.
Values are the mean ± S.E.

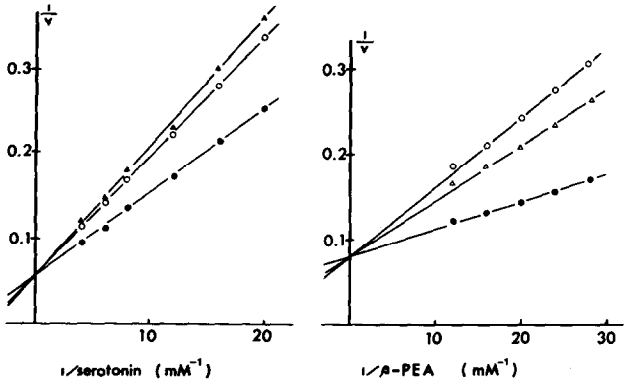


Fig. 2. Inhibition by various 3'-Me-DAB on rat liver mitochondrial MAO. Lineweaver–Burk plots of the reciprocal of the initial velocity of β-PEA and serotonin oxidation against the reciprocal of the substrates concentration in the presence of 3'-Me-DAB. Abscissa: 1/substrate concentration in mM; Ordinate: 1/initial velocity in nmole/min/mg protein. Each point represents the mean of duplicate determinations. Substrates used were β-PEA (right), assayed in the absence (●——●) and presence of $1 \times 10^{-7}$ M (△——△) and $4 \times 10^{-7}$ M (○——○) 3'-Me-DAB. Serotonin (left), assayed in the absence (●——●) and presence of $4 \times 10^{-7}$ M (○——○) and $1 \times 10^{-6}$ M (▲——▲) 3'-Me-DAB.

before dialysis, the activities with 3'-Me-DAB were 81.9% and 67.7% of the control values with serotonin and β-PEA, respectively. After dialysis, the activities were 97.8% and 98.7%, respectively. Similarly, a sample of a suspension of mitochondrial preparation from rats ingesting 3'-Me-DAB for 3 weeks was mixed with an equal volume of 0.1 M phosphate buffer only and dialyzed overnight against

Table 3. Effect of dialysis on the inhibition of monoamine oxidase by 3'-Me-DAB

| | serotonin | | | | β-phenylethylamine | | | |
|---|---|---|---|---|---|---|---|---|
| | before dialysis | | after dialysis | | before dialysis | | after dialysis | |
| | activity* | ( % ) | activity* | ( % ) | activity* | ( % ) | activity* | ( % ) |
| control | 9.15 ± 0.06 | 100 | 8.54 ± 0.77 | 100 | 8.53 ± 0.15 | 100 | 8.49 ± 0.85 | 100 |
| control + DAB** | 7.50 ± 0.21 | 81.9 | 8.36 ± 0.48 | 97.8 | 5.78 ± 0.59 | 67.7 | 8.38 ± 0.88 | 98.7 |
| DAB-fed*** | 8.78 ± 0.30 | 95.9 | 8.23 ± 0.41 | 96.3 | 2.22 ± 0.65 | 26.0 | 2.11 ± 0.68 | 24.8 |

Dialyzed against 0.001 M phosphate buffer (pH 7.2) overnight in cold room.
* Activity: nmole/min/mg of protein.
** Control + DAB: control mitochodria + $4 \times 10^{-7}$ M 3'-Me-DAB (final concn).
*** DAB-fed: mitochondrial preparations of rats ingesting 3'-Me-DAB for 3 weeks.

Table 4. $V_{max}$ and $K_m$ values of MAO of mitochondria of rats

| | serotonin | | β-phenylethylamine | |
|---|---|---|---|---|
| | Km ( μM ) | Vmax ( nmole/min/mg protein ) | Km ( μM ) | Vmax ( nmole/min/mg protein) |
| control | 177.3 ± 5.3 | 18.1 ± 1.3 | 44.1 ± 2.2 | 13.1 ± 0.8 |
| 3'-Me-DAB fed* | 179.0 ± 4.4 | 18.6 ± 2.1 | 44.2 ± 1.5 | 3.2 ± 1.4** |

$K_m$ and $V_{max}$ values are calculated from Lineweaver–Burk plots with six substrates concentrations. Results are expressed as means ± S.E. MAO activity is assayed using an ammonia electrode at 37° for 20 min.
* 3'-Me-DAB fed: mitochondrial preparations of rats ingesting 3'-Me-DAB for 3 weeks.
** $P < 0.05$.

Table 5. Titration of monoamine oxidase in rat liver mitochondria with clorgyline and (−)deprenyl

| | MAO-A content ( pmole/mg protein ) | MAO-B content ( pmole/mg protein ) |
|---|---|---|
| Control diet | 25.1 ± 0.81 | 38.3 ± 1.62 |
| 3'-Me-DAB fed* | 24.2 ± 2.1 | 15.3 ± 1.74** |

Enzyme contents were determined by the line of best fit to data from at least six concentrations of clorgyline or (−)deprenyl (see Materials and Methods). Results are expressed as means ± S.E. Inhibitor final concentrations were used from 1 to 20 nM with (−)deprenyl and 1 to 12 nM with clorgyline.

* 3'-Me-DAB fed: mitochondrial preparation in liver of rats ingesting 3'-Me-DAB for 3 weeks.

** $P < 0.05$.

0.001 M phosphate buffer pH 7.2. Before dialysis, the activities were 95.9% and 26.0% of the control value with serotonin and β-PEA, respectively. While after dialysis, the activities were 96.3% and 24.8%, respectively.

*$K_m$ and $V_{max}$ values of MAO in liver of rats ingesting 3'-Me-DAB.* The $K_m$ and $V_{max}$ values for the control and mitochondrial preparation of rats ingesting 3'-Me-DAB for 3 weeks were determined from Lineweaver–Burk double reciprocal plots of values obtained from graphic representation of kinetic data. The results with serotonin and β-PEA as substrates are shown in Table 4. When serotonin was used as substrate, the $K_m$ and $V_{max}$ values were almost identical using these mitochondrial preparations. When the mitochondrial preparation of rats ingesting 3'-Me-DAB for 3 weeks was used, the $V_{max}$ value was decreased markedly compared with that of the control, although the $K_m$ value was almost identical with β-PEA as substrate.

*Content of MAO in rat liver mitochondria with clorgyline and (−)deprenyl.* When mitochondrial fractions of rat liver were preincubated for 2 hr at 37° with increasing concentrations of inhibitors, plots of percent activity of MAO against inhibitor concentration yield approximately straight lines which passed through at a single point on the abscissa. The concentration of inhibitor required to produce 100% inhibition is equivalent to the enzyme concentration. To determine the relationship between the value of MAO activity and the amount of MAO in liver mitochondria of rats, the titration experiment was performed with clorgyline (for determination of content of A-form MAO) and (−)deprenyl (for determination of B-form MAO). Using the mitochondrial preparation, the content of A-form MAO and B-form MAO was about 25.1 ± 0.81 and 38.3 ± 1.62 pmoles/mg of protein, respectively. However, in the case of mitochondrial preparation from rats ingesting 3'-Me-DAB for 3 weeks, the content of A-form MAO and B-form MAO was 24.2 ± 2.1 and 15.3 ± 1.74 pmole/mg of protein, respectively (Table 5).

*Recovery of MAO activity in rat liver following administration of pargyline.* The MAO activities in rat liver mitochondria were measured with serotonin and β-PEA as substrates after administration of 20 mg/kg of pargyline to rats fed on a diet containing 3'-Me-DAB at a level of 0.06% for several definite periods. As shown in Fig. 3, with serotonin as sub-

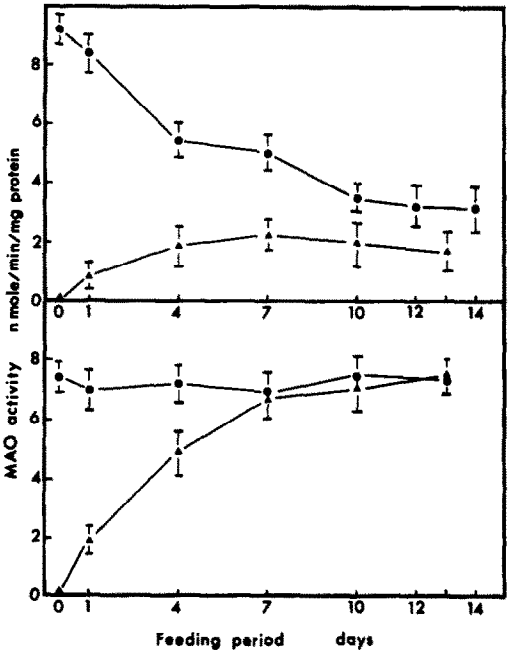


Fig. 3. Recovery of monoamine oxidase activity in rat liver following administration of pargyline. Rats were treated with cycloheximide (1 mg/kg i.p.) twice the first day and next day treated by injection with cycloheximide (1 mg/kg) plus pargyline (20 mg/kg) i.p. The animals fed on a diet containing 3'-Me-DAb at a level of 0.06% were killed and mitochondrial fractions were prepared at various times after administration as indicated in the figure. β-PEA (upper panel) and serotonin (lower panel) were used as substrates. The results without administration of inhibitor (●——●) and with administration of pargyline plus cycloheximide (▲——▲) are as illustrated. Each point represents the mean MAO activity (± S.E., $n = 3$) assayed in triplicate experiments.

strate MAO activity was about 25% on the control value 1 day after administration of pargyline and then increased rapidly; it was restored completely within 14 days. With β-PEA as substrate, MAO activity decreased rapidly and the level of MAO activity was maintained at about 30% without administration of pargyline. MAO activity increased gradually after administration of pargyline, the level of MAO activity was maintained at about 20% of the control value from the 4th to the 13th day.

## DISCUSSION

In a preliminary study we reported that MAO activity in liver mitochondria of rats maintained on a diet containing 0.06% 3′-Me-DAB decreased proportionate to the period of 3′-Me-DAB ingestion with kynuramine as substrate. In the present study, similar results were obtained with tyramine as substrate. When β-PEA as substrate (ıor B-form MAO), MAO activity decreased remarkably and the level of activity was maintained at about 30% of control. But when serotonin was used as substrate (for A-form MAO), a change of MAO activity was not observed during a 5 week period. MAO activities in brain mitochondria of rats did not change with serotonin and β-PEA as substrates.

Both the activity of kynurenine hydroxylase, an outer membrane marker enzyme, and that of malate dehydrogenase, an inner membrane marker enzyme, were also significantly decreased proportionately to the time periods of 3′-Me-DAB ingestion.

To investigate whether the preferential decrease of B-form MAO is due to the inhibition by 3′-Me-DAB itself or by the metabolites of 3′-Me-DAB, MAO activities in rat liver mitochondria were measured after a single or repeated administration (once a day for 3 days) of 100 mg/kg (p.o.) with tyramine, dopamine, serotonin and β-PEA as substrates. Significant changes of MAO activity were not changed with every substrate used during 108 hr following a single administration. However, when the rats were administered once a day for 3 days, MAO activities were decreased with tyramine, dopamine and β-PEA, but were not changed with serotonin. B-form MAO activity in rat liver was also decreased preferentially by administration of 3′-Me-DAB (p.o.). These results indicate that the lag time of at least 3 days is necessary to obtain the decrease of B-form MAO activity and that 3′-Me-DAB itself does not bind to the membrane enzymes of mitochondria directly.

The effects of 3′-Me-DAB on rat liver mitochondrial MAO were investigated *in vitro*. 3′-Me-DAB at a concentration of $1 \times 10^{-6}$ M or $1 \times 10^{-7}$ M inhibited MAO activity about 30% with serotonin and β-PEA as substrates, and MAO activity was inhibited in an apparently competitive fashion by 3′-Me-DAB as determined by Lineweaver–Burk double reciprocal plots, respectively. There was a reversibility of the inhibition of MAO activity by 3′-Me-DAB after dialysis overnight against 0.001 M phosphate buffer pH 7.2 at 4°. These results show that 3′-Me-DAB can be bind to MAO enzyme with a very weak affinity *in vitro* (see Fig. 2).

The inhibiting effects of 3′-Me-DAB were investigated using mitochondrial preparations from livers of rats ingesting 3′-Me-DAB. Even after dialysis overnight, no recovery to the control activity towards β-PEA was obtained (see Table 3). When serotonin was used as substrate, there was no significant difference of $K_m$ and $V_{max}$ values between mitochondrial preparations from livers of control and 3′-Me-DAB fed rats. However, when the mitochondria from livers of 3′-Me-DAB fed rats were used as enzyme preparation, $V_{max}$ decreased to about 75% of the control with β-PEA as substrate.

The relationship between the values of MAO activity and the molecular amount of MAO was examined by titration experiments with clorgyline (for A-form MAO) or (−)deprenyl (for B-form MAO) using both enzyme preparations from livers of rats. Clorgyline and (−)deprenyl inhibit MAO irreversibly at ratio of 1 : 1; i.e. the amount of enzyme inhibited is equal to the amount of inhibitor added on a molar basis. The content of A-form MAO in control rat liver mitochondria was the same as that from animals that were fed a diet containing 3′-Me-DAb with serotonin as substrate. But, the content of B-form MAO decreased about 60% in rat liver mitochondria after 3′-Me-DAB ingestion with β-PEA as substrate. These results indicate that 3′-Me-DAB itself did not decrease MAO activity directly by binding to the B-form MAO, but that 3′-Me-DAB is activated enzymatically in the microsomal fractions and then B-form MAO activity is inhibited by these activated reagents (i.e. *N*-hydroxylated derivatives of 3′-Me-DAB) [17].

Moreover, from these results the lag time of at least 3 days is necessary to obtain the decrease of B-form MAO activity. The level of B-form MAO activity is maintained at about 30% from 2nd to the 5th week. And from results of experiments of Fig. 3, it is considered that *N*-hydroxylated derivatives of 3′-Me-DAB do not bind the B-form MAO in mitochondria but that these bind the newly synthesized B-form MAO [18] in microsomal fractions and that the B-form MAO on microsomes is transferred to the mitochondrial membranes.

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