*Biochemical Pharmacology*, Vol. 40, No. 4, pp. 811–815, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00
© 1990. Pergamon Press plc

# THYROID HORMONE-INDUCIBLE MONOAMINE OXIDASE INHIBITOR IN RAT LIVER CYTOSOL

TOSHIO OBATA,* MINORU TAMURA† and YASUMITSU YAMANAKA*

*Department of Pharmacology and †Department of Biochemistry, Medical College of Oita, 1-1506, Idaigaoka, Hazama-cho, Oita 879-56, Japan

(*Received* 2 *January* 1990; *accepted* 23 *March* 1990)

**Abstract**—An endogeneous inhibitor of monoamine oxidase (MAO) was separated by gel-filtration from 105,000 *g* supernate of $T_4$-treated rat liver cytosol. The inhibition by this inhibitor was concentration-dependent and more potent for A-form MAO than for B-form MAO. The mode of inhibition was competitive either with 5-hydroxytryptamine or β-phenylethylamine. The molecular weight of this inhibitor was estimated to be 600–700 by gel filtration. The pI value was determined to be 3.0 by isoelectric focusing. This inhibitor was proved to be heat-stable and resistant to protease treatment. MAO inhibition activity was much lower in the cytosol of thyroidectomized, non-$T_4$-treated rats than $T_4$-treated rats, suggesting that this inhibitor is induced by thyroid hormone $T_4$. MAO activity in rat liver might be regulated by the level of this inhibitor.

Monoamine oxidase (MAO‡; EC 1.4.3.4) plays a major role in the metabolism of biogenic amines including neurotransmitters such as dopamine, adrenaline and 5-HT. MAO is classified into two forms, form A (MAO-A) and form B (MAO-B), based on the substrate specificity and the sensitivity to inhibitors [1–3]. That is, MAO-A prefers 5-hydroxytryptamine (5-HT) and MAO-B prefers β-phenylethylamine (β-PEA) as a substrate. Clorgyline and deprenyl are two typical inhibitors selective for MAO-A and MAO-B, respectively.

One of the action of thyroid hormones is thought to be the regulation of protein synthesis and enzyme activities [4–8]. Lyles and Callingham [9] reported that thyroid hormones increase the protein synthesis of MAO in rat heart. On the contrary, MAO activity in rat liver was shown to be diminished by thyroid hormone [4, 10] or by a pituitary factor [11], which is known to induce the secretion of thyroid hormones. In the latter cases [4, 10, 11], it is not clear whether thyroid hormones affect the protein synthesis of MAO or act by another mechanism (e.g. induction of specific modulator).

Endogeneous MAO modulators have been detected in human plasma [12–15], urine [16–18] and cerebrospinal fluid [19]. Also, several reports suggest the presence of MAO modulators in the cytosol of rat heart [20, 21] liver [20], and lung [22]. These studies imply that endogeneous MAO modulators might be important in the physiological regulation of MAO activity [20–22].

In the present study, we found an endogenous MAO inhibitor in rat liver cytosol and that this inhibitor could be induced by thyroid hormone $T_4$.

## MATERIALS AND METHODS

*Isolation of MAO Inhibitor.* Thyroidectomized rats (Male Wistar), weighing 100–150 g, were used for experiments. In the case of $T_4$ administered rats, $T_4$ (dissolved in saline) was injected subcutaneously to the rats after 10 days from operation at a dose of 200 μg/kg per day for 2 weeks. The rats were killed by decapitation and livers were quickly removed and homogenized in 10 vol. of 10 mM phosphate buffer, pH 7.4 containing 0.25 M sucrose. The homogenate was centrifuged at 105,000 *g* for 60 min, and the supernate (cytosol fraction) was applied on a Sephadex G-25 column (1.0 × 60 cm), previously equilibrated with 200 mM sodium or potassium phosphate buffer (pH 7.4). The column was eluted with the same buffer at a rate of 10 mL/hr and the fractions were collected in 2.5 mL each. An aliquot of each fraction was assayed for MAO inhibition activity, and active fractions were combined and used for further characterization. This fraction is referred to as "endogeneous MAO inhibitor (EMI)".

*Assay of MAO activity.* Rat liver mitochondrial fraction was used as a source of MAO activity. The mitochondrial fraction suspended in 20 vol. of 10 mM phosphate buffer, pH 7.4, was gently stirred for 60 min at 4° to rupture mitochondria [21]. The mixture was centrifuged at 20,000 *g* for 60 min at 0° and the pellets were resuspended in 10 mM Na or K phosphate buffer pH 7.4 containing 0.25 M sucrose.

MAO activity was assayed radiochemically as described by Fowler *et al.* [23]. Assay mixture contained 20 μL of mitochondrial fraction (100 μg/mL protein), 100 μM [$^{14}$C]5-HT or 10 μM [$^{14}$C]β-PEA, in 200 mM phosphate buffer, pH 7.4 (100 μL) in the presence of EMI (0–180 μL). After a 10- or 20-min preincubation at 37°, the mixture was diluted with the solution of respective unlabeled amines and the reaction was stopped by adding 2 N HCl (200 μL). The reaction products were extracted with ethyl acetate–benzene (1:1, v/v) and the radioactivity was counted.

*Estimation of protein.* Protein was estimated by the method of Lowry *et al.* [24] with bovine serum albumin.

‡ Abbreviations: EMI, endogeneous MAO inhibitor; MAO, monoamine oxidase; β-PEA, β-phenylethylamine; 5-HT, 5-hydroxytryptamine.

*Isoelectric focusing (IEF).* Gel isoelectric focusing was performed by the method of Fawcett [25]. The gel contained 5% acrylamide, 0.2% methylene bisacrylamide, 0.75% Triton X-100, 2% servalyte (pH 2–11), 0.0002% riboflavin, 0.01% ammonium persulfate, 0.05% *N,N,N'N'*-tetramethylenediamine. The gel was mounted on a vertical apparatus containing 0.01 M $H_3PO_4$ in the upper tank (anode) and 0.02 M NaOH in the lower tank (cathode). The voltage was fixed at 100 V for the first 1 hr, 200 V for the next 2 hr and then 300 V for 2 hr. After the electrophoresis, the gel was cut into 4 mm thick slices and each sliced gel was placed in a test tube, suspended in 1 mL of distilled water and bubbled with $N_2$ gas for 1 hr at room temperature. After the measurement of the pH of gel suspension, a minimum amount of 0.5 M $H_3PO_4$ was added to adjust the pH to 7.4. An aliquot of each slice suspension was then assayed for MAO inhibition activity with β-PEA as a substrate.

*Chemicals.* $T_4$ sodium salt, subtilisin (protease Type III) and pronase E (protease Type XIV) were purchased from the Sigma Chemical Co. (St Louis, MO). 5[2-$^{14}$C]Hydroxytryptamine binoxalate (5-HT) (2.0 GBq/mmol), β-[1-$^{14}$C]phenylethylamine hydrochloride (β-PEA) (2.1 GBq/mmol) were purchased from New England Nuclear (Boston MA). Sephadex G-25 (Medium) was obtained from Pharmacia Fine Chemicals (Piscataway, NJ). All other reagents were of the highest grade commercially available.

## RESULTS

### *Separation of EMI*

The gel-filtration of $T_4$-treated rat liver cytosol with Sephacryl S-200 column showed that the fractions to inhibit MAO activity were eluted in a low molecular weight area (<1300) (data not shown). Therefore, we used Sephadex G-25 column to separate the inhibitor and determine the molecular size (Fig. 1.). The fractions 18–20 were found to inhibit MAO activity either with β-PEA or 5-HT as a substrate. The molecular weight of the inhibitor was estimated to be 600–700. Some endogeneous MAO activity was observed in fractions 8–10. We assume that this activity is due to cytosolic MAO [26] rather than to the contamination of the mitochondrial enzyme.

### *The effect of $T_4$ treatment on MAO inhibitory activity in rat liver cytosol*

Table 1 summarizes the effect of $T_4$ administration to rats on MAO inhibitor. When thyroidectomized, non-$T_4$-treated rats were used (EMI-control), the inhibition activities for β-PEA and 5-HT were very low. $T_4$-treatment remarkably enhanced MAO inhibition activities for both substrates. This results suggests that the inhibitor could be induced by $T_4$ treatment.

### *The inhibition of MAO activity by EMI*

The effect of concentration of the inhibitor on MAO activity was examined using β-PEA or 5-HT as a substrate. As shown in Fig. 2, the inhibition curves of MAO activity by EMI were concentration-dependent, and $IC_{50}$ values for β-PEA and 5-HT

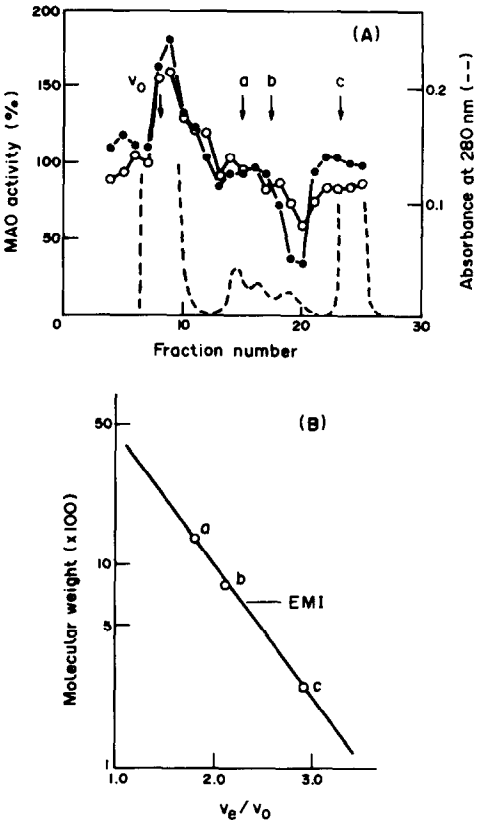


Fig. 1. Sephadex G-25 chromatography of cytosolic fraction from $T_4$-treated rat liver. Cytosol fraction (10 mg protein) was applied to a Sephadex G-25 column (1.0 × 60 cm). (A) The activity of MAO in mitochondria was assayed with β-PEA (○—○) and 5-HT (●—●) as a substrate. Arrows indicate the position of marker compounds. The molecular for markers used were as follows: a, Cyanocobalamin (MW, 1355); b, FAD (MW, 786); c, DNP-Alanine (MW, 255). $v_0$; dextran (MW, 2,000,000). The broken line shows the absorbance at 280 nm. (B) Estimation of molecular weight of EMI by gel filtration with Sephadex G-25. The arrow shows the mobility of the peak of inhibition activity.

Table 1. Inhibition of MAO activity by the endogeneous MAO inhibitor (EMI)

| G-25 fraction added | Inhibition of MAO activity (%) β-PEA | 5-HT |
|---|---|---|
| Buffer | 0.0 | 0.0 |
| EMI-control | 2.6 ± 0.3 | 1.3 ± 1.2 |
| EMI-$T_4$ | 40.0 ± 3.4 | 68.5 ± 3.9 |

EMI-control or EMI-$T_4$ was obtained from thyroidectomized rat or thyroidectomized, $T_4$-treated rat, respectively, as described in Materials and Methods. After incubation at 37° for 30 min with 160 μL of EMI-control or EMI-$T_4$, MAO activity was assayed with 10 μM β-PEA or 100 μM 5-HT as a substrate at 37° for 10 and 20 min. The control activities (buffer) for β-PEA and 5-HT were 2.21 ± 0.15 and 3.15 ± 0.20 nmol/min/mg protein, respectively. The values represent the mean percentages (±SE) of the control MAO activity in triplicate determinations.

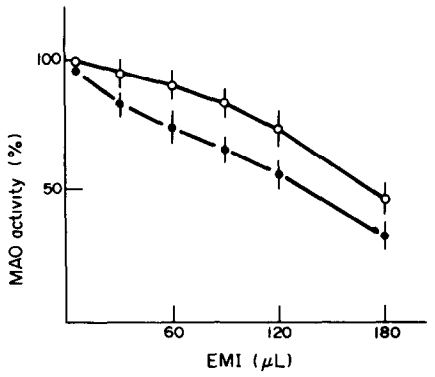


Fig. 2. Inhibition of MAO activity by EMI. After incubation with various amounts of EMI at 37° for 40 min, MAO activity was assayed using 100 μM 5-HT (●—●) or 10 μM β-PEA (○—○) as substrate in 200 μL of 200 mM Na-phosphate buffer, pH 7.4 at 37° for 10 min. The control activities for β-PEA and 5-HT were 3.15 ± 0.20 and 2.21 ± 0.15 nmol/min/mg protein, respectively. Each point represents the mean percentages (±SE) on the control MAO activity in triplicate experiments.

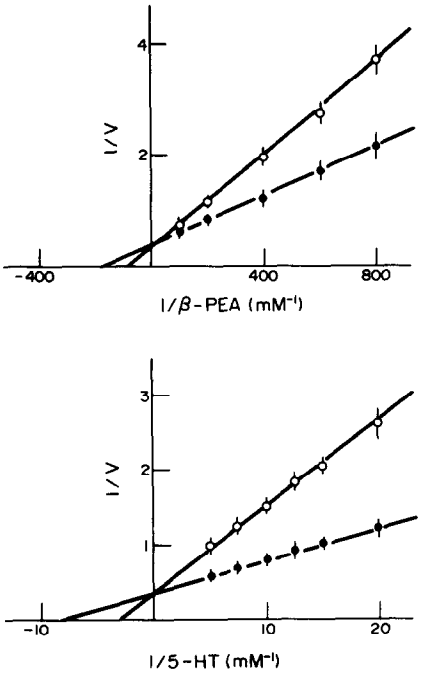


Fig. 3. Lineweaver–Burk plots of the velocity of β-PEA and 5-HT oxidation and substrate concentration in the presence and absence of EMI. $V$ (velocity) is expressed as dpm × $10^{-4}$ of the products formed in 20-min reaction time. Substrates used were β-PEA (upper) and 5-HT (bottom). The assay was carried out in the absence (●—●) and presence (○—○) of 120 μL EMI.

oxidation were 126 and 176 μL, respectively. This result shows that EMI inhibits A-form MAO somewhat more strongly than B-form MAO.

*The inhibition by EMI of MAO activity with different substrate concentrations*

To know the mode of EMI inhibition, the inhibition was investigated with different substrate concentrations, Fig. 3 shows Lineweaver–Burk plots of the results with 5-HT or β-PEA as a substrate. EMI increased the $K_m$ values for β-PEA from 7.0 to 12.4 μM and that for 5-HT from 116.4 to 306.5 μM, but did not alter $V_{max}$ values. Time-course study on the preincubation time showed that the extent of the inhibition was not changed when the incubation was prolonged up to 90 min (data not shown). These results indicates that the inhibition was competitive both with 5-HT and β-PEA.

*Isoelectric point (pI) of EMI*

pI value of EMI was determined by gel isoelectric-focusing (Fig. 4). MAO inhibition activity was found as a single peak at a position 1.2 cm from the top of the gel. The pH measurement after isoelectric focusing revealed that EMI has a pI value of about 3.0.

*Effect of heat or protease treatment of EMI*

When EMI were treated at 100° for 10 min, the inhibition activity was not changed. Also, treatment of EMI with subtilisin or pronase at 37° for 12 hr did not affect the inhibition activity of EMI. These results suggest that EMI is a non-peptide inhibitor.

## DISCUSSION

There have been many reports on the effects of thyromimetic compounds on MAO activity: some indicated that hepatic MAO activity was decreased by thyroid hormone [27–29], while the others

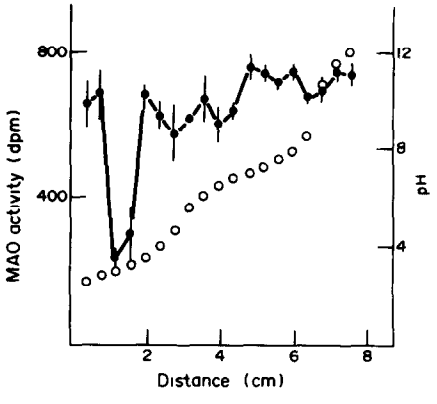


Fig. 4. Estimation of pI value of EMI in rat liver cytosol by gel isoelectric focusing. EMI fraction was collected and was solubilized with 0.75% Triton X-100. Isoelectric focusing was performed as described in Materials and Methods. After IEF-gel electrophoresis, the gel was cut into 4-mm slices, and the pH (○) of each gel slice was determined. After adjusting pH to 7.4, the MAO activity for β-PEA was determined in the presence of each gel slice (●). The control activity for β-PEA was 0.233 ± 0.014 nmol/min/mg protein. Each point represents the mean percentages (±SE) on the control MAO activity in triplicate experiments.

reported that the cardiac MAO activity was increased in hyperthyroid rats or by administration of thyromimetic compound [9, 20].

In the present study, we found an endogeneous MAO modulator of a low molecular weight (600–700) in liver cytosol of $T_4$-treated rats. This molecular weight is much smaller than that reported for rat heart cytosol [21] (8500–35,000), human CSF [19] (3000–35,000) or human plasma [14, 15] (4000–30,000).

When thyroidectomized, non treated rats were used (EMI-control), inhibition activities for β-PEA and 5-HT were very low. $T_4$-treatment remarkably enhanced MAO inhibition activities for both substrates (Table 1). The fact that the inhibitory activity increased in the hyperthyroid state suggests the possibility that thyroid hormone may regulate the mitochondrial MAO activity by induction of this inhibitory modulator.

Dilution experiments showed that the dilution of the reaction mixture restored the activity to the level at final concentration of the inhibitor (data not shown), suggesting that the inhibition is reversible. A kinetical study showed that endogeneous MAO inhibitor (EMI) increased the $K_m$ values for β-PEA and for 5-HT but did not alter $V_{max}$ values. This result showed that the property of EMI is different from endogeneous MAO inhibitor so far reported in plasma [12–15], urine [16–18], heart [21] and CSF [19] because the inhibition by these modulators was competitive to type A MAO and non-competitive to type B MAO. The pI value of EMI was determined by IEF-gel electrophoresis to about 3.0. This result indicates that EMI is an acidic compound. Although the nature of this inhibitor is not clear at present, the present study showed that this compound is heat-stable and resistant to protease treatment.

In the present study, we found a new MAO inhibitory modulator in $T_4$-treated rat liver cytosol and that the level of this modulator is regulated by thyroid hormone. Although the physiological role of this inhibitor still remains unclear, we consider that this modulator may play some role in regulating the MAO activity in rat liver. Further study will be necessary to clarify this point.

*Acknowledgement*—We are grateful to Dr Masasumi Takeshita (Department of Biochemistry) and Mr Yasuo Kodama (Department of Hospital Pharmacy) for their helpful discussions.

## REFERENCES

1. Johnstoe JP, Some observations upon a new inhibitor of monoamine oxidase in brain tissue. *Biochem Pharmacol* **17**: 1285–1297, 1968.
2. Knoll J and Magyar K, Some puzzling pharmacological effects of monoamine oxidase inhibitors. *Adv Biochem Psychopharmacol* **5**: 393–408, 1972.
3. Neff NH and Yang H-YT, Another look at the monoamine oxidase and the monoamine oxidase inhibitor drugs. *Life Sci* **14**: 2061–2074, 1974.
4. Okamoto H, Influence of *l*-thyroxine on kynurenine-3-hydroxylase, monoamine oxidase, and rotenone-insensitive NADPH-cytochrome *c* reductase in mitochondrial outer membrane. *Biochem Biophys Res Commun* **43**: 827–833, 1971.
5. Ivarie RD, Baxter JD and Morris JA, Interaction of thyroid and glucocorticoid hormones in rat pituitary tumor cells. *J Biol Chem* **256**: 4520–4528, 1981.
6. Brucker Rf and Choen PP, Alterations in enzyme and cytochrome profiles of rana catesbeiana liver organelles during thyroxine-induced metamorphosis. *J Biol Chem* **251**: 6161–6169, 1976.
7. Barsano CP, DeGroot LJ and Getz GS, The effect of thyroid hormone on *in vitro* rat liver mitochondrial RNA synthesis. *Endocrinology* **100**: 52–60, 1977.
8. Assad MM and Clarke DE, Modulation *in vitro* monoamine oxidase activity by thyroid hormones. *Biochem Pharmacol* **27**: 751–756, 1978.
9. Lyles GA and Callingham BA, The effects of thyroid hormones on monoamine oxidase in the rat heart. *J Pharm Pharmacol* **25**: 921–930, 1974.
10. Moonat LBS, Asaad MM and Clarke DE, L-Thyroxine and monoamine oxidase activity in the kidney and some other organs of the rat. *Res Commun Chem Path Pharmacol* **12**: 765–779, 1975.
11. Illselly NP, Kita E and Lamartiniere CA, Role of the pituitary in modulating hepatic monoamine oxidase activity. *Endocrinology* **106**: 798–804, 1980.
12. Yu PH and Boulton AA, Activation of platelet monoamine oxidase by plasma in the human. *Life Sci* **25**: 31–36, 1979.
13. Giambalvo CT and Becker RE, Modulators of monoamine oxidase in plasma. *Life Sci* **29**: 2017–2024, 1981.
14. Becker RE and Giambalvo CT, Endogenous modulation of monoamine oxidase in schizophrenic and normal humans. *Am J Psychiatry* **139**: 1567–1570, 1982.
15. Giambalvo CT, Purification of endogeneous modulators of monoamine oxidase from plasma. *Biochem Pharmacol* **33**: 3929–3932, 1984.
16. Glover V, Reveley MA and Sandler M, A monoamine oxidase inhibitor in human urine. *Biochem Pharmacol* **29**: 467–470, 1980.
17. Petursson H, Reveley MA, Glover V and Sandler M, Urinary MAO inhibitor in psychiatric illness. *Psychiat Res* **5**: 355–340, 1981.
18. Glover V, Bhattacharya SK and Sandler M, Benzodiazepines reduce stress-augmented increase in rat urine monoamine oxidase inhibitor. *Nature (Lond)* **292**: 347–349, 1981.
19. Becker RE, Giambalvo CT, Fox RA and Macho M, Endogeneous inhibitors of monoamine oxidase present in human cerebrospinal fluid. *Science* **221**: 476–478, 1983.
20. Tong JH and D'Iorio A, Differential effects of *l*-thyroxine on cardiac and hepatic monoamine oxidase activity toward Benzylamine and serotonin. *Endocrinology* **98**: 761–766, 1976.
21. Ichikawa K, Hashizume K and Yamada T, Monoamine oxidase inhibitory modulators in rat heart cytosol. *Endocrinology* **111**: 1803–1809, 1982.
22. Egashira T and Yamanaka Y, Changes in MAO activities in several organs of rats after administration of *l*-thyroxine. *Jap J Pharmacol* **45**: 135–142, 1987.
23. Fowler CJ, Ekkstedt B, Egashira T, Kinemuchi H and Oreland L, The interaction between human platelet monoamine oxidase its monoamine substrates and oxygen. *Biochem Pharmacol* **28**: 3063–3068, 1979.
24. Lowry OH, Rosebrough, NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol regent. *J Biol Chem* **193**: 265–275, 1951.
25. Fawcett JS, Isoelectric fractionation of proteins on polyacrylamide gels. *FEBS Lett* **1**: 81–82, 1968.
26. Mayanil CSK and Baquer NZ, Comparison of the properties of semipurified mitochondrial and cytosolic monoamine oxidase from rat brain. *J Neurochem* **43**: 906–912, 1984.
27. Zile M and Lardy HA, Monoamine oxidase activity in

liver of thyroid-fed rats. *Arch Biochem Biophys* **82**: 411–421, 1959.
28. Utley HG, Effect of thyromimetic compounds on myocardial and hepatic monoamine oxidase activity in the rat. *Endocrinology* **75**: 975–977, 1964.
29. Proulx L, D'Iorio A and Begnak M, The metabolism of catecholamines in hyperthyroid and vitamin V deficient rats. *Can J Biochem* **44**: 1577–1585, 1966.