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Effects of Inhibitors on Monoamine Oxidase Activity in Several Organs of Croaker *Nibea mitsukurii* and Ayu *Plecoglossus altivelis*

Takeshi Nagai*, Moritsugu Hamada, Norihisa Kai, and Yasuhiro Tanoue

The effects of inhibitors on monoamine oxidase activity in several organs of croaker *Nibea mitsukurii* and ayu *Plecoglossus altivelis* have been tested. In most organs of the croaker, a similar pattern of inhibition was obtained by inhibitors such as deprenyl and clorgyline. In the stomach, a slightly different pattern of inhibition was obtained by these inhibitors. Moreover, in most organs of the ayu, a similar pattern was obtained by the two inhibitors. In particular, a simple-phase sigmoid curve was obtained in the heart and the stomach. On the other hand, in the liver, each inhibitor produced a different-phase sigmoid inhibition curve. These results suggested the possible existence of two types of monoamine oxidases in the croaker's stomach and ayu's liver.

1 Introduction

Monoamine oxidase (monoamine: oxygen oxidoreductase, deaminating; EC 1. 4. 3. 4.; MAO) is found in many different tissues and organs of vertebrates.[1] Its major roles are the metabolism of monoamine neurotransmitters, regulation of intraneuronal amine concentrations and detoxification of endogenous and exogenous amines.[2] MAO has been studied in mammals.[3-7] In particular, mammalian MAO exists in two isozyme forms, A and B, and these forms are characterized by different substrate specificities and inhibitor sensitivities. MAO-A deaminizes 5-hydroxytryptamine and epinephrine, and MAO-B deaminizes norepinephrine, 2-phenylethylamine, and benzylamine. Moreover, MAO-A is inhibited by clorgyline, amphetamine, and harmaline. On the other hand, MAO-B is inhibited by deprenyl and lilly 54781. Both forms of MAO deaminize tyramine, noradrenaline, dopamine, kynuramine, and tryptamine.

By the way, a few is known of MAO in non-mammalian vertebrates.[8-11] In our recent paper, we found that MAO-A and -B existed in some tissues of skipjack and the properties of MAO in the liver resembled to mammalian MAO-A and -B.[12-14]

The aim here is to report the effects of inhibitors on the MAO activity in several organs of croaker and ayu.

2 Materials and Methods

2.1 Materials

Croaker *Nibea mitsukurii* and ayu *Plecoglossus altivelis* were obtained from a fish market and

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Department of Food Science and Technology, National Fisheries University, Shimonoseki, Yamaguchi 759-6595, Japan.
(永井 毅・浜田盛承・甲斐徳久・田上保博：水産大学校食品化学科)
*To whom all correspondence should be addressed: T. Nagai, e-mail: machin@fish-u.ac.jp or machin@agr.kyushu-u.ac.jp

immediately transported to the laboratory. The organs were excised, and stored at -85℃ until use.

2.2 Preparation of Enzyme

Each organ was homogenized with 3 volumes of 50 mM sodium phosphate buffer (pH 8.0) containing 0.2 mM ethylenediaminetetraacetic acid (EDTA). The homogenate was centrifuged at 28,800×g for 30 min. The precipitate was dissolved in 3 volumes of 50 mM sodium phosphate buffer (pH 8.0) containing 1.0% (W/V) Triton X-100. After gentle stirring for 2 h, the solution was centrifuged at 225,000×g for 1 h and the supernatant was used as the source of the enzyme. Triton X-100 was removed from the supernatant by means of Bio-Beads SM-2 previously equilibrated in 10 mM sodium phosphate buffer (pH 8.0) containing 0.2 mM EDTA and 1 mM dithiothreitol (DTT). After the Bio-Beads were washed with the same buffer, the filtrate and washings were combined and this solution was used as an enzyme solution.

2.3 Enzyme Assay

MAO activity was measured by the method of Nagai *et al.*[12] Namely, MAO activity was measured spectrophotometrically by monitoring the increase in absorbance at 314 nm on the oxidation of kynuramine with the formation of 4-hydroxyquinoline.

2.4 Inhibition Experiment

Clorgyline or deprenyl was added to the assay mixture without substrate, and preincubated at 25℃ for 10 min; it was confirmed that 10 min of preincubation with 10^{-7} M clorgyline or 10^{-7} M deprenyl resulted in almost the same extent of inhibition as did 30 min of preincubation. Seven different concentrations of each inhibitor were employed over the range of 10^{-10} to 10^{-4} M. The inhibition was carried out at a substrate concentration of 200 μl.

3 Results and Discussion

When different concentrations of deprenyl and clorgyline have been tested in several organs of croaker and ayu, the results have been expressed as inhibition percentages against MAO activity of control sample, and plotted against the negative log of molar concentrations of inhibitor. In most organs of croaker, the similar pattern of inhibition was obtained with deprenyl and clorgyline (Fig. 1). With both inhibitors, a simple-phase curve was produced. MAO activity was inhibited approximately 20% by 10^{-9} M inhibitors, about 50% by 10^{-7} to 10^{-6} M inhibitors, and about 80% by 10^{-5} M inhibitors. There was no enzymatic activity at a concentration of 10^{-4} M inhibitors. In croaker stomach, the slightly different pattern of inhibition was obtained by deprenyl and clorgyline (Fig. 1E). On the other hand, in most organs of ayu, the similar pattern of inhibition was obtained by two inhibitors (Fig. 2). In particular, a simple-phase sigmoid curve was obtained in the heart and the stomach (Fig. 2E, 2F). MAO activity was inhibited about 20% by 10^{-9} M inhibitiors, about 50% by 10^{-7} to 10^{-6} M inhibitors, and more than about 80% by 10^{-5} M inhibitors. There were almost no activity in 10^{-4} M inhibitors. In the liver, each inhibitor produced a different-phase sigmoid inhibition curve. That is, it was suggested that ayu liver containes two MAO types, which appear to be similar to mammalian MAO types A and B. So far, there was no report that MAO-B is present in fish. But in our previous paper, we reported that MAO-A and -B existed in some tissues of skipjack and the properties of MAO in the liver resembled to

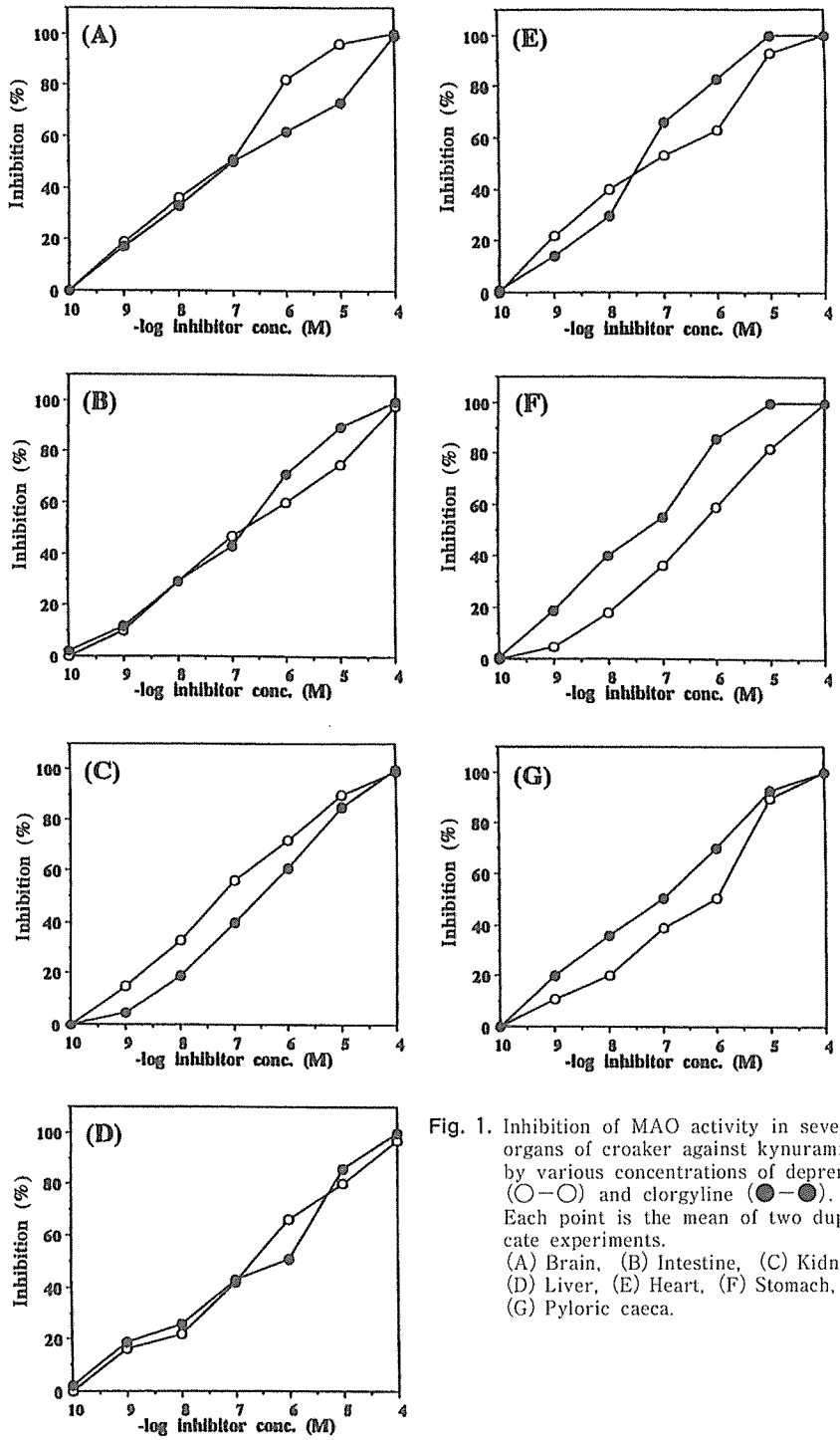

Fig. 1. Inhibition of MAO activity in several organs of croaker against kynuramine by various concentrations of deprenyl (○—○) and clorgyline (●—●). Each point is the mean of two duplicate experiments.
(A) Brain, (B) Intestine, (C) Kidney, (D) Liver, (E) Heart, (F) Stomach, (G) Pyloric caeca.

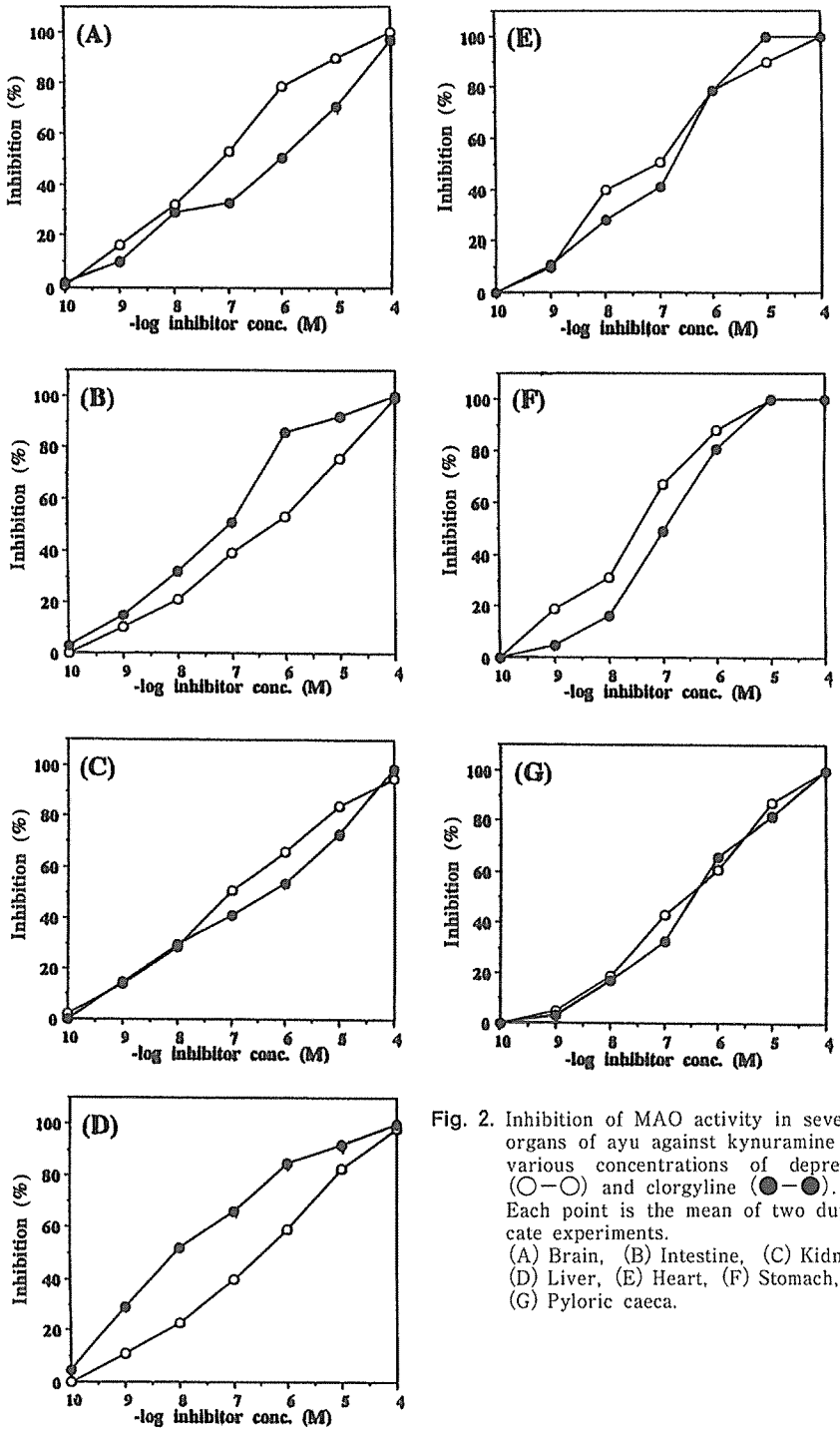

Fig. 2. Inhibition of MAO activity in several organs of ayu against kynuramine by various concentrations of deprenyl (○—○) and clorgyline (●—●). Each point is the mean of two duplicate experiments.
(A) Brain, (B) Intestine, (C) Kidney, (D) Liver, (E) Heart, (F) Stomach, (G) Pyloric caeca.

mammalian MAO-A and -B each other.[12-14] In the present investigation, the possibility of the existence of two types MAO was indicated in croaker stomach and ayu liver.

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ニベとアユ器官中のモノアミンオキシダーゼ活性に及ぼす阻害剤の影響

永井　毅・浜田盛承・甲斐徳久・田上保博

ニベ(*Nibea mitsukurii*)とアユ(*Plecoglossus altivelis*)の数種器官のモノアミンオキシダーゼ活性に対する阻害剤の影響について検討した。ニベとアユの多くの器官において、デプレニルとクロルジリンに対して同様の阻害剤感受性を示した。ニベ胃においてはこれらの阻害剤に対して互いに若干異なった感受性を示した。また、アユ肝臓では全く異なった感受性を示した。これらの結果から、ニベ胃とアユ肝臓では2種類のモノアミンオキシダーゼが存在する可能性のあることが示唆された。