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MONOAMINE OXIDASE ACTIVITIES IN CATFISH (*PARASILURUS ASOTUS*) TISSUES

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The substrate- and inhibitor-related characteristics of monoamine oxidase (MAO) were studied for catfish brain and liver. The kinetic constants for MAO in both tissues were determined using 5-hydroxytryptamine (5-HT), tyramine and β-phenylethylamine (PEA) as substrates. For both tissues, the V_{max} values were highest with 5-HT and lowest with PEA. The K_m value for the brain was highest with 5-HT, followed by tyramine and PEA; but for the liver its value was highest with PEA, followed by 5-HT and tyramine, although all values were in the same order of magnitude. The inhibition of MAO by clorgyline and deprenyl by use of 5-HT, tyramine and PEA as substrates showed that the MAO-A inhibitor clorgyline was more effective than the MAO-B inhibitor deprenyl for both catfish tissues; a single form was present since inhibition by clorgyline or deprenyl with 1000 μM PEA showed single phase sigmoid curves. It is concluded that catfish brain and liver contain a single form of MAO, relatively similar to mammalian MAO-A.

Keywords: Monoamine oxidase; 5-Hydroxytryptamine; β-Phenylethylamine; Tyramine; Clorgyline; Deprenyl; Catfish

INTRODUCTION

Monoamine oxidase (MAO; EC 1.4.3.4) is known to exist in tissue mitochondria of many species.[1,2] Its major roles are the metabolism of monoamine neurotransmitters, regulation of intraneuronal amine concentrations

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and detoxification of endogenous and exogenous amines.[3,4] It is known that MAO exists in mammalian tissues in two functional forms, MAO-A and MAO-B, which differ in their substrate specificity and inhibitor sensitivity. In mammals, MAO-A is active toward 5-hydroxytryptamine (5-HT) and norepinephrine as substrates, and is inhibited by a low concentration of clorgyline. MAO-B is active toward β-phenylethylamine (PEA) and benzylamine, and is inhibited by a low concentration of deprenyl. Tyramine and kynuramine are common substrates for both forms of the enzyme.[3,5]

For MAO of teleostean species, only a small number of reports have appeared; goldfish (*Carassius auratus*), perch (*Perca flavescens*), carp (*Cyprinus carpio*) and rainbow trout (*Salmo gairdneri*).[6–9] In most of these studies, only kynuramine was used as common substrate for MAO-A and MAO-B. However, inhibition curves with non-specific substrates, such as kynuramine and tyramine, are not sensitive enough to detect two forms of MAO, if the activity of one form accounts for less than 10% of total activity.[10] In the present study, we have carefully studied the substrate- and inhibitor-related characteristics of MAO in catfish brain and liver using 5-HT, tyramine and PEA as substrates. This is the first report dealing with MAO in the catfish.

MATERIALS AND METHODS

Chemicals

Homovanillic acid, horseradish peroxidase (type I), 5-HT creatine sulfate, tyramine-HCl, PEA-HCl and pargyline-HCl were obtained from the Sigma Chemical (St. Louis, MO, USA); tyramine-HCl from Nakarai Tesque (Kyoto, Japan); semicarbazide-HCl from Kanto Chemical (Tokyo, Japan); and 2′,7′-dichlorofluorescin diacetate from Eastman Kodak (Rochester, NY, USA).

Clorgyline, a selective inhibitor of type A MAO, was supplied by May & Baker Ltd. (Dagenham, England). Deprenyl, a selective inhibitor of type B MAO, was obtained from Research Biochemicals Inc. (Natick, MA, USA).

Enzyme Preparations

Both sexes of the siluroid catfish, *Parasilurus asotus*, were used. Fish with body lengths of about 25 cm were selected for the experiments. After decapitation, the brains and livers were rapidly removed and frozen at −80°C

until assayed. The tissues were thawed, weighed and homogenized in 0.01 M potassium phosphate buffer solution (pH 7.4). The crude homogenates of both tissues were used as an enzyme source.

MAO Assays

MAO activities toward tyramine and PEA were measured fluorometrically by the method of Matsumoto *et al.*[11] In this method, hydrogen peroxide formed in the MAO reaction is measured fluorometrically, by converting homovanillic acid to a highly fluorescent compound in the presence of peroxidase. The assay mixture consisted of 0.1 ml potassium phosphate buffer solution (final 0.083 M, pH 7.4), 0.1 ml horseradish peroxidase solution (0.5 mg/ml), 0.1 ml homovanillic acid solution (1.0 mg/ml), 0.1 ml of the homogenate, 0.05 ml of substrate solution, 0.05 ml semicarbazide (final 1 mM) and 0.1 ml distilled water, to give a total volume of 0.6 ml. The incubation mixture was preincubated for 10 min prior to addition of the substrate solution. After incubation at 37°C for 30 min, the activity was stopped by adding 2.0 ml of 0.1 M NaOH solution. The fluorescence intensity was measured with excitation at 323 nm and emission at 426 nm. As blank tests, assay mixtures without substrates were incubated; the substrates were mixed after adding the NaOH solution. Standards were prepared by adding the appropriate amounts of hydrogen peroxide to the mixtures prior to incubation. The fluorometric measurements were made on a Hitachi 650-10S fluorescence spectrophotometer.

MAO activities toward 5-HT were measured by a photometric assays.[12] In this method, hydrogen peroxide, formed in the MAO reaction, is measured by converting 2′,7′-dichlorofuorescin to 2′,7′-dichlorofluorescein in the presence of peroxidase. The assay mixture consisted of 0.5 ml potassium phosphate buffer solution (final 0.083 M, pH 7.4), 0.5 ml horseradish peroxidase solution (0.5 mg/ml), 0.5 ml of the homogenate, 0.5 ml 2′,7′dichlorofuorescin solution, 0.5 ml 5-HT solution and 0.5 ml distilled water, to give a total volume of 3.0 ml. The incubation mixture was preincubated for 10 min prior to addition of the substrate solution. After incubation at 37°C for 30 min, the activity was stopped by adding of 0.1 ml pargyline solution (2 mg/ml). The mixture was subjected to spectrophotometric analysis at 502 nm. Blank assays differed from controls only in the respect that the mixture was incubated in the presence of pargyline. Standards were prepared by adding appropriate amounts of hydrogen peroxide to the mixtures prior to incubation. The photometric measurements were made on a Shimadzu double beam spectrophotometer UV-200S.

Inhibition Studies

Clorgyline and deprenyl were dissolved in distilled water, added to the assay mixture without substrate, and preincubated at 37°C for 10 min to ensure reproducibility of enzyme inhibition. Seven different concentrations for each inhibitor were employed over the range of 10^{-10}–10^{-4} M. It was confirmed that each inhibitor did not interfere with the fluorometry or the photometry.

RESULTS

Apparent Kinetic Constants for MAO

The apparent Michaelis–Menten kinetics constants for MAO in catfish brain and liver were determined from Lineweaver–Burk plots by use of 5-HT, tyramine and PEA as substrates. The results are summarized in Table I. For all tissues, the V_{max} values were highest with 5-HT and lowest with PEA. With tyramine and PEA, the V_{max} values for the liver were higher than that for the brain. The K_m value for the brain were highest with 5-HT, followed by tyramine and PEA; the K_m value for the liver were highest with PEA, followed by 5-HT and tyramine. However, all K_m values were of the same order of magnitude.

Inhibition of MAO

Figures 1 and 2 show the inhibition of MAO in catfish brain and liver by clorgyline and deprenyl using two different concentrations (20 and 1000 μM) of 5-HT, tyramine and PEA as substrates. MAO activities for both tissues were inhibited by clorgyline and deprenyl in a concentration dependent fashion producing single phase sigmoid curves. The inhibition by clorgyline was higher than that by deprenyl for all substrates and for both brain and liver.

TABLE I Apparent kinetic constants for MAO in brain and liver of the catfish

Tissue	*5-HT*		*Tyramine*		*PEA*	
	K_m (μM)	V_{max} (μmol/g wet tissue/ 30 min)	K_m (μM)	V_{max} (μmol/g wet tissue/ 30 min)	K_m (μM)	V_{max} (μmol/g wet tissue/ 30 min)
Brain	473	231	203	2.95	195	1.12
Liver	235	95.3	160	43.9	239	11.8

Each kinetic constant was determined from Lineweaver–Burk plots, using seven substrate concentrations and assayed in duplicate, with a single enzyme source (crude homogenate) prepared from the pooled tissues of more than five catfish. The assay methods are described in the text.

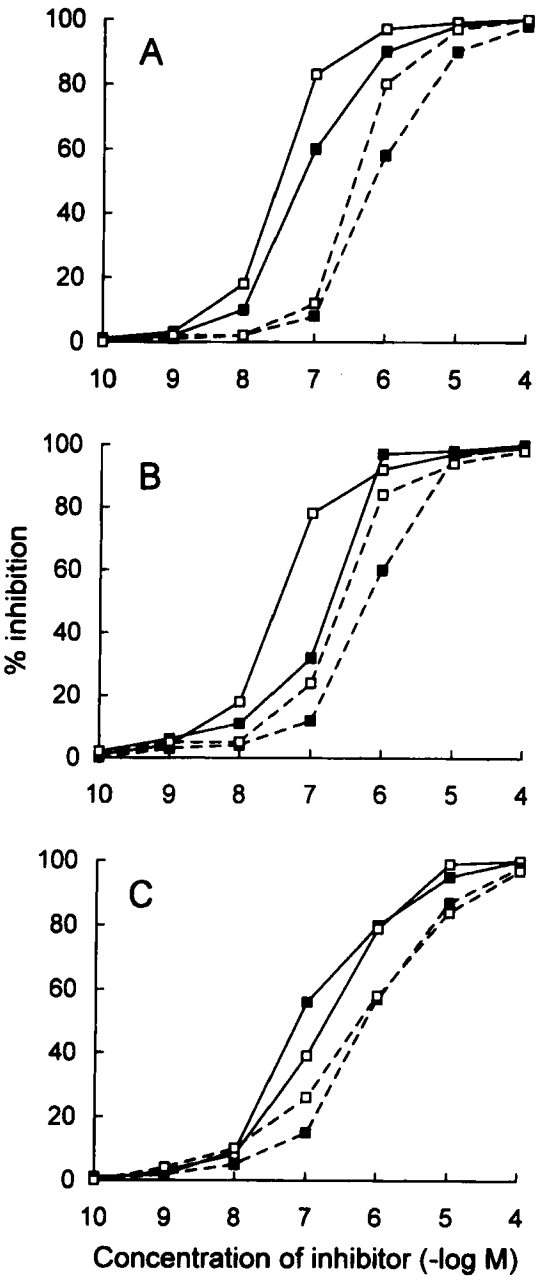

FIGURE 1 Inhibition of MAO in catfish brain by clorgyline (—) and deprenyl (----) using two concentrations of 5-HT (A), tyramine (B) and PEA (C) as substrates. The concentrations of the substrate were 20 μM (□) and 1000 μM (■). Each point is the mean obtained from duplicate determinations.

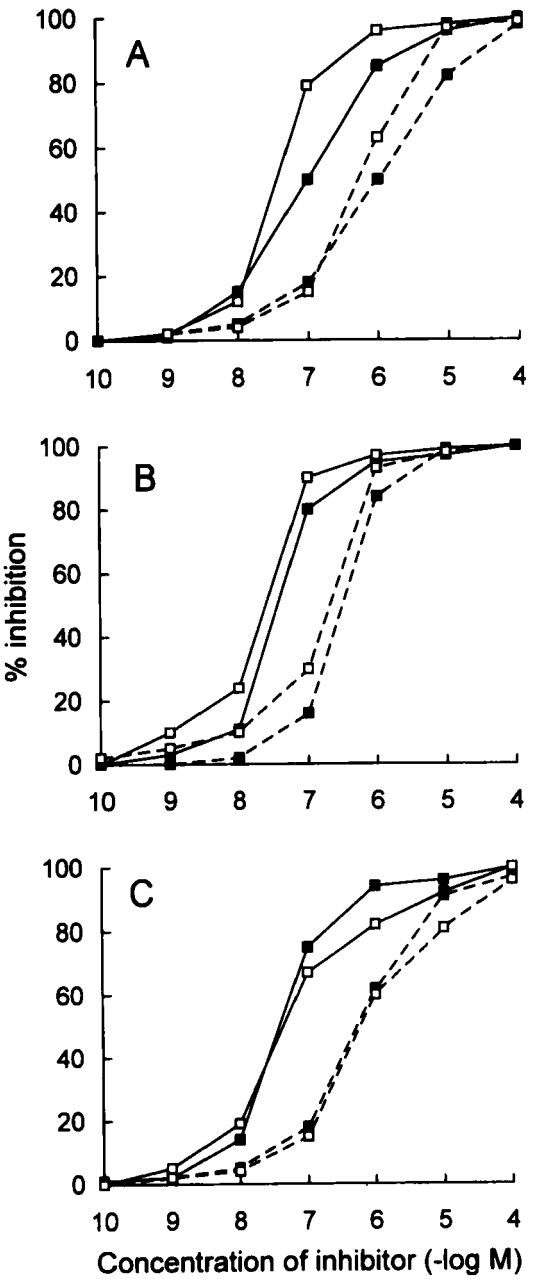

FIGURE 2 Inhibition of MAO in catfish liver by clorgyline (—) and deprenyl (----) using two concentrations of 5-HT (A), tyramine (B) and PEA (C) as substrates. The concentrations of the substrate were 20 μM (□) and 1000 μM (■). Each point is the mean obtained from duplicate determinations.

DISCUSSION

In this study catfish brain and liver MAO provide single sigmoid inhibition curves for both clorgyline and deprenyl (Figures 1 and 2), in contrast to the biphasic inhibition curves usually observed for MAO of mammalian, avian and amphibian tissues.[13-15] The enzyme from catfish brain and liver was more sensitive to clorgyline than to deprenyl, showing that MAO in catfish brain and liver is closely related to MAO-A. The MAO-A like characteristic of the catfish enzyme is supported by the result that the V_{max} value for 5-HT is much higher than that for PEA for both tissues (Table I).

We have used 20 and 1000 μM of each substrate, in the inhibition studies since the specificity of a substrate sometimes depends on its concentration; substrate concentrations more than 1000 μM give less specificity.[16] Therefore, if a small amount of MAO-B was contained in the catfish enzyme preparations, the inhibition curves with 1000 μM PEA should have appeared as biphasic ones by this method, a minor part (less than 10%) of one form can be amplified for its identification.[10] The monophasic inhibition curves observed for PEA (Panels C for both figures) show that MAO in catfish brain and liver is of a single form, which is relatively similar to type A MAO. Our data seems similar to those for MAO in the goldfish (*Carassius auratus*), perch (*Perca flavescens*) and rainbow trout (*Salmo gairdneri*) though only kynuramine was used as substrate,[6,7,9] but seems different from those for MAO in the carp,[8] which showed no difference in the degree of inhibition between clorgyline and deprenyl.

The distribution of adrenergic and serotonergic neurons has been investigated in teleosts.[17,18] Thus, the MAO-A like enzyme considered in this study seems to serve for the regulation of amine levels in catfish. In this connection, the levels of 5-HT and norepinephrine, MAO-A substrates, in the brains of some fish were reported to be of the order of 100 ng and 1000 ng per gram wet weight, respectively, although no data are available for catfish.[19,20] The concentration of PEA, a MAO-B substrate, in catfish brain was only 8.5 ng/g wet weight.[21]

In a previous study, we reported the presence of a semicarbazide-sensitive amine oxidase (SSAO) activity in catfish tissues;[22] the enzyme being active toward PEA, tryptamine, benzylamine and tyramine in catfish intestine. Therefore, we added 1 mM semicarbazide to each assay mixture to suppress the activity of this component; at this concentration, the inhibitor inhibits SSAO almost completely, but not MAO (Figures 1 and 2).

Human liver MAO-A and MAO-B cDNAs were isolated by Shih's group.[23] The deduced amino acid sequences showed that the A and B forms

had subunit molecular weights of 59,700 and 58,800, respectively, and shared 70% sequence homology, although functional domains for both forms were not identified. These two forms are derived from separate genes on the X chromosomes.[24] Recently, trout liver MAO has also been cloned by screening a cDNA library with human MAO-A cDNA probe.[25] The trout MAO cDNA gave 499 amino acid with a molecular weights of 56,600. The deduced amino acid sequence of trout MAO shows 70% and 71% identity with those of human MAO-A and MAO-B, respectively. Trout MAO expressed in transfected COS cells was less sensitive to inhibition by clorgyline than was human MAO-A, and less sensitive by deprenyl than was human MAO-B. Therefore, it has been suggested that the trout enzyme is a novel type of MAO.

In conclusion, this study has shown that catfish brain and liver contain a single form of MAO, relatively similar to mammalian MAO-A, with use of conventional substrate- and inhibitor-related experiments. A study on cloning of the catfish MAO is now in progress in our laboratory.

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