Evidence for Existence of A and B Form Monoamine Oxidase in Mitochondria from Dog Platelets

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Abstract—It is known that platelet MAO appears to behave more like the B-form enzyme than the A-form enzyme based on inhibitor sensitivity and substrate specificity. However, dog platelets showed a different substrate specificity such as high activity with 5-HT and β -PEA as substrates. Moreover, dog platelet MAO was sensitive to the drugs clorgyline and harmaline with 5-HT as the substrate, while it was sensitive to the drug deprenyl with β -PEA as the substrate. These results also indicate the existence of two forms of MAO in dog platelets unlike in other platelets such as those from humans. A-form MAO from dog platelets was more stable against heat treatment at 55°C than A-form MAO from dog liver and brain. On the other hand, there was no difference in the heat resistance of the three enzymes with β -PEA as the substrate. After digestion with trypsin at 37°C for 30 min, it was found that MAO from dog platelets, brain and liver were mostly inhibited with 5-HT as the substrate. In contrast, MAO in brain and liver were inhibited about 10%, but platelet MAO was inhibited about 50% with β -PEA as the substrate. From these results, it is considered that dog platelet MAO exists as the two forms of the enzyme and has different enzymic properties in comparison with those of MAO from dog liver and brain mitochondria.

Monoamine oxidase (MAO) occurs in many tissues (1-3) including heart, liver, kidney, spleen, platelets and the peripheral and central nervous system (4). MAO oxidatively deaminates biogenic amines such as norepinephrine, dopamine and 5-HT (4) and may play an important role in the regulation of the intracellular metabolism of these neurotransmitters. Recently, alterations in activity of serotonergic and catecholaminergic neurons have been suggested in affective disorder and schizophrenia (5-7). In addition, alterations in platelet MAO activity in affective disorder and schizophrenia were described (8, 9). However, there have been few studies on the possible physiological relationship between platelet MAO activity and any psychiatric diagnosis. So, it is considered necessary to investigate the enzymic properties of platelet MAO. Recently, it has been reported that human platelet MAO is a single enzyme and appears to behave

more like the B-form enzyme than the A-form enzyme (10, 11). None of these investigations, however, provided any information about the existence of multiple forms of MAO in dog platelets. In this present study, to determine whether or not dog platelet MAO exists as the two forms of the enzyme, the substrate specificities and sensitivities to selective MAO inhibitors or trypsin and heat treatment were studied.

Materials and Methods

1. Preparation of platelets: Blood samples were obtained from a dog. After 3.8% sodium citrate was added to the sample, the blood was centrifuged at 1100 r.p.m. for 10 min to remove the red cells, and then the supernatant was centrifuged at 3000 g for 30 min. The pellet was suspended in 10 volumes of 0.01 M potassium phosphate buffer (pH 7.4). This suspension was recentrifuged at 20000 g for 30 min, and the pellet was resuspended in

3 volume of 0.01 M potassium phosphate buffer (pH 7.4) and used as the enzyme preparation. Dog brain and liver mitochondria were prepared as described earlier by differential centrifugation (12). Protein contents in the enzyme preparations were assayed by the method of Lowry et al. (13) with bovine serum albumin as the standard.

2. Monoamine oxidase assay: MAO activity was assayed radiochemically (14), as described previously, with [14 C]-tyramine, [14 C]-5-HT or [14 C]- β -phenylethylamine (β -PEA) after dilution with the respective unlabelled amines as substrates. The standard assay mixture contained 20 μ l of mitochondrial suspension, 20 μ l of 0.1 M phosphate buffer, pH 7.4, and 140 μ l distilled water. The reaction was started by adding 20 μ l of substrate solution. The incubation was carried out at 37°C for 80 min, and then 200 μ l of 2 N HCl was added to stop the reaction. The final substrate concentrations used were approx. 100 μ M tyramine, 100 μ M 5-HT and 10 μ M β -PEA. Metabolites formed in this enzyme reaction were extracted with an ethyl acetate-benzene mixture (1:1 V/V) saturated with water, and radioactivity in the extract was measured in a Beckman LS-9000 scintillation spectrometer. In inhibition studies, enzyme preparations were pre-incubated at 37°C for 1 hr with clorgyline and deprenyl or for 10 min with harmaline to inhibit either A-form MAO or B-form MAO before addition of the substrate. Then, the remaining MAO activity was determined. Specific activity was expressed as nmoles/min/mg protein.

3. Thermal inactivation of dog platelet MAO: Blood platelet suspensions were pre-incubated at 55°C for various incubation times, and then quickly chilled, and residual MAO activity was determined with tyramine, 5-HT or β -PEA as the substrate. The values were expressed as percent of the control.

4. Sensitivity to trypsin digestion of dog platelet MAO: Dog platelets were pre-incubated in the presence of trypsin (0.05 mg/mg protein) at 37°C, pH 7.4, for various time intervals prior to the determination of MAO activity. MAO activities in aliquots of the control (untreated) and trypsin treated mitochondria were determined at 37°C, pH

7.4, with tyramine, 5-HT and β -PEA as substrates, respectively. The values were expressed as percent of the control.

5. Chemicals: 5[2- 14 C]-Hydroxytryptamine binoxalate (51.5 mCi/mmol), [1- 14 C]-tyramine hydrochloride (55.0 mCi/mmol) and β -[1- 14 C]-phenylethylamine hydrochloride (50.2 mCi/mmol) were purchased from New England Nuclear, Boston, MA, U.S.A. Clorgyline was a gift from May & Baker, Ltd., Dagenham, Essex, England. Deprenyl was a gift from Dr. J. Knoll, Dept. of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary. 5-Hydroxytryptamine creatinine sulphate, tyramine hydrochloride, harmaline hydrochloride and trypsin were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. β -Phenylethylamine hydrochloride was obtained from Tokyo Chemical Industries, Co., Ltd., Japan.

Results

1. Substrate specificity of dog brain, liver and platelet mitochondrial MAO: Comparisons of the substrate specificities of dog brain, liver and platelet mitochondrial MAO with tyramine, 5-HT and β -PEA as substrates are shown in Table 1. When brain and liver mitochondria from dogs were used as the enzyme preparation, the highest MAO activity was obtained with β -PEA as the substrate, followed by tyramine and 5-HT; in platelet mitochondria, the highest MAO activity was obtained with 5-HT as a substrate.

2. Inhibition of MAO activity in dog platelet mitochondria by clorgyline and deprenyl: Inhibition of MAO in mitochondria from dog platelets by clorgyline as the A-form MAO inhibitor with 5-HT as the A-form MAO substrate and inhibition by deprenyl as the B-form MAO inhibitor with β -PEA as the B-form MAO substrate are shown in Fig. 1. After incubation at 37°C for 60 min with clorgyline, MAO activity with 5-HT as the substrate was highly sensitive, and the activity with β -PEA was less sensitive (Fig. 1, upper). When corresponding experiments were performed with deprenyl, the opposite results were obtained (Fig. 1, lower).

3. Thermal inactivation of dog platelet MAO: When dog platelets were treated at

Table 1. Substrate specificities of dog liver, brain and platelet mitochondrial MAO

Substrate	MAO activity $\times 10^{-2}$ nmoles/min/mg protein		
	liver	brain	platelet
Tyramine	6.32	4.30	0.10
5-HT	1.08	3.49	0.14
β -PEA	46.46	15.29	0.09

MAO activity was determined by radiometric assay. Experimental conditions were described in the Methods. Substrate concentrations used were 100 μ M tyramine, 100 μ M 5-HT and 10 μ M β -PEA as a final concentration. MAO activities were expressed as nmoles/min/mg protein.

Fig. 1. Inhibition of MAO activity in dog platelet mitochondria toward 5-HT and β -PEA by deprenyl and clorgyline. The mitochondrial preparation was preincubated with clorgyline (upper) and deprenyl (bottom) at 37°C for 1 hr in a total volume of 200 μ l of phosphate buffer. MAO activity was assayed radiometrically by addition of substrate 5-HT ($\circ$ — $\circ$) and β -PEA ($\bullet$ — $\bullet$) at 37°C for 30 min.

Fig. 2. Effects of heat treatment on the activity of mitochondrial MAO in dog platelets, brain and liver toward 5-HT and β -PEA. Each of the mitochondrial preparations, platelet ($\bullet$ — $\bullet$), brain ($\square$ — $\square$) and liver ($\circ$ — $\circ$), were incubated at 55°C for 5 to 30 min. The remaining activity is expressed as % of the control MAO activity with 5-HT (upper) and β -PEA (bottom) as substrate.

55°C, progressive inhibition of activity was observed, and inhibition was 45% at 30 min with 5-HT as the substrate. On the other hand, in the case of dog brain and liver, 85% and 95% inhibition were observed at 30 min, respectively. When β -PEA was used as a substrate, the dog platelet MAO was heat labile in comparison to when 5-HT was used as the substrate (Fig. 2).

4. Sensitivity to trypsin digestion of dog platelet MAO: Dog platelet, liver and brain mitochondria were preincubated in the

presence of trypsin for various time intervals prior to determination of MAO activity. After the treatment by trypsin for 30 min, MAO activities in dog brain, liver and platelet were inhibited 85, 80%, 90% with 5-HT as the substrate, respectively (Fig. 3, upper). On the other hand, in the case of β -PEA as the substrate, it was found that MAO activities in the dog brain and liver were lost by only 25%, but MAO activity in dog platelets remained 35% at 4 hr (Fig. 3, lower).

Fig. 3. Sensitivity to trypsin digestion of mitochondrial MAO in dog platelets, brain and liver toward 5-HT and β -PEA. Each of the mitochondrial preparations, platelet (●—●), brain (□—□) and liver (○—○), were preincubated in the presence of trypsin (0.05 mg/mg protein) at 37°C, pH 7.4, for the various time intervals indicated prior to the determination of MAO activity. At the time indicated, MAO activities in aliquots of the control (untreated) and trypsin treated mitochondria were determined by radiochemically by addition of 5-HT (upper) and β -PEA (bottom) at 37°C for 30 min. The results are expressed as % of the control MAO activity.

5. Kinetics of inhibition of dog platelet MAO by clorgyline: The inhibitions by clorgyline of dog platelet MAO with tyramine, 5-HT and β -PEA as substrates are shown by Lineweaver-Burk plots (Fig. 4). The inhibition was noncompetitive, and the K_m value for 5-HT was 145 μ M. As shown in Fig. 5, the inhibitions by clorgyline of dog brain and liver MAO were also noncompetitive. These K_m values for 5-HT were 80 μ M and 145 μ M, respectively.

Discussion

MAO is classified into two forms, A-form and B-form MAO (15–17). It has recently been reported that MAO may exist in multiple forms depending on tissue and species (18, 19). Rat organs such as spleen and testis

Fig. 4. Lineweaver-Burk plots of dog platelet mitochondrial MAO toward 5-HT. Lineweaver-Burk plots of the reciprocal of the initial velocity of 5-HT oxidation against the reciprocal of the 5-HT concentration in the absence (○—○) and presence (●—●) of 1×10^{-8} M clorgyline.

(20), and the neuroblastoma and glioma of mouse (21, 22) and human placenta (12, 23) contain predominantly a single species of the A-form MAO. In contrast, B-form MAO activity is found in the mouse and rabbit liver or human platelets (11, 24). In general, it is known that the various platelet MAO's appear to behave more like the B-form enzyme than the A-form enzyme based on inhibitor sensitivity and substrate specificity (11, 24). However, dog platelet MAO showed a different substrate specificity, that is, 5-HT was also deaminated highly. Moreover, dog platelet MAO was sensitive to the drugs clorgyline and harmaline (preferentially A-form MAO inhibitors) and was resistant to the drug deprenyl (preferentially a B-form MAO inhibitor) with 5-HT as the substrate. When corresponding experiments were performed with β -PEA (substrates for B-form MAO), the opposite results were obtained. These results also indicate the existence of two forms of MAO in dog platelets unlike in other platelets such as those from humans.

In general, A-form MAO is readily digested by trypsin and is heat stable as compared to the B-form MAO (12, 25, 26). To determine whether or not A-form MAO in dog platelets also show similar properties to A-form MAO in brain and liver, the thermostability and trypsin digestion of dog platelet MAO were compared with those of MAO from dog liver and brain. A-form MAO from platelets was more stable against 55°C incubation than

Fig. 5. Lineweaver-Burk plots of dog brain (upper) and liver (bottom) mitochondrial MAO toward 5-HT. The method of plotting is the same as that of Fig. 4.

A-form MAO from liver and brain. On the other hand, there were no differences in the abilities of the three enzymes to resist heat with β -PEA as the substrate. After treatment with trypsin at 37°C for 30 min, it was found that MAO in platelets, brain and liver were mostly inhibited with 5-HT as the substrate. In contrast, MAO in brain and liver were inhibited by about 10%, but MAO in platelets was inhibited by about 50% with β -PEA as the substrate. From these results, A-form MAO in platelets also was heat stable and sensitive to trypsin digestion, like the A-form in other organs. These findings are in line with the results of Houslay and Tipton (27) and Ekstedt and Orelund (26) that A-form MAO is more dependent on phospholipids for its activity and that the thermostability and resistance to trypsin digestion of the enzyme are influenced by the chemical nature of the environment surrounding the enzyme protein.

Recent studies using platelet MAO activity as a possible index of central nervous

system events have found altered activity of platelet MAO in depressed and schizophrenic patients (28). In addition, Murphy and Wyatt (29) reported reduced platelet MAO activity in chronic schizophrenic individuals (30, 31). Human platelets contains solely B-form MAO (11). However, B-form MAO can not deaminate 5-HT, although 5-HT is present at high concentration in platelets. So, in the case of human platelets, we must consider the possibilities that 5-HT is metabolized by other enzymic processes or is deaminated by a clorgyline/deprenyl insensitive form of MAO (11). At present, however, dog platelet MAO deaminated 5-HT and β -PEA, and it is sensitive to low concentrations of clorgyline and deprenyl, that is, contain also A-form and B-form MAO. We do not have any information which provides an explanation of this difference.

It seems likely that dog platelet MAO is different from other species and that it has different physiological properties. Further in vivo studies using MAO inhibitors are under

way in our laboratory to elucidate the physiological function of MAO in platelets.

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