Biochemical Pharmacology, Vol. 40, No. 10, pp. 2215–2218, 1990.
Printed in Great Britain.

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

ENHANCEMENT OF RAT BRAIN CYTOSOLIC MONOAMINE OXIDASE ACTIVITY BY CLORGYLINE

COMPARISON WITH (−)-DEPRENYL AND MDL 72145

MOHD. AZAM, SADHANA JAIN and NAJMA Z. BAQUER*
Hormone and Drug Research Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India

(*Received* 30 *June* 1989; *accepted* 14 *March* 1990)

Abstract—The presence of unsedimentable forms of monoamine oxidase (EC 1.4.3.4) in liver and brain homogenates has prompted fresh studies on the effects of inhibitors on this cytosolic monoamine oxidase. Clorgyline is a specific monoamine oxidase A inhibitor and (−)-deprenyl and MDL 72145 are specific monoamine oxidase B inhibitors. We investigated the effects of (−)-deprenyl, MDL 72145 and clorgyline on the purified enzyme from mitochondria and cytosolic monoamine oxidase along with high speed cytosol and 1% Triton X-100 treated mitochondrial preparations. Clorgyline enhanced the activity of the purified enzyme several-fold. (−)-Deprenyl and MDL 72145 also enhanced and inhibited the activity of cytosolic monoamine oxidase in a concentration-dependent manner.

Monoamine oxidase (MAO) [monoamine:oxygen oxidoreductase (deaminating) (flavin containing) EC 1.4.3.4] has been studied widely and well characterized in different tissues and in several species and recently was the subject of extensive reviews [1–3]. Previously, MAO was considered to be a marker enzyme of outer mitochondrial membrane. A new form of this enzyme which is present in the cytosolic fraction [4, 5] has also been reported. This new form of enzyme is different from the mitochondrial forms, namely MAO A and MAO B [6].

The continuing theme of research is to develop specific MAO inhibitors directed towards one particular enzyme form in the brain and to study their use in achieving specific pharmacological effects; these include the alleviation of depression and the treatment of Parkinson's disease, without causing the "cheese effect." We are still very limited in our knowledge about the physiological function and regulation of cytosolic MAO. In the present study, the effects of clorgyline, (−)-deprenyl and MDL 72145 on the MAO activity in high speed cytosol and purified cytosolic MAO are reported. A comparison has also been made of the effects of these inhibitors on high speed cytosol and purified cytosolic MAO, with crude mitochondrial detergent extract and purified mitochondrial MAO.

MATERIALS AND METHODS

Subcellular fractionation and purification. Rat brain homogenate (1:10, w/v) from 200–300 g male Wistar strain rats was prepared as described earlier [4], and high speed cytosol was collected at 105,000 *g* centrifugation for 1 hr [7]. The high speed cytosol was used as the starting material for the purification of cytosolic MAO, and the enzyme was purified to homogenity [8]. Mitochondria were isolated by sucrose density gradient centrifugation [9], and the mitochondrial enzyme was purified to homogenity [8]. The crude high speed cytosol enzyme preparation (0.45 units/mg protein) and the purified cytosolic MAO (2000 units/mg protein) were used for MAO assay. The mitochondrial crude enzyme preparation had a specific activity of 0.56 units/mg protein and the purified mitochondrial MAO 3471 units/mg protein.

Protein assay. Protein was estimated by the method of Bradford [10] using bovine serum albumin as standard.

Monoamine oxidase assay. MAO activity was determined by the method of Tipton and Spires [11] with either 5-hydroxytryptamine (200 μM) or β-phenylethylamine (30 μM). At such concentrations, these amines have been shown to behave as essential specific substrates for the A and B forms of MAO respectively. The final reaction volume was 3.1 mL containing 0.2 mL each of semicarbazide (0.1 M) and potassium cyanide (0.01 M). Enzyme activity was expressed as microgram atoms of oxygen consumed per minute per milligram of protein.

Inhibition and enhancement curves with clorgyline, (−)-deprenyl, and MDL 72145 were carried out by incubating samples of enzyme at different inhibitor concentrations for 30 min at 25° for concentration–response curves and for different time intervals for the inhibition–enhancement time-dependent curves.

Materials. 5-Hydroxytryptamine-creatinine sulfate, β-phenylethylamine hydrochloride and catalase were purchased from Sigma (St. Louis, MO, U.S.A.). All other chemicals were standard analytical grade laboratory reagents. MDL 72145 [(E)-2-2-(3′,4′-dimethoxyphenyl)-3-flurallylamine A-08] was a gift from the Merrell Dow Research Institute, Strasbourg, France. (−)-Deprenyl (phenylisopropylmethyl propionylamine hydrochloride E-250) was

* To whom correspondence should be addressed.

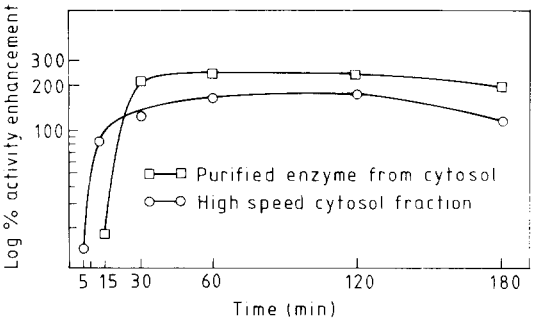

Fig. 1. Time-dependent enhancement of cytosolic monoamine oxidase activity by clorgyline (10^{-5} M) using 5-hydroxytryptamine as substrate (200 μM). Enzyme activity was measured as described [11]. Clorgyline + enzyme preparation were incubated and assayed at the specified time intervals. Protein concentration was 25 μg/mL, and specific activity was 0.45 units/mg; the reaction mixture was incubated at 25°. Purification of the enzyme for high speed cytosol was performed up to homogeneity [8], and 0.12 μg of the purified protein (sp. act. 2000 units/mg) was used for the assay. The units of enzyme activity = μg atoms of oxygen consumed/min/mg protein. Each value is the mean of at least four separate determinations.

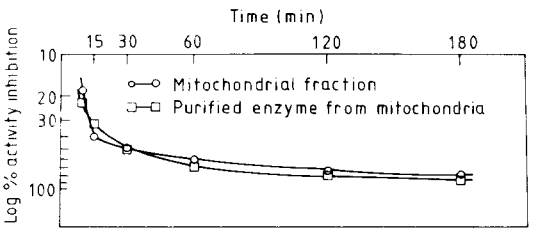

Fig. 2. Time-dependent inhibition of mitochondrial monoamine oxidase activity by clorgyline (10^{-5} M) using 5-hydroxytryptamine as substrate (200 μM). Enzyme activity was measured as described in the legend of Fig. 1. Mitochondria were lysed by Triton X-100 at 4° for 1 hr and then centrifuged at 40,000 g, and the supernatant fraction was used for the assay. Protein concentration was 1 mg/mL and specific activity 0.56 units/mg. Incubation was carried out at 25°. Purification of monoamine oxidase from mitochondria was performed up to homogeneity [8], and 10 μg of the purified protein (sp. act. 3400 units/mg) was used for the assay. The units of enzyme activity = μg atoms of oxygen consumed/min/mg protein. Each value is the mean of at least four separate determinations.

a gift from Dr. J. Knoll, Sammelweis University of Medicine, Budapest, Hungary. Clorgyline [(*N*-methyl *N*-propargyl-3)2,4-dichlorophenoxypropylamine hydrochloride M + B 9302] in two lots was provided by Dr. R. A. Robinson of May & Baker, Dagenham, U.K.

RESULTS

Effect of incubation time with inhibitors. The activity of purified cytosolic MAO followed a time-dependent course (Fig. 1). The activity was enhanced rapidly to 220% of the initial activity after the first 30 min when incubated with clorgyline. Similarly, the activity of the high speed cytosol fraction was enhanced by 125% of the initial activity and reached a plateau at 120 min. The activity of the purified MAO from mitochondria showed an inhibition of up to 50% of the initial activity in the first 30 min with clorgyline (Fig. 2). It declined further at 120 min. The activity in 1% Triton X-100 treated mitochondrial extract was also measured. There was an inhibition of up to 50% in the first 30 min following the same pattern as the purified enzyme. It reached a plateau after 120 min. These results agree with earlier published observations [12–14].

The inhibition and enhancement of high speed cytosol as well as purified cytosolic MAO by MDL 72145 (10^{-5} M) towards β-phenylethylamine (30 μM) was also time dependent (data not shown). After 1 hr of incubation, there was an inhibition of 76% of the initial enzyme activity in the case of the high speed cytosol, whereas (−)-deprenyl (10^{-5} M) enhanced the enzyme activity to 150% in comparison to the initial enzyme activity after 15 min. On the other hand, purified cytosolic MAO was not inhibited by MDL 72145 in the first 5 min, but after 10 min there was enhancement up to 33%. This

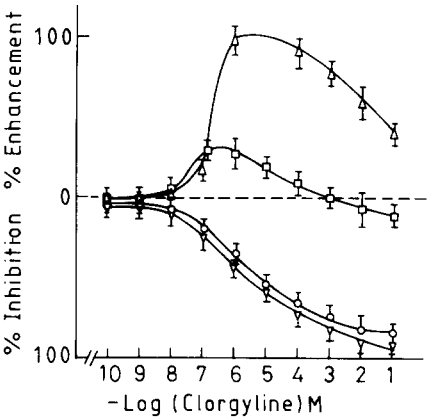

Fig. 3. Percent inhibition and enhancement of monoamine oxidase activity in high speed cytosolic (□), purified cytosolic (△), 1% Triton X-100 treated mitochondrial (○) and purified mitochondrial (▽) preparations of rat brain vs the clorgyline concentrations with 5-hydroxytryptamine (200 μM) as substrate. Enzyme preparations were pre-incubated for 30 min at 25° with different inhibitor concentrations (10^{-1}–10^{-10} M). The resultant activity is expressed as percent of the control activity determined in the absence of inhibitor. Absolute values for the control enzyme activity (μg atoms of oxygen consumed/min/mg protein): high speed cytosol, 8.6; purified cytosolic, 23.2; Triton X-100 treated, 13.7; and purified mitochondrial, 34.9. Each value is the mean ± SE of four determinations.

difference in the behaviour of inhibitors with the purified cytosolic MAO in comparison to the high speed cytosolic fraction may be attributed to the modification of protein during purification.

Sensitivity to inhibitors. The enhancing and inhibitory effects of different concentrations of clorgyline, deprenyl and MDL 72145 on purified cytosolic as well as high speed cytosolic fractions towards both 5-hydroxytryptamine and β-phenylethylamine as substrates were measured. The results obtained are shown in Figs 3 and 4. The pattern followed was the

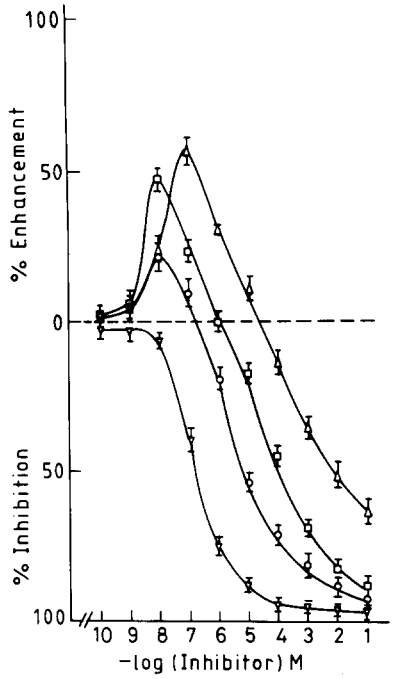

Fig. 4. Percent inhibition and enhancement of monoamine oxidase activity in high speed cytosolic [□] and purified cytosolic (△) preparations vs deprenyl with β-phenylethylamine (30 μM) as substrate and in high speed cytosolic (▽) and purified cytosolic (○) preparations vs MDL 72145 with β-phenylethylamine (20 μM) as substrate. Details of the experiment are the same as in the legend of Fig. 3. Each value is the mean ± SE of four determinations.

same as with the other inhibitors, i.e. a decrease of mitochondrial MAO and an enhancement in the soluble MAO activity.

DISCUSSION

An enhancement in the activity of cytosolic MAO by clorgyline at a fixed substrate concentration could be due to a low K_m value towards the substrate, an increased molecular turnover number (moles of substrate deaminated/mole MAO/min) of the enzyme towards the substrate, an increased concentration of available enzyme active centres or a combination of these effects [15]. The enhancement by clorgyline of MAO activity has been noted previously [16] where low concentrations of clorgyline were found to enhance the activity of MAO B, but that author used a higher concentration of the inhibitor. This phenomenon of enhancement is rather intriguing because MAO A is most likely a neuronal enzyme in brain, whereas MAO B is extraneuronal [7, 17, 18] and is known to play a minor role in the deamination of the biogenic amines in rat brain [19, 20]. Moreover, (−)-deprenyl, like clorgyline, is an acetylene containing inhibitor and attaches to the N5 of the flavin cofactor [21]; in the case of MDL 72145, the covalent adduct has not been identified yet [22, 23]. In the present paper though there was always an enhancement in the activity of cytosolic MAO by clorgyline, the extent of enhancement was different in the concentration–response curves and the time–course data. The basis for the observed differences may be related to the differences in the batches of clorgyline and purified proteins. However, it can be argued on the basis of the present study that MDL 72145 is a more effective inhibitor than (−)-deprenyl.

The ineffectiveness of clorgyline and (−)-deprenyl in inhibiting the cytosolic MAO may be due to the absence of drug receptors on the cytosolic MAO or to the masking of the inhibitor site environment by lipids affecting the interaction of the inhibitor with the enzyme [16]. Differences in the immunochemical crossreactivity [8] give support to the idea that cytosolic MAO is probably different in structure and function from mitochondrial MAO. The modes of action of clorgyline, MDL 72145 and (−)-deprenyl on cytosolic MAO need further study. The search for an effective inhibitor for soluble MAO may be a worthwhile area of research in looking for an effective antidepressant drug.

REFERENCES

1. Tipton KF, Enzymology of monoamine oxidase. *Cell Biochem Funct* **4**: 79–87, 1986.
2. Callingham BA, Some aspects of monoamine oxidase pharmacology. *Cell Biochem Funct* **4**: 99–108, 1986.
3. Glover V and Sandler M, Clinical chemistry of monoamine oxidase. *Cell Biochem Funct* **4**: 89–97, 1986.
4. Mayanil CSK and Baquer NZ, Comparison of the properties of semipurified mitochondrial and cytosolic monoamine oxidases from rat brain. *J Neurochem* **43**: 906–912, 1984.
5. Copeland ES, Campbell JC and Murphy DL, Interaction between cytosolic monoamine oxidase and spin labelled amphetamine and its modification by clorgyline and pargyline. *Biochim Biophys Acta* **743**: 186–194, 1983.
6. Johnston JP, Some observations upon a new inhibitor of monoamine oxidase in brain tissue. *Biochem Pharmacol* **17**: 1285–1297, 1968.
7. Student AK and Edwards DJ, Subcellular localization of types A and B monoamine oxidase in rat brain. *Biochem Pharmacol* **26**: 2337–2342, 1977.
8. Azam M and Baquer NZ, Purification and immunochemical interrelationship of cytosolic and mitochondrial monoamine oxidase from rat brain. *Neurochem Int* **14**: 321–326, 1989.
9. Whittaker VP and Barker LA, The subcellular fractionation of brain tissue with special reference to the preparation of synaptosomes and their component organelles. In: *Methods in Neurochemistry* (Ed. Fied R), pp. 1–52. Marcel Dekker, New York, 1972.
10. Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein dye binding. *Anal Biochem* **72**: 248–254, 1976.
11. Tipton KF and Spires IPG, The kinetics of 2-phenylmethylhydrazine oxidation by monoamine oxidase. *Biochem J* **125**: 521–524, 1972.
12. Knoll J, The pharmacology of (−)-deprenyl. *J Neural Transm Suppl* **22**: 75–89, 1986.
13. Fowler CJ, Mantel TJ and Tipton KF, The nature of the inhibition of liver monoamine oxidase types A and B by the acetylenic inhibitors clorgyline, deprenyl and pargyline. *Biochem Pharmacol* **31**: 3555–3561, 1982.
14. Egashira T, Yamamoto T and Yamanaka Y, Effect of pH on inhibitors of monkey brain monoamine oxidase

by clorgyline and deprenyl. In: *Monoamine Oxidase. Basic and Clinical Frontiers* (Eds. Kamijok K, Usdin E and Nagatsu T), pp. 147–159. Excerpta Medica, Amsterdam, 1982.
15. Benedetti MS and Dostert P, Monoamine oxidase, brain ageing and degenerative diseases. *Biochem Pharmacol* **38**: 555–561, 1989.
16. Houslay MD, A model for the selective mode of action of the irreversible monoamine oxidase inhibitors clorgyline and deprenyl, based on studies of their ability to activate a Ca^{2+}–Mg^{2+} ATPase in defined lipid environments. *J Pharm Pharmacol* **29**: 664–669, 1977.
17. Stenström A, Arai Y and Oreland L, Intra- and extraneuronal monoamineoxidase-A and -B activities after central axotomy (hemisection) on rats. *J Neural Transm* **61**: 105–113, 1985.
18. Demarest KT, Smith DJ and Azzaro AJ, The presence of the type A form of monoamine oxidase within nigrostriatal dopamine-containing neurons. *J Pharmacol Exp Ther* **215**: 461–468, 1980.
19. Fagervall I and Ross SB, A and B forms of monoamine oxidase within the monoaminergic neurons of the rat brain. *J Neurochem* **47**: 569–576, 1986.
20. Fagervall I and Ross SB, Inhibition of monoamine oxidase within monoaminergic neurons in the rat brain by (*E*)-β-fluoromethylene-*m*-tyrosine (MDL 72394). *J Neurochem* **52**: 467–471, 1989.
21. Salach JI, Detmer K and Youdim MBH, The reaction of bovine and rat liver monoamine oxidase with [^{14}C]-clorgyline and [^{14}C]-deprenyl. *Mol Pharmacol* **16**: 234–241, 1979.
22. Zreika M, McDonald IA, Bey P and Palfreyman HG, MDL 72145, an enzyme-activated irreversible inhibitor with selectivity for monoamine oxidase type B. *J Neurochem* **43**: 448–454, 1984.
23. Palfreyman MG, Bey P and Sjorendsma A, Enzyme activated mechanism based inhibitors. In: *Essays in Biochemistry* (Ed. Tipton KF), pp. 28–80. Biochemical Society, London, 1987.