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PROPERTIES OF MONOAMINE OXIDASE IN MOUSE NEUROBLASTOMA N1E-115 CELLS

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Abstract—Monoamine oxidase (MAO) from adrenergic mouse neuroblastoma N1E-115 cells was compared to MAO found in rat and mouse brain, rat superior cervical ganglion, and human platelet. In comparison to MAO from brain and ganglion, mouse neuroblastoma MAO deaminated 5-hydroxytryptamine (5-HT) to a proportionately greater extent than all other substrates studied, with benzylamine deamination representing only 1 per cent that of 5-HT. Neuroblastoma MAO was over 1000 times more sensitive to inhibition by clorgyline than by deprenyl. With increasing concentrations of clorgyline, inhibition of tyramine deamination was represented by a simple sigmoid curve, suggesting the presence of primarily one form of MAO. Our results are consistent with evidence for a specific form of MAO associated with sympathetic neurons and suggest that neuroblastoma N1E-115 cells are highly enriched in MAO type A.

Multiple forms of monoamine oxidase (MAO) [monoamine-O_2 oxidoreductase (deaminating) EC 1.4.3.4] may be differentiated in mammalian tissues on the basis of properties such as thermal stability [1,2], antibody reactivity [3,4], electrophoretic mobility [5–13], substrate specificity and inhibitor sensitivity [14–17]. Differences in thermal stability and electrophoretic mobility have often distinguished more than two forms of MAO; however, only two basic types, MAO A and MAO B, have been identified on the basis of substrate specificity and inhibitor sensitivity [14]. MAO A appears relatively more active with serotonin and norepinephrine as substrates, and is sensitive to inhibition by low concentrations of clorgyline [14–16]. MAO B, however, is relatively more active with benzylamine and phenylethylamine, and is sensitive to inhibition by low concentrations of deprenyl [2,15,18]. As other amines such as tyramine, tryptamine and dopamine appear to be deaminated by both forms of MAO [4,14,15], tyramine has been used as substrate in the presence of various concentrations of clorgyline to ascertain the relative amounts of deamination due to MAO A vs MAO B in various tissues. In a plot of per cent inhibition of MAO activity vs concentration of clorgyline ($-\log$ M), the initial plateau region, where inhibition is constant, has been interpreted as representing 100 per cent inhibition of MAO A, and further inhibition has been attributed to the MAO B present in the preparation [14]. Rat brain, like most other tissues, has been reported to possess both MAO A and B [19]; in contrast, rat superior cervical ganglion and rat C6 glial cells contain predominantly MAO A [19],* while the human platelet appears to contain exclusively MAO B.†

We have compared the MAO activity characteristics of an adrenergic clone of mouse neuroblastoma, N1E-115 [20], to that found in brain, ganglion and platelet preparations. Clone N1E-115 has high levels of tyrosine 3-hydroxylase [20,21] and acetylcholinesterase [20], moderate levels of dopamine-β-hydroxylase [22], and electrically excitable membranes [23] which are sensitive to acetylcholine [24].

MATERIALS AND METHODS

Cell culture. N1E-115 cells (subcultures 14–21) were cultured in the Dulbecco-Vogt modification of Eagle's medium (high glucose, no pyruvate, Grand Island Biological Co., Grand Island N.Y.) supplemented with 10% (v/v) fetal calf serum (Colorado Serum Co., Denver, Colo.) without antibiotics at 37° in an atmosphere of 10% CO_2 and 90% humidified air. Cells were first grown in monolayer culture in flasks (75 cm^2/250 ml, Falcon Plastics, Oxnard, Calif.) before subculture into Petri dishes (150 mm × 25 mm, Falcon Plastics) using a modified Puck's D_1 solution (137 mM NaCl, 5.4 mM KCl, 0.17 mM Na_2HPO_4, 0.22 mM KH_2PO_4, 5.5 mM glucose, and 59 mM sucrose), pH 7.2, 340 mosM [21]. The medium was changed every other day during the log phase of growth and daily during the stationary phase. For enzyme assays, cells were maintained in the stationary phase of growth for several days prior to harvesting. For harvesting, the growth medium was carefully poured from the Petri dish, and the cell monolayer was washed twice with 10-ml aliquots of modified Puck's D_1 solution containing 0.14 mM $CaCl_2$ and with one 10-ml aliquot of modified Puck's D_1 solution at 4°. The Petri dish was then allowed to drain for 90 sec and the cells were harvested by scraping with a Teflon-covered spatula [21]. Cells were negative for mycoplasma (PPLO) by bacteriologic criteria.

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The cells were sonicated approximately 15 sec in 0.08 M phosphate buffer, pH 7.2, using a Sonifier cell disruptor (model W140, Heat Systems-Ultrasonics, Inc., Plainview, L.I., N.Y.). The sonicate was distributed in 50-μl aliquots (0.3 to 0.6 mg protein), and stored at $-70°$ until assayed for MAO activity.

Brain, ganglion and platelet. Wistar and Sprague–Dawley male rats, 150–250 g, were obtained from Microbiological Associates, Walkersville, Md., and Taconic Farms, Germantown, N.Y. respectively. Male A/J mice, 7 to 8-weeks-old, were obtained from Jackson Laboratories, Bar Harbor, Me. Only small differences in substrate specificity and inhibitor sensitivity were observed between mouse and rat brain in initial experiments; therefore, rat brain was utilized for most subsequent comparative purposes with neuroblastoma, ganglion and platelet.

Whole brains were homogenized in 10% (w/v) 0.08 M phosphate buffer, pH 7.2. The homogenate was centrifuged at 900 *g* for 10 min at 4°. The resultant supernatant was sonicated approximately 15 sec and distributed in 50-μl aliquots (0.2 to 0.7 mg protein).

Superior cervical ganglia, obtained from Sprague–Dawley male rats, were homogenized in 0.08 M phosphate buffer, pH 7.2. The homogenates were sonicated approximately 15 sec and distributed in 50-μl aliquots (0.06 to 0.07 mg protein).

Human blood platelet concentrates, prepared from venous blood collected in ACD solution (USP formula A, 75 ml/500 ml of whole blood), were obtained from Community Blood and Plasma, Baltimore, Md. The concentrates were centrifuged at 175 *g* for 5 min at 4°. The supernatant was removed and centrifuged at 2000 *g* for 20 min to obtain a platelet pellet. The pellet was washed once with ice-cold saline, resuspended in distilled water, and sonicated approximately 15 sec. The sonicated preparation was then distributed in 50-μl aliquots (0.2 to 0.9 mg protein).

All preparations were stored at $-70°$ until assayed for MAO activity.

MAO activity studies. MAO activity was determined by a modification of the method used by Robinson *et al.* [25] using the following substrates: [^{14}C]5-hydroxytryptamine creatinine sulfate (55 mCi/m-mole), [^{14}C]dopamine HCl (57.3 mCi/m-mole), [^{14}C-1]noradrenaline bitartrate (57 mCi/m-mole) (Amersham/Searle Corp., Arlington Heights, Ill.); [^{14}C]tyramine HCl (9.2 mCi/m-mole), [^{14}C]tryptamine bisuccinate (47 mCi/m-mole), [^{14}C]β-phenylethylamine HCl (9.86 mCi/m-mole) (New England Nuclear, Boston, Mass.); and [^{14}C]benzylamine HCl (3.5 mCi/m-mole) (Mallinkrodt Chemical Works, St. Louis, Mo.). The sonicated preparations were assayed in duplicate by incubating 50-μl aliquots in 0.5 ml of 0.08 M phosphate buffer (Na_2HPO_4–KH_2PO_4), pH 7.2, with 25 μl ^{14}C-labeled substrate for 20 min at 37°. Aliquots heated to 100° for 10 min were assayed simultaneously to establish blank values which were subtracted from those of the active enzyme preparation. Final concentrations (and specific activities) of substrates used were 5-hydroxytryptamine (serotonin, 5-HT, 1.67 mCi/m-mole) and tyramine (TYR, 0.5 mCi/m-mole), 10^{-3} M; norepinephrine (noradrenaline, NE, 3.3 mCi–m-mole) and dopamine (DA, 3.3 mCi/m-mole), 5×10^{-4} M; benzylamine (BA, 3.5 mCi/m-mole), 2×10^{-4} M; tryptamine (TA, 4.46 mCi/m-mole), 8×10^{-5} M; and phenylethylamine (PEA, 9.86 mCi/m-mole), 2×10^{-5} M. Nonradiolabeled amines [5-hydroxytryptamine creatinine sulfate complex; tyramine, monohydrochloride; (−)arterenol bitartrate hydrate (NE); 3-hydroxytyramine HCl (DA); tryptamine HCl, (CalBiochem, San Diego, Calif.)] were added when required to obtain the desired final concentrations. When norepinephrine, dopamine or tyramine was used as the substrate, ascorbic acid (10^{-4} M) and EDTA (10^{-4} M) were added to the phosphate buffer to prevent nonenzymatic alteration of the substrate. In cases where inhibitors were used, the samples were preincubated at 25° for 30 min in the presence of either clorgyline (May & Baker, Ltd., Essex, England) or deprenyl (Professor J. Knoll, Semmelweis University of Medicine, Budapest, Hungary), and then incubated at 37° in the presence of substrate as described above. After incubation, the samples were placed on ice and then transferred to pasteur pipettes containing 0.5 × 2.5 cm Amberlite resin (CG-50, 100–200 mesh, Mallinkrodt Chemical Works). The columns were washed twice with 1 ml of distilled water, and the entire 2.5 ml was collected in glass vials containing 17.5 ml Aquasol (New England Nuclear). The radioactivity of the products was determined by liquid scintillation spectrometry. In all of the tissues, monoamine oxidase activity was found to be linear with respect to the previously described protein concentration and time of incubation. K_m values were determined according to the method of Lineweaver and Burk [26] over a concentration range of 0.05 to 1.0×10^{-3} M tyramine. Protein was determined by a modification of the method of Lowry *et al.* [27].

RESULTS

The MAO specific activities of neuroblastoma, brain and ganglion preparations with the various substrates at concentrations previously described are listed in Table 1. In all preparations, 5-HT and tyramine were most actively metabolized, although in neuroblastoma, 5-HT deamination was almost twice that of tyramine. Deamination of β-phenylethylamine and benzylamine was lowest in neuroblastoma, representing approximately 1 per cent of 5-HT; in ganglion and brain their deamination averaged approximately 6 and 18 per cent of 5-HT respectively. In comparison to brain, the specific activities of neuroblastoma MAO were lowest for phenylethylamine and benzylamine, which represented only about 2 per cent that of brain, while deamination of the other substrates represented between 15 and 27 per cent that of brain. While norepinephrine deamination was regularly present in the brain preparations, only slight and variable activity was obtained with the ganglion and neuroblastoma preparations.

Inhibition of MAO activity by various concentrations of clorgyline using 1 mM tyramine as substrate is illustrated in Fig. 1. In contrast to the plateau-shaped curve obtained with brain, a simple sigmoid curve was obtained with neuroblastoma, with 100 per cent inhibition of MAO activity occurring at 10^{-7} M clorgyline. Curves similar to those previously reported [19] were obtained with ganglion.

Table 1. Monoamine oxidase specific activities of mouse neuroblastoma N1E-115, rat brain and superior cervical ganglion preparations

Substrate	MAO specific activities (nmoles/mg protein/hr)			Specific activities of Neuroblastoma cells as per cent of brain
	Brain	Ganglion	Neuroblastoma	
Tyramine	174	192	26	15
Serotonin	165	204	45	27
Dopamine	84	87	15	18
Tryptamine	60	78	13	22
Norepinephrine	30			
Phenylethylamine	30	10	0.60	2.0
Benzylamine	28	15	0.45	1.6

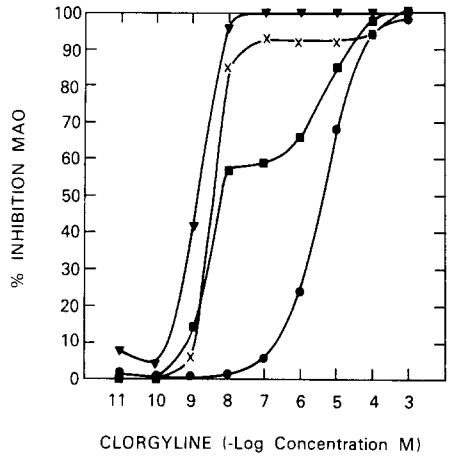

Fig. 1. Clorgyline inhibition of MAO activity using 10^{-3} M tyramine as substrate in neuroblastoma N1E-115 cells (▼), Sprague–Dawley superior cervical ganglion (×) and brain (■), and human blood platelets (●).

Human platelet MAO, which appears to be MAO B*, was included for comparative purposes.

Deprenyl inhibition of MAO activity is shown in Fig. 2. Neuroblastoma and ganglion MAO, which were most sensitive to inhibition by clorgyline, were least sensitive to inhibition by deprenyl. In contrast, platelet MAO was maximally inhibited by low concentrations of deprenyl. A plateau region (midpoint at 44 per cent inhibition) was still observable with deprenyl in brain although it was not as distinct as that observed with clorgyline.

The pI_{50} values (−log of the concentration of inhibitor resulting in 50 per cent inhibition) were calculated from curves presented in Figs. 1 and 2 and are listed in Table 2. The pI_{50} for clorgyline represents a 1000-fold lower concentration required to inhibit neuroblastoma MAO than that required to inhibit platelet MAO. Conversely, the pI_{50} for deprenyl represents a 1000-fold lower concentration required to inhibit platelet MAO compared to that required for neuroblastoma MAO. When the inhibitor curves for brain were broken into segments at the plateau region and separate pI_{50} values calculated for each segment, the resultant pI_{50} values closely resembled those for neuroblastoma and platelet, respectively, regardless of whether clorgyline or deprenyl was used as an inhibitor. Similar pI_{50} values were previously reported for brain [14,19].

Inhibitor concentrations of 10^{-7} M clorgyline and 10^{-6} M deprenyl were chosen on the basis of these data as those which most completely and selectively inhibited MAO A and B using 1 mM tyramine as substrate. In comparing inhibition due to clorgyline (Fig. 3) and to deprenyl (Fig. 4), substrate concentrations are important, as our preliminary studies have shown that the amount of deamination attributable to MAO A or B may be influenced by the choice of substrate and the substrate concentration.

Clorgyline (10^{-7} M) inhibited 100 per cent of the MAO activity in neuroblastoma and ganglion with 5-HT, dopamine and tyramine as substrates, and approximately 95 per cent of that with tryptamine; however, the deamination of benzylamine and phenylethylamine was inhibited 81 and 100 per cent, respectively, in neuroblastoma, but only 22 and 45 per cent, respectively, in ganglion. In brain, inhibition by clorgyline of 5-HT and norepinephrine deamination was essentially complete; however, clorgyline inhibition of other substrates was less complete, especially with benzylamine and phenylethylamine, whose deamination was inhibited by less than 10 per cent. Deprenyl

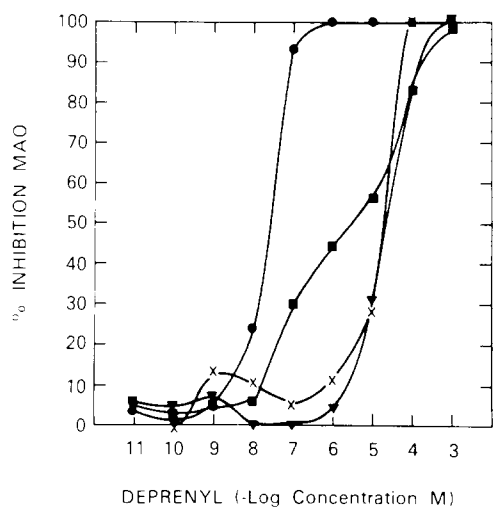

Fig. 2. Deprenyl inhibition of MAO activity using 10^{-3} M tyramine as substrate in neuroblastoma N1E-115 cells (▼), Sprague–Dawley superior cervical ganglion (×) and brain (■), and human blood platelets (●).

*C. H. Donnelly and D. L. Murphy, manuscript in preparation.

Table 2. Clorgyline and deprenyl inhibition of tyramine deamination by monoamine oxidase in mouse neuroblastoma N1E-115, rat superior cervical ganglion and brain, and human platelet preparations

Preparation	Clorgyline pI_{50}	Deprenyl pI_{50}
Neuroblastoma N1E-115 cells	8.8	4.7
Rat superior cervical ganglion	8.5	4.7
Rat brain	8.6, 5.3*	7.3, 4.3*
Human platelet	5.4	7.6

* Estimated separately for the two phases of the inhibition curves presented in Figs. 1 and 2.

(10^{-6} M) inhibited benzylamine and phenylethylamine deamination in brain by approximately 90 per cent while 5-HT deamination was inhibited by only 8 per cent. MAO activity in ganglion was less affected by deprenyl, with phenylethylamine deamination inhibited 45 per cent and other substrates less than 20 per cent. In neuroblastoma, only phenylethylamine and dopamine deamination was inhibited (28 and 18 per cent respectively) by deprenyl.

The apparent K_m values for tyramine were similar in all three preparations. Neuroblastoma and Sprague–Dawley rat brain had K_m values of 1.0×10^{-4} M and the K_m for ganglion was found to be 0.8×10^{-4} M.

DISCUSSION

These results indicating the occurrence of predominantly MAO A in cultured neuroblastoma cells support indirect evidence provided by denervation experiments [19,28–30], which suggested that neuronal MAO in peripheral sympathetic nerves has A-type characteristics. Fujiwara *et al.* [29] have shown that denervation of dog submaxillary gland results in a significant reduction in MAO activity when serotonin (5-HT), but not tyramine, is used as substrate. Jarrott [30] similarly found reductions in MAO activity in the sympathetically denervated rabbit submaxillary gland and vas deferens of several species, with a greater loss in activity using 5-HT as substrate than with benzylamine as substrate. Jarrott observed that 5-HT appeared to be deaminated more extensively by neuronal MAO in contrast to benzylamine, which appeared to be deaminated to a greater extent by extraneuronal MAO.

Further support for a neuronal specific form of MAO was suggested by Goridis and Neff [19], who

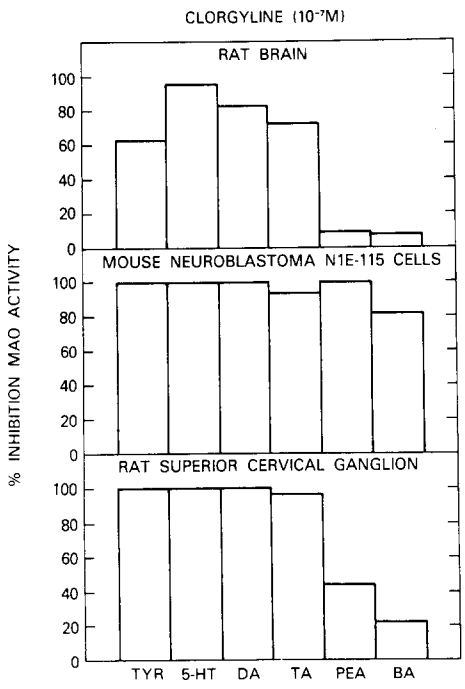

Fig. 3. Clorgyline (10^{-7} M) inhibition of MAO activity in neuroblastoma N1E-115 cells, Sprague–Dawley superior cervical ganglion and brain using 10^{-3} M tyramine (TYR), 10^{-3} M 5-hydroxytryptamine (5-HT), 5×10^{-4} M dopamine (DA), 8×10^{-5} M tryptamine (TA), 2×10^{-5} M phenylethylamine (PEA), and 2×10^{-4} M benzylamine (BA) as substrates.

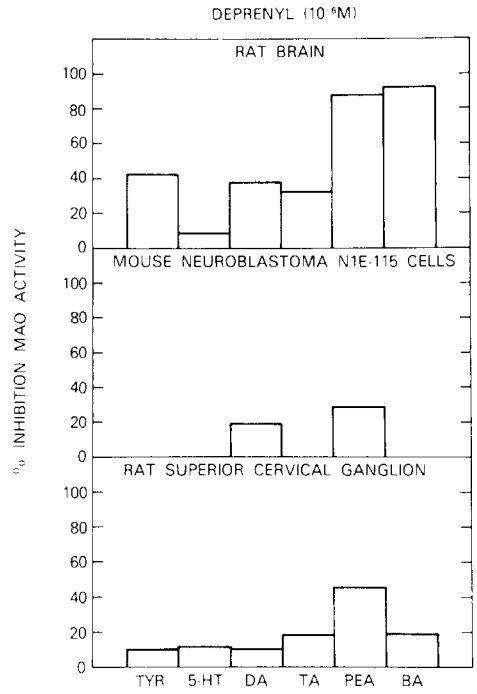

Fig. 4. Deprenyl (10^{-6} M) inhibition of MAO activity in neuroblastoma N1E-115 cells, Sprague–Dawley superior cervical ganglion and brain using 10^{-3} M tyramine (TYR), 10^{-3} M 5-hydroxytryptamine (5-HT), 5×10^{-4} M dopamine (DA), 8×10^{-5} M tryptamine (TA), 2×10^{-5} M phenylethylamine (PEA), and 2×10^{-4} M benzylamine (BA) as substrates.

showed that denervation of rat pineal gland resulted in a 70 per cent loss of MAO activity with serotonin as substrate, while activity with tyramine was not significantly changed. The reduction in MAO activity with serotonin as substrate was attributed to loss of MAO A associated with sympathetic nerves while the nonsignificant reduction in MAO activity with tyramine as substrate suggested that the MAO B, which contributed up to 85 per cent of tyramine deamination in the pineal gland remained unchanged.

Different proportions of MAO A and B occur in various preparations [16,19]; however, few tissues are as relatively free of MAO B as the neuroblastoma preparation. Goridis and Neff [16] have shown that the ratio of MAO A/B activity in superior cervical ganglion of rat is 9/1 in contrast to brain, which is 6/4 when 2.1 mM tyramine is used as substrate. Using 1 mM tyramine as substrate, we have shown that inhibition of neuroblastoma MAO with increasing concentrations of clorgyline or deprenyl results in a simple sigmoid curve, suggesting that MAO A is predominant in the neuroblastoma N1E-115 cell line.

Although differences may possibly exist between neuroblastoma N1E-115 cells and adrenergic neurons in brain, N1E-115 cells contain enzymes for the synthesis of catecholamines and have other properties characteristic of differentiated neurons [20]. We have found that neuroblastoma (N1E-115) MAO preferentially deaminates the A-type substrate, 5-HT. Deamination of the B-type substrates, benzylamine and phenylethylamine, by neuroblastoma represents approximately 1 per cent that of 5-HT compared to ganglion and brain, which average 6 and 18 per cent that of 5-HT, respectively. However, in contrast to ganglion and brain, deamination of benzylamine and phenylethylamine by neuroblastoma MAO is almost completely inhibited by 10^{-7} M clorgyline, which suggests that deamination of these two substrates in neuroblastoma may be largely attributed to a clorgyline-sensitive MAO of the A-type.

The substrate- and inhibitor-related characteristics observed with neuroblastoma MAO in comparison with the MAO of other preparations studied suggest that neuroblastoma contains primarily one form of MAO, MAO A, and is consistent with evidence that MAO A is the predominant form of MAO associated with sympathetic neurons [16,19,28–31].

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