Table 2. Competition between mebendazole and [^{3}H]colchicine for binding to sheep brain microtubule protein

[Colchicine](μM)	[Mebendazole](μM)	Colchicine : tubulin Molar ratio	Per cent of control
5	0	0.431	100
5	50	0.240	56
50	0	0.980	100
50	50	0.795	81
50	100	0.639	65

Microtubule protein was incubated with mebendazole (or buffer for controls) for 60 min at 37°, and then for a further 90 min with [^{3}H]colchicine. Other procedures were as in the text.

Note added in proof. Since this manuscript was prepared, Friedman and Platzer (*Biochim. biophys. Acta* **544**, 605 1978) have described similar results with calf brain tubulin.

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Biochemical Pharmacology, Vol. 28, pp. 2682–2684

0006-2952/79/0901-2682 $02.00/0

Oxidation of *p*-, *m*- and *o*-tyramine by type A and type B monoamine oxidase

(*Received* 21 *February* 1979; *accepted* 29 *March* 1979)

Mitochondrial monoamine oxidase [amine: oxygen oxidoreductase (deaminating, flavin-containing); EC 1.4.3.4] (MAO) is believed to exist in many animal tissues in two functional forms called type A and type B [1–3], based primarily on its sensitivity to clorgyline [1]. Recently, many monoamines have been characterized as substrates for type A and type B MAO; 5-hydroxytryptamine [4], 5-methoxytryptamine [5] norepinephrine and epinephrine [4] are specific for type A MAO, while β-phenylethylamine [2, 6], phenylethanolamine [7, 8] at low substrate concentrations, and benzylamine [4] are specific for type B MAO. Some substrates such as kynuramine [9], *p*-tyramine, tryptamine [1], dopamine [4], *p*-octopamine [8, 10] and *p*-synephrine [11] are oxidized by either type of MAO. Recently, *m*-tyramine was found in the brains of various species of mammals [12–15]. *o*-Tyramine was also identified in rat and human urine [16–18]. These findings have led us to characterize *m*- and *o*-tyramine as substrates for type A and type B MAO. The results on *p*-tyramine are also presented for comparison.

A crude mitochondrial fraction was isolated from whole

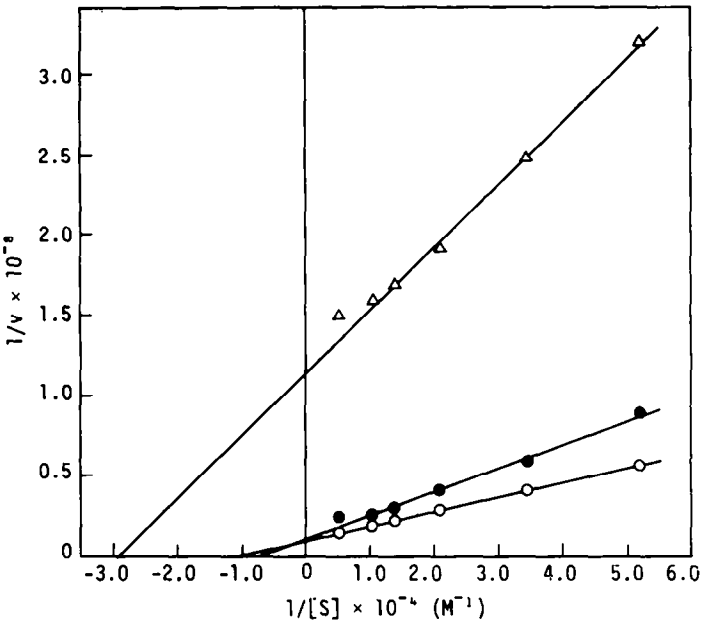

Fig. 1. Lineweaver–Burk plots for MAO in rat brain mitochondria with *p*-(○——○), *m*-(●——●) and *o*-tyramine (△——△) as substrates. The symbol *v* indicates moles H_2O_2 formed/mg of protein/30 min. The K_m values for *p*-, *m*- and *o*-tyramine were 90.9, 133 and 34.5 μM; the V_{max} values were 100, 90.9 and 8.85 nmoles/mg of protein/30 min respectively. Each point represents the mean obtained from duplicate determinations upon a single enzyme source prepared from the pooled brains of seven rats.

brains of male Sprague–Dawley rats, weighing 100–150 g, as described previously [19]. MAO activities were determined fluorometrically by the method of Guilbault *et al.* [20] and Snyder and Hendley [21]. For each assay (final volume, 3.0 ml), 0.079 to 0.525 mg of mitochondrial protein was used. The assays were carried out at 37° and pH 7.4 for 30 or 60 min. Under the conditions used, the assays were linear during incubation for at least 60 min. Care was taken not to convert more than 20 per cent of the substrate to reaction product. For kinetic analyses, substrate concentrations over the range of 19.2 to 192 μM were used. For the inhibition studies with clorgyline, a selective inhibitor of type A MAO [1], and with deprenyl, a selective inhibitor of type B MAO [22], the assay mixture was preincubated with each inhibitor at 37° for 10 min to ensure maximal enzyme inhibition. It was confirmed that each inhibitor neither interfered with the formation of the fluorescent compound nor quenched its fluorescence when hydrogen peroxide was added directly.

Protein was measured by a slight modification [23] of the conventional biuret method.

Both *m*- and *o*-tyramine were substrates for MAO. The Michaelis–Menten kinetic constants for the tyramine isomers were determined from the Lineweaver–Burk plots as shown in Fig. 1. The kinetic constants for *m*-tyramine were generally similar to those for *p*-tyramine; both K_m and V_{max} values for *o*-tyramine were much lower than those for *p*- and *m*-tyramine.

To determine the specific type of MAO involved in the metabolism of the tyramines, the susceptibility of MAO to clorgyline and deprenyl was studied. Since we reported that the sensitivity to both inhibitors was affected by substrate concentration, especially when β-phenylethylamine and phenylethanolamine were used as substrates [6, 8], the inhibition experiments with tyramines were carried out at their K_m values. As can be seen in Fig. 2, the inhibition patterns with both inhibitors for *m*-tyramine were extremely similar to those for the *p*-isomer; clear plateaus were observed at 10^{-8}–10^{-7} M of both inhibitors. The susceptibility of *o*-tyramine deamination to clorgyline was much lower than that of *p*- or *m*-tyramine deamination, while deprenyl showed much more potent inhibition for *o*-tyramine than for the other two. In addition, the inhibition patterns with *o*-tyramine were almost single sigmoidal for both inhibitors. These results show that *p*- and *m*-tyramine are common substrates for both types of MAO, while *o*-tyramine is almost specific for type B MAO.

Houslay and Tipton [4] previously suggested that type A substrates must carry a *p*-hydroxyl group. Our results with the tyramine isomers show that a *m*-hydroxyl group also contributes to being a substrate for type A enzyme, but an *o*-hydroxyl group does not.

Although physiological roles of tyramines are not well-defined now, the possibility has been presented that they may act as neurotransmitters or neuromodulators [24]. The substrate specificity of tyramines for type A and type B MAO may serve for the explanation of their metabolism in neural tissues. Boulton [24] reported that the administration of clorgyline to rats resulted in an increase of *p*-tyramine by 643 per cent and *m*-tyramine by 208 per cent in the brain, while the administration of deprenyl resulted in an increase of *p*-tyramine by 179 per cent and *m*-tyramine by 487 per cent. In the present study, we have demonstrated that the substrate specificity of *m*-tyramine for type A and type B MAO is extremely similar to that of *p*-tyramine (Fig. 2). Therefore, the data of Boulton, considered along with our own, suggest a different localization of the oxidation sites of *p*- and *m*-tyramine in the brain.

In summary, the present study shows that *m*-tyramine, like *p*-tyramine, is a common substrate for type A and type B MAO, while the *o*-isomer is almost specific for type B MAO.

Acknowledgements—We are very grateful to Prof. A. A. Boulton and Dr. A. V. Juorio, Psychiatric Research Unit, University Hospital, Saskatoon, Saskatchewan, Canada, for kindly supplying *m*- and *o*-tyramine. Clorgyline and deprenyl were obtained as gifts from May & Baker, Ltd., Dagenham, England and from Prof. J. Knoll, Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary, respectively.

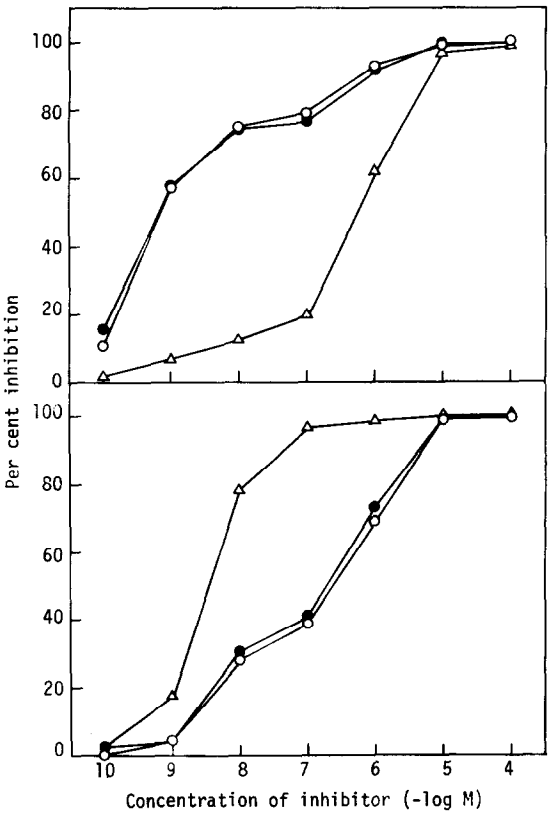

Fig. 2. Inhibition of MAO in rat brain mitochondria by clorgyline (upper panel) and deprenyl (lower panel) using *p*- (○——○), *m*- (●——●) and *o*-tyramine (△——△) as substrates. The concentrations of the substrates were at their K_m values, viz. 90.9, 133 and 34.5 μM respectively. Each point represents the mean obtained from two to four determinations upon a single enzyme source prepared from the pooled brains of seven rats. For no point was the S.D. > 5 per cent of the mean.

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