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Differences between monoamine oxidase concentrations in striatum and forebrain of aged and young rats

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Summary. The MAO-A and MAO-B activities in the striatum and the rest of the forebrain of young adult and aged rats were determined and compared. There was no significant difference in K_m values of MAO-A for 5-HT or of -B for benzylamine in any of the brain regions of both rat groups. With increase in age, the V_{max} value of MAO-A in the forebrain decreased; in the striatum the V_{max} values of MAO-A and -B increased with age. The MAO-A concentrations, measured by enzyme titration with clorgyline or 1-deprenyl as the titre, were the same in both brain regions of young and aged rats, but in both brain regions of aged rats, MAO-B concentrations were greater than those in young rats. The MAO-B concentrations increased in parallel with the increases in V_{max} indicating that the increase with age was due to increase in the number of MAO molecules.

Keywords: Monoamine oxidase (MAO), MAO-A and MAO-B, aging, titration, enzyme concentration.

Introduction

The enzyme, monoamine oxidase (MAO, EC 1.4.3.4) is divided into two distinct forms, MAO-A and MAO-B, based on difference in inhibitor sensitivities to clorgyline and l-deprenyl (Johnston, 1968; Knoll and Magyar, 1972). In rat brain, MAO-A preferentially oxidizes 5-hydroxytryptamine (5-HT) at low concentrations, and MAO-B oxidizes benzylamine (BZ) and beta-phenylethylamine (PEA), at low concentrations, whereas both forms of MAO oxidize tyramine and tryptamine at various concentrations (Kinemuchi et al., 1984).

Much data suggest that MAO-B activity in brains from patients with Alzheimer's disease, Huntington's disease and Parkinson's disease is higher than that in age-matched controls, whereas this activity is lower in brains of alcoholics, suicidal subjects and cycloid psychotics (for review, see Oreland et al., 1984). Regardless of these diseases, human (Robinson et al., 1972; Horita, 1978;

Fowler et al., 1980a) and rat (Mantle et al., 1976; Strolin Benedetti and Keane, 1980; Leung et al., 1981) brain MAO-B activity increases with increasing age, while increase in MAO-A activity is negligible. Thus the MAO-A/-B activity ratio gradually decreases with age. These ratios, however, have seldom been determined from actual measurement of the quantities of MAO-A and -B. Recent results have shown that neither 5-HT nor PEA are entirely specific substrates for only MAO-A and -B, respectively (for review, see Kinemuchi et al., 1984). Thus, the notion that either substrate can be specific for one or the other form of MAO may not be valid.

In the present study, to determine whether changes in MAO-B activity in aged rat brain are due to increased enzyme concentration, or qualitative change (kinetic parameters, K_m and V_{max}), we measured and compared concentrations of both forms of MAO in striatum and the rest of the forebrain of young and aged rats.

Materials and methods

Male Sprague-Dawley young adult (about 9 weeks, weighing 220–250 g, N = 8) and aged (about 100 weeks, weighing 600–700 g, N = 4) rats were used in the present study. They were housed in the room which temperature was maintained at 23.0 ± 1.0 °C with constant humidity. The room light was controlled on a 12 hr light/dark cycle. The brains of both groups were quickly removed after sacrifice by decapitation and disected into the corpus striatum and the rest of the forebrain. These separate parts were homogenized in 9 vol. of ice-cold 0.32 M sucrose and the resulting homogenates were used as enzyme sources for assay of MAO activity. MAO-A and -B activity was radiochemically assayed as reported previously (Kinemuchi et al., 1985). They were incubated with their respective substrates, 0.1 mM [^{14}C]5-hydroxytryptamine (5-HT) and 0.1 mM [^{14}C]benzylamine (BZ) at 37 °C and pH 7.4 for 5 min. The concentration of MAO-A or -B was determined by enzyme titration, as reported previously (Fowler et al., 1979, 1980c). However, to minimize increase of non-specific binding of the inhibitor at increased concentrations (Gomez et al., 1986), we varied the amount of homogenate, instead of the inhibitor. A fixed concentration of the selective, active centre directed, irreversible MAO-A or -B inhibitor, clorgyline or l-deprenyl was used as the respective titre (Egashira et al., 1976). To selectively and irreversibly inhibit activity of either form of MAO, the preparations were preincubated for 1 hr with 3 nM clorgyline or 4 hrs with 3 nM l-deprenyl at 37 °C before adding the substrate (see, Results and discussion). Control MAO-A or -B activity was determined by preincubating the same size sample for the same time (1 or 4 hr) with water instead of the inhibitor. During the control preincubation, no appreciable decrease in MAO-A (1 hr) or -B (4 hr) activity was observed. Protein contents were assayed by the method of Markwell et al. (1978) with bovine serum albumin as standard.

The radioactive substrates for MAO, [side chain-2-^{14}C]5-HT creatinine sulphate and [7-^{14}C]BZ hydrochloride were purchased from Amersham International plc. Buckinghamshire, U.K. Clorgyline and l-deprenyl as hydrochloride salts were kindly provided by May & Baker Ltd., Dagenham, U.K. and by Dr. J. Knoll, Semmelweis University, Budapest, Hungary. All other chemicals used were of the highest grade commercially available.

Results and discussion

In preliminary experiments, the time courses of inhibition of 5-HT oxidation (MAO-A) by different concentrations of clorgyline (1–3 nM) or BZ oxidation

by different concentrations of l-deprenyl (1–4 nM) were studied. Data are not shown here, but the time to reach the plateau of inhibition varied with clorgyline concentration and amount of striatal or forebrain homogenate used, but was always less than 1 hr. With l-deprenyl, the time to reach the inhibition plateau was less than 4 hr. Neither plateau of inhibition changed when preincubation was lengthened to 2 hr for clorgyline or 5 hr for l-deprenyl. Based on these results, preincubation of the striatal or forebrain homogenates was performed for 1 hr with 3 nM clorgyline or 4 hr with 3 nM 1-deprenyl at 37 °C, in subsequent titration experiments.

The acetylenic MAO inhibitors, a class to which clorgyline and l-deprenyl belong, appear to inhibit MAO activity by a suicide reaction, whereby an initial competitive reaction between inhibitor and MAO is followed by formation of an irreversible adduct in a 1:1 ratio (Fowler et al., 1981). Recent studies indicate that the free concentrations of these two inhibitors are exhausted by the formation of irreversible enzyme-inhibitor adducts upon completion of the reactions between enzyme and inhibitor (Fowler et al., 1981). Under these conditions, if there is no non-specific binding, titration with various amounts of enzyme preparation should produce a line that intercects the x-axis at a point to the right of the origin, which depends on the amount of enzyme bound by the inhibitor (Egashira et al., 1976). The slope of the line should be the same as that obtained with the uninhibited enzyme, which should pass through the origin.

Data obtained in the present study, based on enzyme titration and other methods, are summarized in Tables 1 and 2. Km values of 5-HT (MAO-A) or BZ (-B) for young and aged rat forebrain, were not significantly different

Table 1. Effects of aging on kinetic parameters, amounts and molecular turnover numbers (MTN) of MAO-A and -B in rat forebrain

	MAO-A		MAO-B	
	Young	Aged	Young	Aged
K_m (μM)	72 ± 2.9	63 ± 2.3	73 ± 3.0	68 ± 2.7
V_{max} (pmol/mg protein/min)	413 ± 11.6	375 ± 5.51* (91%)	505 ± 6.8	552 ± 21.4 (110%)
Amount of MAO	4.4 ± 0.24	4.9 ± 0.28 (111%)	4.2 ± 0.31	5.2 ± 0.28* (124%)
MTN	94.1 ± 7.67	81.7 ± 2.75	118.7 ± 7.10	110.1 ± 6.04

Values are expressed as mean ± S.E.M. (N = 4). Amount of MAO is expressed as pmole MAO/mg protein and MTN as mol product formed/mol MAO/min. For estimation of amount of MAO-A or -B, various amounts of forebrain homogenates (young, 0.052–0.416 mg; aged, 0.047–0.376 mg protein) were used. * $p < 0.05$, statistically significant, the young vs the aged rats

Table 2. Effects of aging on kinetic parameters, amounts and molecular turnover numbers (MTN) or MAO-A and MAO-B in rat striatum

	MAO-A		MAO-B	
	Young	Aged	Young	Aged
K_m (μM)	100	100	83	83
V_{max} (pmol/mg protein/min)	423	497 (117%)	394	646 (164%)
Amount of MAO	5.4±0.27	4.9±0.34 (91%)	2.1±0.21	3.6±0.61* (171%)
MTN	78.3	101.4	187.6	179.4

Values are means ± S.E.M. (N=4), where appropriate. The other values are means for two duplicate determinations. Amount of MAO is expressed as pmole MAO/mg protein and MTN as mol product formed/mol MAO/min. For estimation of the amount of MAO-A or -B, various amounts of striatal homogenates (both young and aged, 0.05–0.4 mg protein) were used. * $p<0.01$, statistically significant, the young vs the aged rats

(Table 1). In the striatum there were no difference (Table 2), indicating no difference in enzymatic properties of either form of MAO with aging. In contrast, V_{max} of the aged rat forebrain MAO-A towards 5-HT was slightly, but significantly decreased by aging without significant change in the value of MAO-B towards BZ (Table 1). In the striatum, in contrast to the forebrain, V_{max} of MAO-A in the aged rat increased to about 120% of V_{max} of the young rat. Moreover, as observed in the forebrain, the value for MAO-B increased to 164% of that of the young rat striatum (Table 2). These results are similar to finding of increased MAO-B activity in rat brain by aging with little increase in MAO-A activity, when these activities were assayed with the respective substrates (Oreland et al., 1984; Arai et al., 1985). To verify that these changes were caused by change in the concentrations of MAO molecules, we directly measured the MAO-A and -B concentrations in both brain regions of young and aged rat by titration with clorgyline and l-deprenyl. Results shown in the tables indicate that the concentrations of MAO-A molecules in both the brain regions of both groups were not significantly different. The MAO-B concentrations in the forebrain and striatum of aged rats were 124% and 171%, respectively, of those in young rats. Thus, in the striatum of aged rats, the increase (164%) in the V_{max} value of MAO-B activity corresponded well to the increase (171%) in the enzyme concentration. This was also found here for MAO-B in the aged rat forebrain (V_{max}, 110% and concentration, 124%) even with age-related independent changes in total protein, MAO-A and -B activity in rat brain (Strolin Benedetti and Keane, 1980). The present results thus lead us hypothesize that the increase in rat brain MAO-B activity by treatment with

hemitransection (Oreland et al., 1980; Stenstrom et al., 1985) might be a result of increased numbers of MAO-B molecules. This correlation between the increase of V_{max} and the concentration of MAO-B was also found for aged human brain (Fowler et al., 1980c).

The degree of non-specific binding of the acetylenic irreversible MAO inhibitors used as titres differs from titre to titre and/or tissue to tissue used in the titration (Fowler et al., 1981). The present correlation between increase of V_{max} value and MAO-B concentration would suggest that, under the present conditions, the titre, l-deprenyl, did not appreciably bind to sites other than the enzyme active centres. However, this was not true for clorgyline, where its V_{max} values towards 5-HT and concentration were not exactly parallel. Reason(s) for this difference remain be clarified. At present it seems to be due, at least in part, to non-specific binding to other sites, selective metabolism of clorgyline by enzyme(s) other than the SKF-525A-sensitive enzyme (Fowler, 1980b) and/or, if it appreciably occurs in the brain preparations used, formation of irreversible clorgyline-cytochrome P-450-dependent enzyme adducts (Dupont et al., 1987).

The molecular turnover numbers of MAO-A and -B obtained in the present study, calculated as V_{max}/concentration of appropriate enzyme form, were similar to those previously determined in rat tissues (Egashira et al., 1976), but lower than those in rat liver (Gomez et al., 1986). Different values were reported for the same MAO form in various tissues from different species, even if this number is independent of protein concentration or purity of the enzyme source tested (Fowler et al., 1981; Gomez et al., 1986). Thus the differences might be due to the methods and/or to the different experimental conditions (e.g. pH, buffer, preincubation time with titre: etc.).

A previous study (Langston et al., 1987) suggested that older rodents are more efficient than younger ones in converting the nigrostriatal dopamine neurotoxin that produces the Parkinsonian syndrome, l-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, to its active metabolite, l-methyl-4-phenylpyridinium ion, which step is mainly catalyzed by brain MAO-B (Kinemuchi et al., 1985). This should be mainly due to increased MAO-B concentration in the brain, as found in the present study.

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