demonstrated that 5-HT-S_1 autoreceptors on nerve terminals in the cerebral cortex are similar to the pre-synaptic 5-HT-S_1 receptor (Middlemiss 1984).

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Comparative lipophilicities of substrates of monoamine oxidase

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Oil/water partition coefficients of various substrates of monoamine oxidase (MAO) and kinetic parameters of MAO-A and -B of rat liver at two pH values, pH 7 and pH 9, were investigated. Octanol, heptane or benzene were chosen for the oil phases. The deamination of the biogenic amines 5-hydroxytryptamine (5-HT), tyramine, 2-phenethylamine (PEA) and benzylamine was studied at pH 7 and pH 9. Results indicated all four substrates were very hydrophilic, and the oil/water partition coefficients of benzylamine and PEA were higher than those of 5-HT and tyramine. The changes in K_m and V_{max} values at pH 7 and pH 9 indicated that the affinities of MAO-A towards 5-HT and tyramine slightly increased at pH 9 and those of MAO-B towards tyramine and benzylamine also increased at pH 9, while uncharged amines at pH 9 amounted to about a hundred times of those at pH 7. It is concluded that the mitochondrial MAO bound to the membrane may metabolize charged molecules as well as uncharged counterparts.

Monoamine oxidase (monoamine; oxidoreductase deaminating EC 1.4.3.4, MAO) catalyses the oxidative deamination of different monoamines, and is assumed to exist in two forms, MAO-A and MAO-B. This is based primarily upon its substrate specificity and inhibitor sensitivity (Johnston 1968; Houslay & Tipton 1974; Houslay et al 1976; Knoll 1976). MAO-A is more readily inhibited by clorgyline and appears to have a preference for 5-HT and noradrenaline, while MAO-B is more sensitive to selegiline (deprenyl) with its preferred substrate being 2-phenethylamine (PEA) and benzylamine. Other amines, such as tyramine, tryptamine and dopamine, are thought to be substrates acceptable to both enzyme types.

There is considerable experimental support for the existence of two different protein species (Callingham & Parkinson 1979; Cawthon & Breakefield 1979). However, the hypothesis that the enzyme exists in a single form which is modified by its specific membrane environment is also supported by many observations (Houslay & Tipton 1973, 1974; Tipton et al 1976). There is evidence that substrate specificity may be affected differently by the lipid environment (Houslay 1980).

To provide further information about the basic interactions that govern the substrate specificities of the mitochondrial monoamine oxidase of rat liver, the present paper reports studies of the oil/water partition coefficients of substrates and the effect of pH on the kinetic parameters of MAO.

Materials and methods

Oil/water partition coefficients. A 1·0 ml amount of 0·2 M phosphate buffer (pH 7) or 0·2 M carbonate-bicarbonate buffer (pH 10) containing radioactive substrates (0·01–0·10 mM) was mixed with 1·0 ml of one of the organic

* Correspondence.

solvents, octanol, heptane or benzene, for 10 min at 37 °C. After separation of the two phases, the radioactivity of the sample from each phase was measured. The reading obtained after the equilibration was used to calculate apparent partition coefficients at pH 7 and pH 10.

Preparation of samples. Male Wistar rats were killed by a blow to the head, the livers rapidly removed and mitochondria prepared by standard fractionation techniques and used as a MAO preparation.

Assay of MAO activity. MAO activity was determined by radiochemical procedures based on the method of Wurtman & Axelrod (1963). The incubation medium contained radioactive substrates (20–100 μCi mol^{-1}) in a total volume of 100 μl of phosphate buffer (0·2 M, pH 7) or boric acid-borate buffer (pH 9). After incubation at 37 °C the medium was extracted with 1 ml of toluene–ethyl acetate (1:1) and 0·5 ml taken for measurement of radioactivity. To study the activity towards MAO-A, the B-form was inhibited by preincubating samples of the mitochondria for 30 min at 37 °C with 3×10^{-7} M selegiline. To study the activity towards MAO-B, the activity of the A-form was inhibited by treatment in a similar manner with 3×10^{-7} M clorgyline. The remaining activity was determined by the assay method described above.

K_m determination. K_m and V_{max} values were calculated from Lineweaver-Burk plots.

Results

Oil/water partition coefficients of substrates. All substrates tested showed higher oil/water partition coefficients with octanol than with benzene, and with benzene than heptane as shown in Table 1. With each solvent, each substrate was soluble in the oil phase at pH 10 as expected. The values obtained at pH 10 with any substrate were very small, indicating the hydrophilicity of all the four substrates. The oil/water partition coefficients of 5-HT and tyramine were much lower than those of benzylamine and PEA.

Table 1. Comparison of oil/water partition coefficients of each substrate at pH 7 and at pH 10 using three organic solvents. Radioactive substrates containing buffer at pH 7 and pH 10 were mixed with an organic solvent. Apparent oil/water partition coefficient is ratio of the radioactivity of oil phase to that of buffer phase. Values shown are the mean of four experiments.

		pH 7	pH 10
5-HT	Heptane	0·0003	0·0004
	Benzene	0·0005	0·0095
	Octanol	0·0176	0·494
Tyramine	Heptane	0·0004	0·0003
	Benzene	0·0009	0·0076
	Octanol	0·0089	0·381
Benzylamine	Heptane	0·0036	0·210
	Benzene	0·0296	1·05
	Octanol	0·0405	1·96
PEA	Heptane	0·0036	0·277
	Benzene	0·0312	1·04
	Octanol	0·0403	2·25

Table 2. Comparison of K_m values of MAO-A, MAO-B and untreated enzyme from rat liver at pH 7 and pH 9, using various substrates. For measurement of MAO-A and MAO-B activities, enzyme preparations were pretreated with 3×10^{-7} M selegiline and clorgyline, respectively, for 30 min. Values shown are expressed in μM. Data represent means ± s.e., with n = 3.

		pH 7	pH 9
5-HT	Untreated enzyme	145 ± 12	109 ± 24
	MAO-A	138 ± 18	98 ± 3
	MAO-B	210 ± 53	195 ± 21
Tyramine	Untreated enzyme	88 ± 6	81 ± 11
	MAO-A	81 ± 6	74 ± 13
	MAO-B	245 ± 25	186 ± 38
Benzylamine	Untreated enzyme	241 ± 9	111 ± 4
	MAO-A	255 ± 44	263 ± 29
	MAO-B	190 ± 30	84 ± 2
PEA	Untreated enzyme	19 ± 2	14 ± 3
	MAO-A	69 ± 18	18 ± 3
	MAO-B	16 ± 1	12 ± 1

Table 3. Comparison of V_{max} values of MAO-A, MAO-B and untreated enzyme from rat liver at pH 7 and pH 9, using various substrates. Values shown were obtained from the Lineweaver-Burk plots used in Table 2 and expressed in nmol mg $protein^{-1}$ min^{-1}. Data represents means ± s.e., with n = 3.

		pH 7	pH 9
5-HT	Untreated enzyme	1·51 ± 0·09	1·95 ± 0·21
	MAO-A	1·35 ± 0·12	1·65 ± 0·07
	MAO-B	0·15 ± 0·05	0·13 ± 0·01
Tyramine	Untreated enzyme	1·11 ± 0·04	2·78 ± 0·16
	MAO-A	0·97 ± 0·09	1·69 ± 0·28
	MAO-B	0·56 ± 0·16	1·76 ± 0·31
Benzylamine	Untreated enzyme	0·47 ± 0·04	1·20 ± 0·10
	MAO-A	0·16 ± 0·03	0·18 ± 0·04
	MAO-B	0·42 ± 0·04	1·07 ± 0·08
PEA	Untreated enzyme	4·27 ± 0·24	4·32 ± 0·27
	MAO-A	1·55 ± 0·33	2·60 ± 0·21
	MAO-B	3·44 ± 0·26	3·53 ± 1·67

Effects of pH on the kinetic parameters of MAO. The kinetic parameters of MAO were determined for each substrate at pH 7 and pH 9. In almost all cases there were only minor changes in measured K_m or V_{max} values for either MAO-A or -B when the pH was raised from 7 to 9 (Tables 2, 3). With 5-HT and PEA as substrate, the K_m values of MAO-A decreased at pH 9 and with tyramine those of both MAO-A and MAO-B were lower at pH 9 than at pH 7. On the other hand, with benzylamine MAO-B had a lower K_m value at pH 9. The V_{max} value of the untreated enzyme was nearly equal to the sum of that of MAO-A and MAO-B. The V_{max} values of MAO-A with 5-HT, tyramine and PEA as substrate increased at pH 9, and those of MAO-B with tyramine and benzylamine also increased at pH 9.

Discussion

The values of oil/water partition coefficients vary with choice of organic solvent for the oil phase. In this study,

heptane, benzene and octanol, which have different degrees of polarity, were used for oil phases. We obtained a small value for oil/water partition coefficients using any substrate indicating that the four substrates are very hydrophilic. On comparing the data from Table 1, it can be seen that these substrates can be divided into two groups. The values of both species with 5-HT and tyramine are similar and differ from those with benzylamine and PEA. 5-HT and tyramine are much more hydrophilic than benzylamine and PEA. From these facts, we conclude the substrate for MAO-A may be more hydrophilic than that for MAO-B. Furthermore, it is suggested that dopamine, noradrenaline and adrenaline will belong to the 5-HT and tyramine group.

It is possible to estimate the percentage of ionized form by using pK_a values. The percentage of uncharged substrate at pH 9 is approximately a hundred times greater than at pH 7. Table 2 shows the decrease of K_m at pH 9 of MAO-A with 5-HT and tyramine, and that of MAO-B with tyramine and benzylamine. In addition to these results, Table 3 shows the increase of V_{max} at pH 9 of MAO-A with 5-HT and tyramine, and that of MAO-B with tyramine and benzylamine. These facts indicate that when uncharged amine exists, the affinities of MAO-A and MAO-B for their specific substrates increase. Though PEA has long been believed to be a specific substrate for MAO-B (Houslay & Tipton 1974; Houslay et al 1976), it is reported that PEA loses its substrate specificity for MAO-B at relatively high pH (Suzuki et al 1979) and concentration (Kinemuchi et al 1980; Kawaguchi et al 1982). Considering the increase in affinity of MAO-A at pH 9 as shown in Tables 2, 3, PEA may be metabolized by MAO-A as the uncharged species of PEA increases.

Studies by McEwen et al (1968, 1969) on solubilized, partially purified human liver MAO, and by Williams (1974) on purified porcine brain MAO, have indicated that the true substrate for the enzyme is perhaps the unprotonated amine. In the present study, there were only minor changes in K_m or V_{max} values for either MAO-A or -B when the pH was raised from 7 to 9 despite a rise in the concentration of the uncharged amine species of nearly two orders of magnitude over those between two pH values. These results may indicate that the substrate for membrane-bound enzyme is not only the unprotonated amine.

From our experiments, we conclude that though unprotonated amine is mainly metabolized by MAO-A and MAO-B, both forms of MAO bound to the mitochondrial membrane may metabolize not only unprotonated amine but also protonated amine. And the physicochemical properties of substrates may contribute to the substrate specificities of MAO-A and MAO-B.

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