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Deamination of dopamine and its 3-*O*-methylated derivative by human brain monoamine oxidase

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In recent years two forms of the mitochondrial monoamine oxidase, which selectively deaminates 5-hydroxytryptficities have been identified [1–4]. The A form of the oxidase, which selectively deaminates 5-hydroxy-tryptamine (5-HT) and norepinephrine, is selectively inhibited by clorgyline, and the B form of MAO, which selectively degrades phenylethylamine (PEA), is inhibited by deprenyl [5, 6]. Substrates which are deaminated exclusively by either the A or B forms of MAO display simple sigmoidal plots when inhibited by these selective inhibitors. In contrast, amines such as tyramine, which are metabolized by both forms of the oxidase, display a biphasic inhibition pattern when incubated in the presence of clorgyline or deprenyl.

The putative neurotransmitter, dopamine, has been reported to be deaminated in rat liver by both forms of the mitochondrial oxidase when inhibited by clorgyline, as evidenced by biphasic plots [7]. Yang and Neff [8] also reported that dopamine is deaminated by both the A and B forms of rat brain MAO. In contrast, Braestrup *et al.* [9] and Waldmeier *et al.* [10] reported that dopamine is primarily metabolized by the A form of rat brain MAO. However type B MAO of human brain was suggested to be the principle form which degrades this catecholamine [11, 12]. The 3-*O*-methylated derivative of dopamine, 3-methoxy-4-hydroxyphenylethylamine (3-MHPEA) has been suggested to be deaminated by both forms of MAO in rat liver [7], although a similar *O*-methylated derivative of benzylamine is primarily degraded by the B type of MAO [7].

Since an imbalance of brain dopamine has been implicated in the symptomatology of behavioral [13] and neurological disorders [14], the pathway by which this neurotransmitter is degraded may be important in regulating brain dopamine levels. Because conflicting reports [9–12] exist in the literature as to which form of MAO degrades dopamine, it was decided to re-examine the form of human brain MAO which deaminates this catecholamine and its catechol *O*-methyl transferase (COMT) degradative product, 3-MHPEA.

Human brain mitochondria obtained from frontal lobes were isolated as described previously [15]. These preparations were stored frozen at −20° in 0.1 M potassium phosphate buffer, pH 7.4. MAO was assayed by a modification of the procedure of Roth *et al.* [16]. In brief, reaction mixtures consisting of 0.125 mM ascorbic acid and varying amounts of clorgyline or deprenyl were preincubated in a total volume of 0.4 ml of 0.062 M potassium phosphate buffer, pH 7.4, for 12 min with human brain mitochondria prior to addition of radioactively labeled amine substrates. After addition of substrate, reaction mixtures were incubated for an additional 10 min when PEA was substrate, and 60 min when either dopamine, 5-HT or 3-MHPEA was employed as substrate. The concentrations of PEA and 5-HT used are near their reported K_m values [17, 18]. Deamination of amines was terminated by the addition of 50 μl of 0.4 M HCl, and 0.2-ml aliquots of the mixture were chromatographed through Bio-Rex 70 cation-exchange resin columns. The resin was washed with 2.8 ml water which was combined with the original 0.2 ml eluate, and to this solution 10 ml Aquasol was added. The radioactive deaminated product was measured by liquid scintillation spectrometry.

The effects of clorgyline on 5-HT, PEA, dopamine and 3-MHPEA deamination are illustrated in Fig. 1, panels A and B. As reported previously [11], clorgyline inhibits 5-HT and PEA metabolism in a sigmoidal, predictable manner based on the known relative specificities of this drug for the A and B forms of MAO. In contrast, both dopamine and 3-MHPEA are degraded in a biphasic pattern by clor-

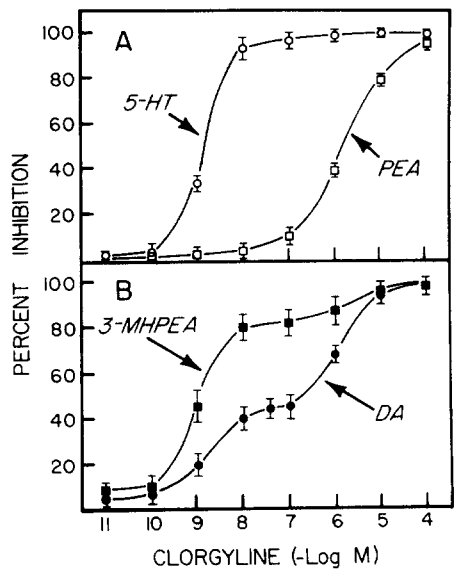

Fig. 1. Per cent inhibition of human brain mitochondrial deamination of (A) 5-hydroxytryptamine (5-HT) and phenylethylamine (PEA) and (B) dopamine (DA) and 3-methoxy-4-hydroxyphenylethylamine (3-MHPEA) at varying concentrations of clorgyline. Reaction mixtures consisting of human brain mitochondria and clorgyline were preincubated for 12 min prior to the addition of 5 μM [^{14}C]PEA or 10^{-4} M [^{14}C]5-HT, [^{14}C]DA or [^{3}H]3-MHPEA. Incubation time was 10 min with PEA and 60 min with the other substrates. In the absence of clorgyline, 82.7, 3.56, 29.3 and 30.4 nmoles of deaminated product were formed from deamination of 5-HT, PEA, DA and 3-MHPEA respectively.

Table 1. Effect of deprenyl on deamination of biogenic amines by human brain mitochondria

Amine	Product formed (μmoles ± S.E.)		
	− Deprenyl	+ Deprenyl	% inhibition
Phenylethylamine*	0.61 ± 0.02	0.02 ± 0.00	96.7
5-Hydroxytryptamine†	6.17 ± 0.42	5.95 ± 0.46	3.6
Dopamine†	9.32 ± 0.49	4.05 ± 0.10	56.5
3-MHPEA†	3.23 ± 0.04	2.99 ± 0.08	7.4

* Reaction mixtures consisting of 0.125 mM ascorbic acid and 0.03 mg protein in 0.062 M potassium phosphate buffer were preincubated for 12 min at 37° in the presence and absence of 10^{-7} M deprenyl prior to the addition of 5 μM [^{14}C]phenylethylamine. Incubations continued for an additional 10 min and were terminated by the addition of 50 μl of 0.4 M HCl. Deaminated product formed was assayed as described in the text.

† Assay conditions were similar to those described above, except that 0.14 mg protein was used, and the concentration of [^{14}C]dopamine, [^{14}C]5-hydroxytryptamine and [^{3}H]3-methoxy-4-hydroxyphenylethylamine (3-MHPEA) was 10^{-4} M. Incubation time with substrate was 60 min at 37°.

gyline. The plateau for inhibition of the latter amines occurs between 10^{-8} and 10^{-7} clorgyline. These plateau regions represent approximately 45 and 80 per cent inhibition of dopamine and 3-MHPEA deamination, respectively.

The above data suggest that both dopamine and its 3-*O*-methyl analog are degraded by both the A and B forms of human brain MAO. To verify this, additional studies were performed using the selective type B MAO inhibitor, deprenyl. Results of these experiments presented in Table 1 reveal that, at 10^{-7} M deprenyl, deamination of the B-selective substrate, PEA, was essentially totally prevented, whereas degradation of the type A MAO substrate, 5-HT, was inhibited less than 5 per cent. The magnitude of deprenyl inhibition of 3-MHPEA was similar to that of 5-HT. Dopamine, on the other hand, was inhibited midway between that of PEA and 5-HT.

The data in Table 1 also indicate that dopamine is more rapidly degraded by human brain MAO in the absence or presence of deprenyl than 3-MHPEA. In the absence of deprenyl, dopamine-deaminating activity is almost three times that of 3-MHPEA, whereas in the presence of this irreversible MAO inhibitor the activity is reduced to less than twice that of the 3-*O*-methylated derivative.

The results of this paper support numerous other reports which demonstrate that PEA and 5-HT are selectively inhibited by clorgyline and deprenyl respectively. In contrast to previous reports [11, 12], the data reported here reveal that dopamine is deaminated by both forms of human brain MAO, as indicated by the biphasic plot with clorgyline (Fig. 1B) and the sensitivity to deprenyl (Table 1). Similar results have been found by White and Glassman [19].

One possible explanation for the discrepancy between the results of Glover *et al.* [11] and those reported here is that Glover *et al.* [11] used caudate mitochondria, whereas mitochondria from the frontal cortex of human brain were used in the present study. The difference may also be due, in part, to the instability of type A of human brain MAO. We found this form of the human brain oxidase to be very sensitive to a number of procedures used to isolate or store mitochondria, including homogenization and freezing and thawing. Accordingly, during our experiments it was often necessary to use a fresh enzyme preparation to measure 5-HT deamination and to obtain biphasic inhibition plots (Fig. 1B) owing to loss of type A MAO activity. In this regard, it was interesting to find that the total diminution of dopamine-deaminating activity on storage of human brain mitochondria was equivalent only to the loss of the clorgyline-sensitive activity of MAO. No apparent conversion of the A form to the B form occurred. This suggests that, by comparison, the B form of MAO is a relatively stable enzyme and resists inactivation by storage at −20° or freezing and thawing procedures. White and Glassman [19] reported similar findings in that the A form of MAO of human brain was more susceptible to inactivation by a number of procedures used to solubilize and purify the oxidases.

In addition to dopamine, the 3-*O*-methyl analog was also examined for its selectivity for either type A or B of human brain MAO. Results reported here clearly demonstrate that 3-MHPEA is deaminated by both types of human MAO, but relative to its parent compound, 3-MHPEA is preferentially degraded by type A of the mitochondrial oxidase. In addition, the data also indicate that the rate of dopamine deamination by either the A or B type of human brain MAO is significantly greater than that for its 3-*O*-methylated derivative.

The concentration of dopamine in the human brain has been found to be inversely related to MAO activity but not to that of COMT [20]. This suggests that MAO may be the principle enzyme that inactivates dopamine in the human brain. Because of differences in the specificity of dopamine and its COMT metabolite, 3-MHPEA, for the two forms of human brain MAO, treatment with selective inhibitors of the oxidases may alter the metabolite pattern derived from dopamine. Since 3-MHPEA is preferentially deaminated by the type A MAO, it may be anticipated that, in the presence of a type A MAO selective inhibitor, this metabolite will increase significantly. On the other hand, dopamine and its deaminated metabolites may be differentially altered to a lesser extent by treatment with either a selective type A or B inhibitor of MAO in humans.

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Metabolism *in vivo* of carbon disulfide to carbonyl sulfide and carbon dioxide in the rat

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Carbon disulfide (CS_2) is metabolized to carbonyl sulfide (COS) by rat hepatic microsomes [1]. The reaction requires NADPH, is inhibited by carbon monoxide, and is stimulated by pretreatment of the rats with phenobarbital. Thus, it appears that the metabolism *in vitro* of CS_2 to COS is catalyzed by the cytochrome P-450 containing mono-oxygenase systems. Additional studies have indicated that COS is metabolized *in vitro* to CO_2, again in a reaction catalyzed by the rat hepatic cytochrome P-450 containing mono-oxygenase system [2]. Studies by DeMatteis and Seawright [3] have shown that $[^{14}C]CS_2$ is metabolized *in vivo* in rats to $[^{14}C]CO_2$. The purpose of the present studies was to determine if $[^{14}C]CS_2$, administered *in vivo* to rats, was also excreted in the breath as $[^{14}C]COS$.

In these experiments, untreated or phenobarbital-pretreated male Sprague–Dawley rats (250–275 g) were given $[^{14}C]CS_2$ dissolved in corn oil by i.p. injection and placed individually in a 3-l. metabolic apparatus. The phenobarbital-pretreated rats received i.p. injections of 50 mg/kg of sodium phenobarbital in distilled water for 5 days followed by administration of the $[^{14}C]CS_2$ 24 hr after the last injection. The expired air was drawn through a trap containing 200 ml of 1 N NaOH and, in turn, through two traps, each containing 35 ml of a 95% ethanol–diethylamine mixture (1:1, v/v). The airflow through the system was approximately 1.5 l./min. Three doses of $[^{14}C]CS_2$ were given; these were 0.0625, 0.125 and 0.250 m-mole/kg, representing 5.64, 11.28 and 22.55 μCi/kg respectively. There were three animals at each dose. The expired air was collected for 4 hr. Preliminary experiments indicated that expiration of radioactivity after administration of $[^{14}C]CS_2$ was essentially complete in 3 hr. DeMatteis and Seawright [3] reported that the exhalation of intraperitoneally administered $[^{14}C]CS_2$ was virtually complete in 4 hr.

Experiments in which $[^{14}C]CS_2$ was introduced into the metabolic apparatus indicated that only a trace of the $[^{14}C]CS_2$ was retained in the first trap (1 N NaOH). The majority was retained in the second trap (95% ethanol–diethylamine, 1:1, v/v) with a small amount appearing in the third trap, which contained the same solution as the second. Similar experiments using $[^{14}C]COS$ showed that about 28 per cent was retained by the first trap and the remainder was found in the second trap. No attempt was made to determine the distribution of $[^{14}C]CO_2$ among the various traps. However, the NaOH trap worked very efficiently for CO_2, and the possibility of spillover of $[^{14}C]CO_2$ into the second and third traps appeared remote. $[^{14}C]CO_2$ formation after administration *in vivo* of $[^{14}C]CS_2$ was determined by liquid scintillation counting of

Table 1. $[^{14}C]CO_2$ and COS content of expired air of untreated rats administered $[^{14}C]CS_2$*

Dose $[^{14}C]CS_2$ (m-mole/kg)	Amount $[^{14}C]CS_2$ administered (μmoles)	Amount $[^{14}C]CO_2$ excreted (μmoles)	Amount $[^{14}C]COS$ excreted (μmoles)	Amount $[^{14}C]CS_2$ excreted (μmoles)	Total recovery of administered dose (%)
0.0625	16.54 ± 0.93	1.82 ± 0.06	4.20 ± 0.19	4.94 ± 0.79	66.8 ± 11.2
0.125	32.52 ± 3.24	2.23 ± 0.08	4.32 ± 0.38	12.59 ± 1.78	59.5 ± 11.4
0.250	60.57 ± 4.86	4.06 ± 0.25	4.48 ± 0.20	43.99 ± 6.89	86.5 ± 4.42

* Each value is the mean ± S.D. of the data obtained from three rats.