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DISTRIBUTION AND INHIBITION CHARACTERISTICS OF HUMAN BRAIN MONOAMINE OXIDASE

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Abstract—Monoamine oxidase (MAO) activity in 14 regions of 10 normal post-mortem human brains using 5-hydroxytryptamine (5HT), benzylamine, tyramine and dopamine as substrates is presented. Regional distribution with 5HT, benzylamine and tyramine was generally similar with the highest activities observed in the hypothalamus. However, with dopamine as substrate, highest MAO activity occurred in the nucleus accumbens. Although there was relatively greater MAO activity towards 5HT than towards benzylamine in all four cerebral cortical areas studied compared with the caudate, putamen, accumbens and hypothalamus this apparently greater proportion of type A MAO in cortex could not be confirmed with the use of the specific inhibitor clorgyline. In some cases inhibition curves with clorgyline (and correspondingly with deprenyl) were not the expected double sigmoid shape. It is suggested that characterisation of MAO by techniques dependent on the use of specific inhibitors in samples of human brain collected and stored in the usual manner may prove difficult to interpret.

Johnston [1] has classified monoamine oxidase (MAO, EC 1.4.3.4) as type A or type B on the basis of *in vitro* studies of rat brain mitochondria with the MAO inhibitor clorgyline (*N*-methyl-*N*-propargyl-3-[2,4-dichlorophenoxypropylamine]). *In vitro* type A MAO is relatively sensitive to inhibition by clorgyline [1] and type B MAO to deprenyl (phenylisopropylmethylproprinylamine) [2]. Noradrenaline [3] and 5-hydroxytryptamine (5HT)[1] are preferentially deaminated by type A MAO and benzylamine [4] and β-phenylethylamine [5] by type B MAO. Tyramine is deaminated by both forms of the enzyme [1]. There is some evidence of species variation; dopamine has been reported to be a substrate for type A MAO in rat [6] and for type B MAO in man [7]. The usefulness of the type A or type B classification of the enzyme has been questioned by Fowler *et al.* [8].

In addition to the evidence for two major forms of MAO several distinct bands of monamine oxidase activity have been separated by polyacrylamide gel electrophoresis [9] and cellulose acetate electrophoresis[10]. One such band seemed to display specific dopamine deaminating characteristics (see Youdim [11]).

The differential *in vivo* metabolism of biogenic amines in rat brain after administration of specific MAO inhibitors has been demonstrated by Neff *et al.* [12] and differential MAO activity towards 5HT and β-phenylethylamine by distinct populations of intact mitochondria from rat brain has been reported by Owen *et al.* [13].

However, Houslay and Tipton [14] have suggested that the substrate preferences and inhibition characteristics of type A and type B MAO as well as the electrophoretically separable forms of the enzyme are attributable to the adherence to the enzyme of differing quantities of lipid or membraneous material.

There have been several studies of the characteristics and distribution of human brain monoamine oxidase in normal and disease states [7, 15–26]. However, it is arguable that in these studies the heterogeneity of MAO has not been taken fully into account or that the investigations have been carried out on a small number of samples or in only a few brain regions. Therefore, as part of a larger comparative study of the characteristics of MAO in controls and schizophrenics, we have investigated the activity of MAO in 14 brain areas in post-mortem tissue from 10 mentally normal subjects using 5HT, benzylamine, tyramine and dopamine as substrates in the assay procedure. in addition we have studied some characteristics of human brain MAO with the specific inhibitors clorgyline and deprenyl.

MATERIALS AND METHODS

Post-mortem material. Brains were obtained at autopsy from 10 (5 females, 5 males) mentally normal individuals (age ± S.D. = 76.4 ± 6.9 yr) who had died from a variety of natural causes. Estimated time (mean ± S.D.) between death and arrival at the mortuary was 2.5 ± 0.6 hr, length of time at 4° in mortuary before autopsy was 47.9 ± 22.3 hr and length of storage of samples at −45° was 258 ± 100 days.

Immediately after removal from the cranium, the brain was frozen at −45° in a polystyrene mould that retained approximate brain shape to facilitate later dissection. Prior to dissection brain temperature was raised to about −10° by placing the brain in a cold room (+2°) for several hours. Brains were then sliced coronally with a domestic meat slicer (Robert Krups (GB) Ltd.) into 0.5–1 cm slices. The sections were placed on aluminium foil in a cooling tray (Chemlab Instruments Ltd) and whilst still frozen the required areas were dissected out. Samples were then finely diced

and thoroughly mixed to ensure homogeneity and stored at −45° until assay.

Homogenates, for assay of MAO activity, were prepared by homogenising 10–20 mg of tissue in 1.5 ml of 0.1 M phosphate buffer pH 7.2 using an MSE motorised homogeniser.

Brain biopsy material. Human temporal cortex was obtained during therapeutic neurological surgery and stored immediately at −45°.

Chemicals. 5-Hydroxytryptamine creatinine sulphate, benzylamine, tyramine HCl and dopamine HCl were obtained from Sigma (London). Benzylamine, free base, was converted to the hydrochloride and re-crystallised from ethanol. [^{14}C]-5-hydroxytryptamine creatinine sulphate, [^{14}C]-tyramine HCl, [^{14}C]-dopamine HCl were obtained from the Radiochemical Centre, Amersham, England and [^{14}C]-benzylamine HCl from ICN Pharmaceuticals, California, U.S.A. Clorgyline was a gift from May and Baker Ltd., Dagenham, Essex and deprenyl from Magyar, Hungary. All other chemicals were obtained through British Drug Houses Ltd., Poole, England, and were of the highest purity available.

Assay of brain MAO. MAO activity was assayed in brain homogenates by a radiometric technique similar to that described by Robinson *et al.* [27] with 5-hydroxytryptamine, benzylamine, tyramine and dopamine as substrates. Michaelis constants of human cortical MAO for the four substrates were determined from double reciprocal plots and were respectively 1.8×10^{-4} M, 1.2×10^{-4} M, 3.4×10^{-4} M, and 1.2×10^{-4} M. Human brain MAO activity determinations were carried out at the following substrate concentrations: 5-hydroxytryptamine, 2 mM; benzylamine, 1.5 mM; tyramine, 3 mM; and dopamine, 1.5 mM. There was no evidence of substrate inhibition at these concentrations. Reaction mixtures consisted of 50 μl of brain homogenate, radioactive substrate at the required concentration (specific activities were 0.3 μCi/μmole for 5-hydroxytryptamine, benzylamine and dopamine and 0.16 μCi/μmole for tyramine) made up to a final volume of 200 μl with 0.1 M phosphate buffer pH 7.2. Incubations were carried out in plastic Eppendorf tubes (open-topped) at 37° for 1 hr. Under these conditions reaction velocity was linear with respect to time for at least 1 hr and with respect to protein concentration of brain homogenates over the range assayed. Reactions were terminated by the addition of 50 μl 6N HCl. Reaction products of 5-hydroxytryptamine, tyramine and dopamine were extracted into 500 μl of ethyl acetate:toluene (1:1, v/v) by vigorous shaking for 5 min and the deaminated products of benzylamine were similarly extracted into 500 μl of toluene. After a brief centrifugation (8000 *g* for 2 min—Eppendorf Centrifuge) 250 μl of solvent was added to 10 ml of Triton based toluene scintillant for quantification of products by scintillation counting. Extraction efficiencies of the solvents were determined by repeated extraction and were: 5-hydroxytryptamine, 83 per cent; benzylamine, 95 per cent; tyramine, 88 per cent; and dopamine, 76 per cent. All results were corrected for losses during the extraction procedure. Homogenate protein was determined by a phenol reagent method [28] and results were expressed as nmoles of product formed/mg protein/hr.

Inhibition studies. MAO activity was determined as described with various concentrations (10^{-3} M–10^{-10} M) of clorgyline or deprenyl. Prior to assay, brain homogenates were incubated at 37° for 30 min in the presence of the inhibitor but in the absence of substrate. Substrate concentration was 1 mM and time of incubation was 30 min.

RESULTS

The activity of MAO towards 5HT, benzylamine, tyramine and dopamine in the 14 brain regions is presented in Table 1.

An interesting feature of the results was the relatively greater MAO activity towards 5HT than towards benzylamine in all cerebral cortical areas studied compared with caudate, putamen, accumbens and hypothalamus. We investigated this strong suggestion of a greater proportion of type A MAO in cortex in samples of occipital cortex and caudate from six brains by using the specific type A MAO inhibitor, clorgyline. The inhibition of MAO activity by various concentrations of clorgyline was determined as described in Materials and Methods and the inhibition curves (mean ± S.D. obtained with six samples of caudate and six samples of

Table 1. MAO activity of various regions of 10 human brains using four substrates*

Brain region	5-Hydroxytryptamine	Benzylamine	Tyramine	Dopamine
Temporal cortex	36 ± 5	43 ± 6	100 ± 22	15 ± 3
Parietal cortex	37 ± 5	30 ± 5	97 ± 15	18 ± 5
Frontal cortex	44 ± 5	41 ± 6	121 ± 28	28 ± 6
Occipital cortex	56 ± 16	46 ± 11	98 ± 25	29 ± 10
Cingulum	53 ± 16	68 ± 19	193 ± 61	19 ± 10
Hypothalamus	82 ± 21	131 ± 32	214 ± 45	34 ± 8
Hippocampus	69 ± 14	82 ± 15	134 ± 20	32 ± 6
Caudate	47 ± 6	86 ± 15	145 ± 27	30 ± 4
Putamen	44 ± 4	85 ± 12	137 ± 34	17 ± 5
Accumbens	68 ± 7	130 ± 24	189 ± 23	51 ± 5
Thalamus	51 ± 4	59 ± 12	112 ± 15	28 ± 5
S. nigra	56 ± 7	58 ± 12	113 ± 18	14 ± 4
Cerebellum	25 ± 8	16 ± 5	38 ± 6	4 ± 1
Amygdala	71 ± 11	76 ± 10	196 ± 23	19 ± 3

* Results are expressed as nmoles of product formed/mg protein/hr.

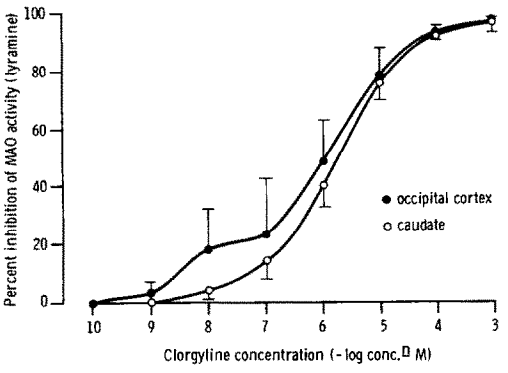

Fig. 1. Mean clorgyline inhibition curves (for human caudate and occipital cortex ($n = 6$, only one S.D. is shown for clarity).

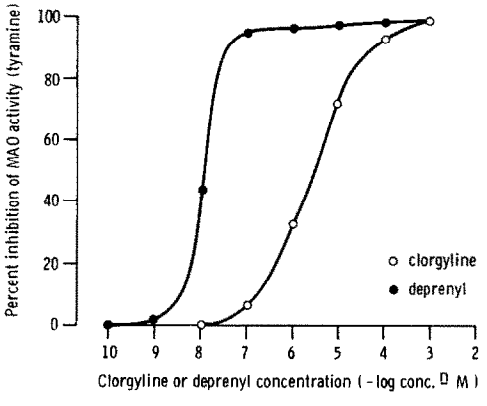

Fig. 2. Single sigmoid clorgyline and deprenyl inhibition curves for human occipital cortex.

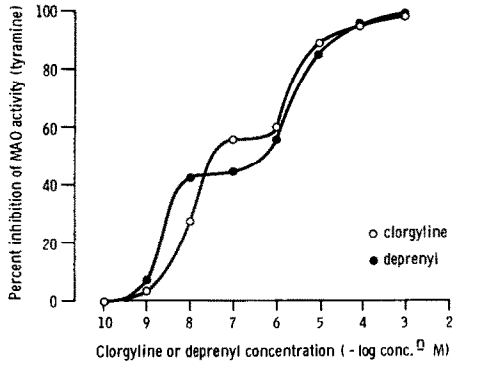

Fig. 3. Double sigmoid clorgyline and deprenyl inhibition curves for human occipital cortex.

Table 2. Correlation matrix of MAO activities using 5HT, benzylamine, tyramine and dopamine as substrates

Benzylamine	0.80†		
Tyramine	0.80†	0.83†	
Dopamine	0.64*	0.73*	0.55(NS)
	5HT	Benzylamine	Tyramine

* $P < 0.05$.
† $P < 0.01$.

occipital cortex are presented in Fig. 1. There was no statistically significant difference between the degree of inhibition of MAO activity in caudate or occipital cortex at any inhibitor concentration. It was noteworthy that the mean clorgyline inhibition curve for samples of caudate was not the expected double sigmoid curve. This resulted from two samples of caudate exhibiting single sigmoid clorgyline inhibition curves. Samples of occipital cortex from these two brains also produced single sigmoid inhibition curves although their effect was not sufficient to abolish the double sigmoid shape of the mean curve for six brain samples. The single and double sigmoid inhibition curves obtained with clorgyline as inhibitor were verified in similar experiments with deprenyl as inhibitor. Figures 2 and 3 illustrate examples of the results which showed that when single or double sigmoid inhibition curves were obtained with clorgyline as inhibitor, similar shaped curves were respectively obtained with deprenyl as the inhibitor. The two samples of occipital cortex which produced uncharacteristic inhibition curves had MAO activities (nmoles/mg protein/hr) towards 5HT of 61.9 and 68.2 and towards benzylamine of 45.2 and 52.0. For the respective samples of caudate, the MAO activities towards 5HT were 58.8 and 45.8 and towards benzylamine were 84.2 and 81.2 (nmoles/mg protein/hr). When these activities were compared with the group as a whole it was clear that there was no selective loss of a particular type of MAO although Fig. 2 shows inhibition curves similar to those expected of type B MAO only. In addition a sample of human cortical biopsy material, deep frozen immediately after surgical removal yielded MAO activities with 5HT and benzylamine as substrates (43.2 and 46.7 nmoles/mg protein/hr respectively) closely similar to the post mortem results.

DISCUSSION

In the present study the distribution of brain MAO activity observed was in broad agreement with earlier reports [16, 20, 22, 23] and was generally similar with 5HT, benzylamine and tyramine as substrates, with highest activities in the hypothalamus and nucleus accumbens with lowest activity in the cerebellum. With dopamine as substrate there were some differences in the distribution of activity with the highest activity in the nucleus accumbens. Table 2 is a correlation matrix of the activity of MAO towards the four substrates. With the exception of dopamine vs tyramine, MAO activity towards all four substrates taken in pairs was significantly correlated. MAO activity with dopamine as substrate correlated best with enzyme activity with benzylamine as substrate ($r = 0.73$, $P < .01$) which lends some support to the suggestion that dopamine is a type B MAO substrate in man [7].

Schwartz *et al.* [23] using 5HT and phenylethylamine as substrates for type A and type B MAO respectively found a constant ratio of MAO activity towards the two substrates in several regions of human brain. In our study using 5HT and benzylamine as type A and type B substrates respectively we noted that in four areas of cortex the benzylamine 5HT ratio was approximately 1 whereas in the caudate, putamen and accumbens the ratio was about 2. We could not, however, confirm this relatively greater proportion of type

Table 3. Post-mortem brains data

Sex	F	F	F	M	M	F
Age (yr)	77	73	90	79	74	80
Time at room temp. (hr)	2	2	3	3	2	3
Time at 4° (hr)	75	35	23	26	44	72
Time at −40° (days)	369	356	331	335	342	361
Cause of death	a	b	c	a	d	d
Plateau	no	no	yes	yes	yes	yes

a—myocardial infarction; b—alveolitis; c—cancer; d—pneumonia.

A MAO in cortex with the use of the specific inhibitor clorgyline. Moreover, we did not always obtain the characteristic double sigmoid curve when percentage MAO inhibition was plotted against clorgyline concentration. This unexpected finding was verified using deprenyl as the specific inhibitor (Figs. 2 and 3). Johnston [1] pointed out that dialysis of rat brain mitochondria for up to 72 hr resulted in a gradual disappearance of the double sigmoid curve consistent with a loss of one form of the enzyme. Johnston also noted a large overall drop in enzyme activity. In the present study this did not seem to be the case since activities towards 5HT and benzylamine were similar in post mortem samples producing single or double sigmoid inhibition curves and also in one sample of human brain biopsy material. There was insufficient biopsy sample to carry out inhibition experiments. Details of the six brains on which inhibition studies were carried out are presented in Table 3.

There was no obvious relationship between cause of death, age, sex or storage parameters and whether or not a characteristic inhibition curve with a plateau resulted. In this respect it is interesting to note that Schwartz *et al.* [23] published an uncharacteristic inhibition curve for clorgyline inhibition of the deamination of β-phenylethamine by human brain homogenates. It seems likely that events after death, i.e., collection and storage, cause alterations in the lipid or membraneous environment of monoamine oxidase in some brain samples but not in all, which is sufficient to result in uncharacteristic inhibition curves with clorgyline and deprenyl as the inhibitor. Nevertheless, the conditions of collection and storage of the samples in the present study are not unusual and using specific inhibitors to study the characteristics of monoamine oxidase in human brains collected in this manner may produce results that are difficult or impossible to interpret in terms of the type A, type B classification of the enzyme.

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