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FURTHER STUDIES ON THE INHIBITION OF MONOAMINE OXIDASE BY M & B 9302 (CLORGYLINE)*—I

SUBSTRATE SPECIFICITY IN VARIOUS MAMMALIAN SPECIES

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Abstract—Evidence is presented concerning the nature of the substrate specificity of the monoamine oxidase (MAO) complex from rat liver and brain, utilising a non-hydrazine inhibitor, M & B 9302. The binary nature of the MAO complex is discussed in the light of the results.

MAO from various mammalian species has been examined. It was possible to demonstrate the presence of two MAO enzymes from the brains of rat, man, rabbit, ox, dog and cat and from the livers of rat and man. The MAO from the brain of the pig and the livers of all species examined other than rat and man, appeared, from the evidence, to be homogeneous.

JOHNSTON[1] reported some observations with the new inhibitor of monoamine oxidase (MAO), M & B 9302. He described how the plot of percentage inhibition against concentration of M & B 9302 does not show a simple sigmoid curve, but a pair of sigmoid curves joined by a horizontal section where the inhibition is invariant. He concluded from these curves that rat brain MAO is a binary system of enzymes each of which has a detectably different sensitivity to this particular inhibitor.

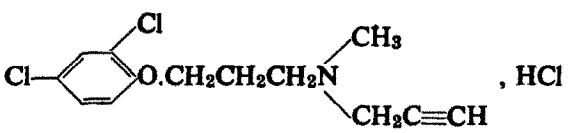
Observations are continually being reported, which indicate that MAO is not a single enzyme, but a multiplicity of amine oxidases which catalyse the oxidation of various substrates.[2–4]

There have also been a number of recent reports that certain inhibitors only inhibit a part of the MAO complex from various tissues.[5–8] Recently Yondrin and Sandler[9] have separated purified rat liver MAO into three bands of enzyme activity.

The authors have made some further observations on the multiplicity and substrate specificity of MAO from various mammalian species as they are elucidated by the inhibitor M & B 9302.

MATERIALS AND METHODS

M & B 9302, *N*-methyl-*N*-propargyl-3(2,4-dichlorophenoxy)propylamine hydrochloride (I) was prepared in the research laboratories of May & Baker Ltd., Dagenham.



(I)

* *N*-Methyl-*N*-propargyl-3(2,4-dichlorophenoxy)propylamine hydrochloride

[1-^{14}C]Tyramine (specific activity, 43·7 mc/mM) was obtained from the Radiochemical Centre, Amersham, Bucks.

Benzylamine hydrochloride (Ward Blenkinsop Ltd.) was recrystallised twice from alcohol.

Preparation of MAO

Partially purified preparations of rat tissue were used for the Warburg assays. Rats were killed by a blow on the head and the brain and liver were removed and plunged immediately into liquid nitrogen. The tissues were homogenised with 5 volumes (w/v) of 0·067 M phosphate buffer, pH 7·2 for 3 min in an MSE top drive homogeniser. The homogenate was centrifuged for 20 min at 35,000 *g* and the precipitate was resuspended in the same buffer (2 volumes w/v). All operations were carried out at 2–4° and the partially purified preparation was stored at 2°. The preparation contained particulate MAO as well as cell debris, nuclei and mitochondria.

For the radiochemical assays a crude homogenate of brain or liver was used. The tissue was homogenised as described above, and then diluted with the same phosphate buffer (1:20 for brain, 1:100 for liver).

Warburg assay of MAO

The Warburg assay of Johnston[1] was used.

Radiochemical assay of MAO

The method used is a modification of that of Wurtman and Axelrod.[10] It is based upon the estimation of the deaminated metabolites formed when [1-^{14}C] tyramine is incubated with MAO. The tissue homogenate (5 ml) was preincubated for 16 min at room temperature with 1 ml of the appropriate inhibitor solution. 1 ml of the mixture was then added to a stoppered test tube containing 25 μl [1-^{14}C] tyramine (5×10^{-2} M, 62·5 mμc) and incubated for 20 min at 37°. The reaction was stopped by the addition of 0·3 ml 2 N hydrochloric acid. The deaminated metabolites were extracted into 7 ml toluene by vigorous shaking for 1 min. After being centrifuged to separate the emulsion, 4 ml of the upper, toluene layer was added to 5 ml of NE 213 scintillation fluid (Nuclear Enterprises Ltd.) and the activity was counted in a liquid scintillation spectrometer. The activity was then expressed as a percentage of a control sample that contained no inhibitor. From this the percentage inhibition was calculated and plotted against inhibitor concentration.

RESULTS

Johnston[1] suggested that the MAO complex from rat brain is composed of two enzymes designated arbitrarily as enzyme A and enzyme B, where enzyme A is highly sensitive to M & B 9302 and enzyme B is relatively insensitive. He indicated that 5-hydroxytryptamine is a substrate for enzyme A only. In a search for a substrate which is acted on only by enzyme B a number of amines were tested by the Warburg method of assay for substrate activity. (Table 1)

The more active substrates were tested with different concentrations of M & B 9302 and the enzyme inhibition curve was plotted. Where a double enzyme curve consisting of two sigmoid curves joined by a horizontal plateau was obtained (plateau-shaped curve) the substrate was presumed to have been oxidised by both enzymes. Where a

TABLE 1. COMPARISON OF VARIOUS SUBSTRATES FOR RAT BRAIN PARTICULATE MAO

Substrate*	Activity (μl O_2/10 min/g brain)
Dopamine	34·0
Tyramine	30·0
5-Hydroxytryptamine	42·0
Tryptamine	40·0
Benzylamine	10·0
2-Phenethylamine†	22·0
1-Phenethylamine†	12·0
1,1-Diphenylethylamine	4·0
2-Methylbenzylamine†	14·0
4-Methylbenzylamine†	18·0

* All concentrations were 5×10^{-2}M, except tryptamine (5×10^{-3}M).
† Rates decreased rapidly after 10 min.

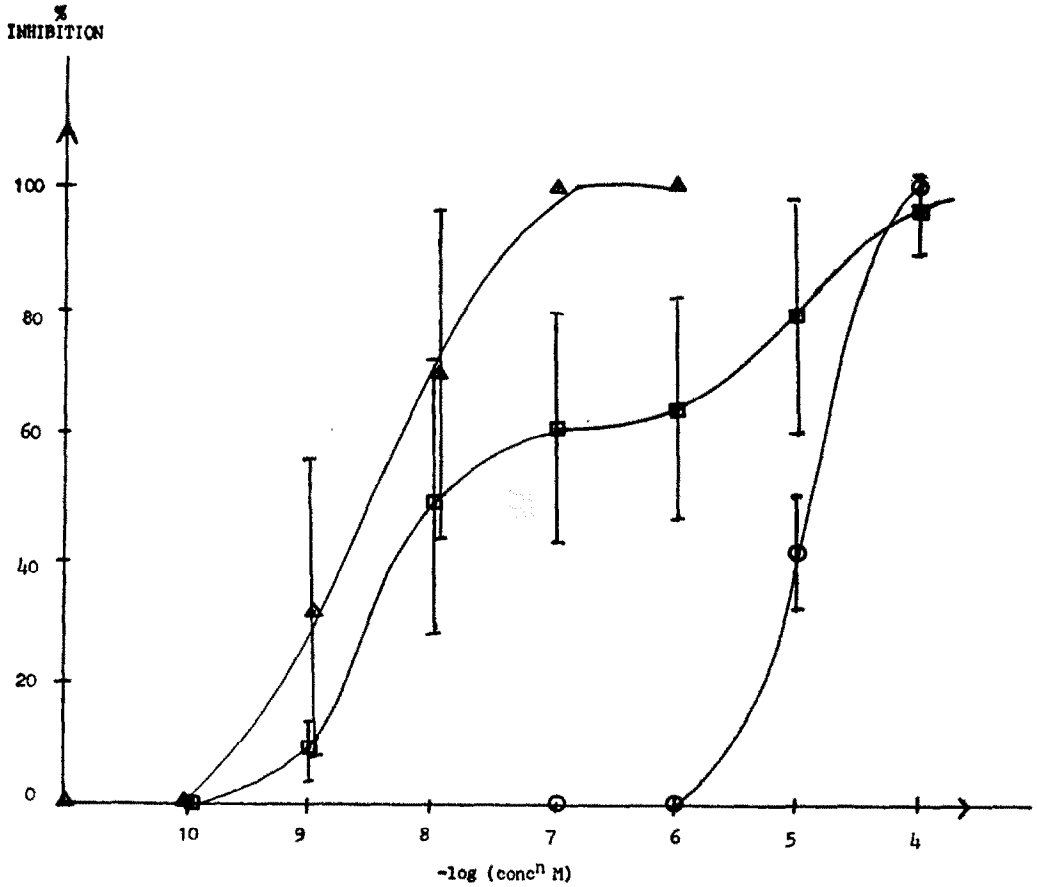


FIG. 1. Inhibition of M & B 9302 for rat brain MAO (standard deviations shown). Key: ○ Substrate —benzylamine; □ Substrate—tyramine; △ Substrate—5-hydroxytryptamine.

TABLE 2. THE TYPE OF ENZYME INHIBITION CURVE PRODUCED BY THE ACTION OF M & B 9302 ON RAT BRAIN PARTICULATE MAO WITH DIFFERENT SUBSTRATES

Substrate	Type of inhibition curve
Dopamine	Plateau-shaped
Tyramine	Plateau-shaped
5-Hydroxytryptamine	Sigmoid (Enzyme A)
Tryptamine	Plateau-shaped
Benzylamine	Sigmoid (Enzyme B)

(See text for explanation of types of inhibition curves.)

single sigmoid curve was obtained the sensitivity to M & B 9302 indicated whether the substrate had been oxidised by enzyme A or enzyme B. (Table 2.)

Benzylamine hydrochloride was found to be the best substrate for enzyme B only. Figures 1 and 2 show the enzyme inhibition curves for tyramine, 5-hydroxytryptamine and benzylamine with rat brain and rat liver MAO and M & B 9302.

Inhibition curves using equimolecular mixtures of benzylamine hydrochloride and

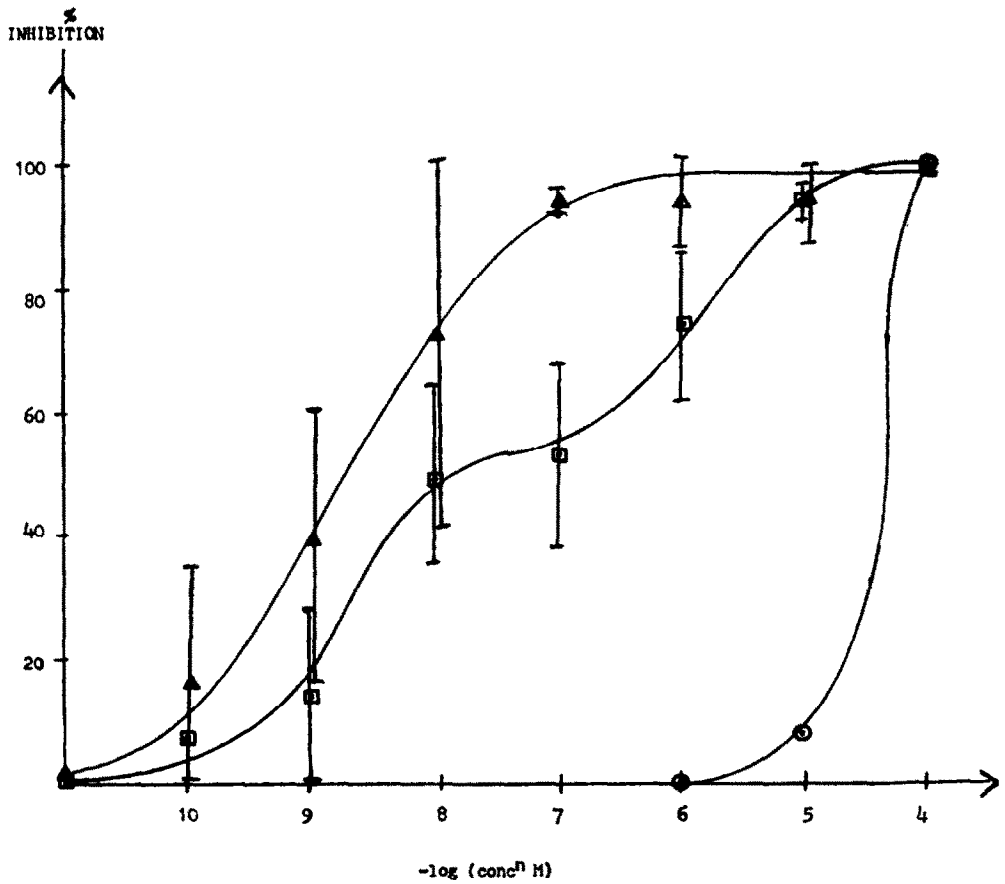


FIG. 2. Inhibition curves of M & B 9302 for rat liver MAO (standard deviations shown). Key as in Fig. 1.

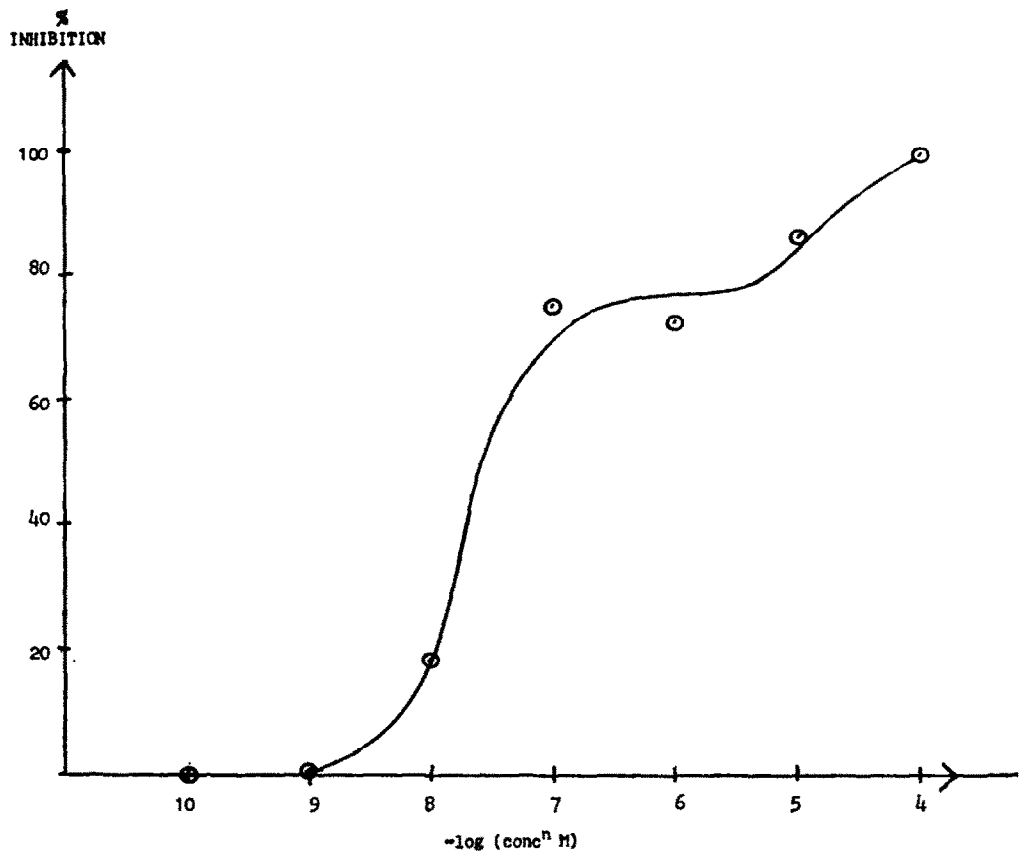


FIG. 3. Double substrate inhibition curves of M & B 9302 for rat brain MAO. Benzylamine hydrochloride (5×10^{-2}M) and 5-hydroxytryptamine creatinine sulphate (5×10^{-2}M) were substrates. Activity of MAO was measured by oxygen consumption using 'total particulate' preparations. (Average of two experiments.)

TABLE 3. TYPE OF ENZYME INHIBITION CURVE PRODUCED BY THE ACTION OF M & B 9302 ON HOMOGENATE MAO FROM VARIOUS SPECIES

Animal	Tissue	Type of inhibition curve
Rat	Brain	Plateau-shaped
Rat	Liver	Plateau-shaped
Cat	Brain	Plateau-shaped
Cat	Liver	Sigmoid
Dog	Brain	Plateau-shaped
Dog	Liver	Sigmoid
Human	Brain	Plateau-shaped
Human	Liver	Plateau-shaped
Pig	Brain	Sigmoid
Pig	Liver	Sigmoid
Ox	Brain	Plateau-shaped
Ox	Liver	Sigmoid
Rabbit	Brain	Plateau-shaped
Rabbit	Liver	Sigmoid

(See text for explanation of types of inhibition curves.)

5-hydroxytryptamine (5×10^{-2} M) are shown in Figs. 3 and 4. With both rat brain and rat liver a plateau-shaped curve was found with the plateau appearing at a much higher percentage inhibition level than was found with other single substrates.

Variation of MAO between species

Tissue homogenates of various species were assayed with different concentrations of M & B 9302 by the radiochemical method. Tyramine was used as substrate. Table 3 indicates the types of inhibition curves obtained.

From Table 3 it can be seen that the composition of MAO's from some tissues and species varies from that from others. Brain and liver from man, as well as brain from

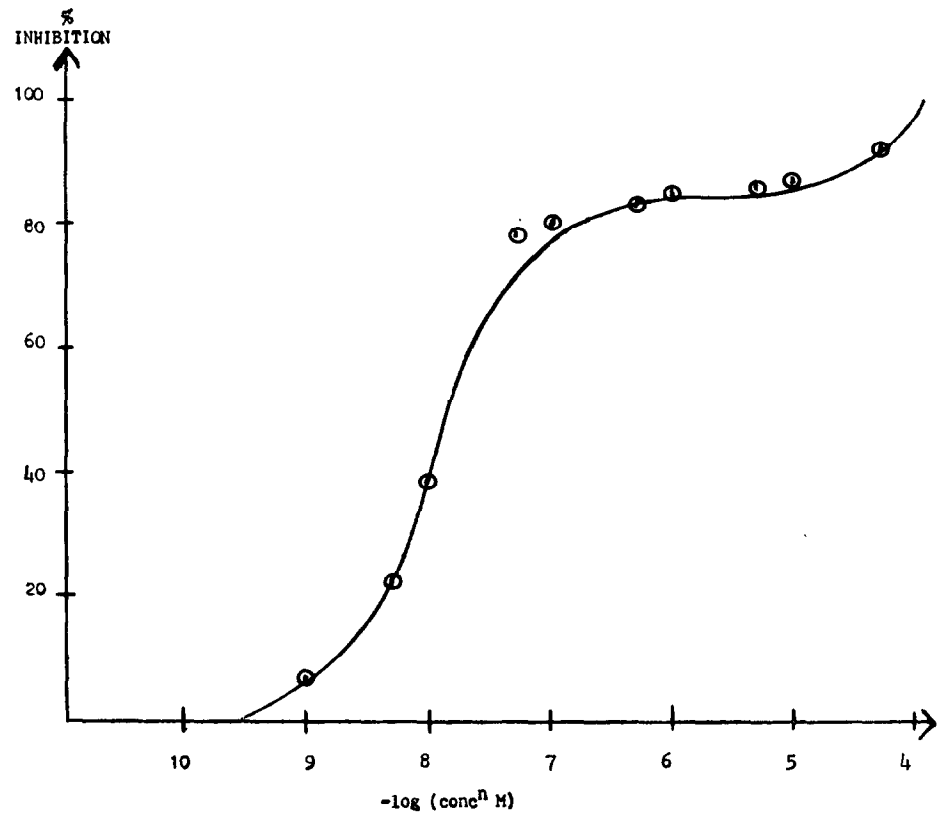


FIG. 4. Double substrate inhibition curves of M & B 9302 for rat liver MAO. Benzylamine hydrochloride (5×10^{-2}M) and 5-hydroxytryptamine creatinine sulphate (5×10^{-2}M) were substrates. Activity of MAO was measured by oxygen consumption using 'total particulate' preparations. (Average of two experiments.)

cat, dog, rabbit and ox gave the familiar plateau-shaped curve that was obtained with rat brain and liver. Both brain and liver of pig and the livers of cat, dog, rabbit and ox gave a single sigmoid curve between concentrations of M & B 9302 from 10^{-4} M to 10^{-7} M. These tissue homogenates, however, were able to oxidise 5-hydroxytryptamine. When this 5-hydroxytryptamine oxidation was inhibited by M & B 9302, a sigmoid curve was obtained with 10^{-5} M to 10^{-8} M M & B 9302.

DISCUSSION

These results on the effect of M & B 9302 on the oxidation of various substrates by MAO confirm the conclusions of Johnston[1] that MAO from rat brain and also from rat liver, and cat, dog, rabbit and ox brain are a binary system of enzymes, each of which has a different sensitivity to M & B 9302 and therefore gives a plateau-shaped inhibition curve. Figures 1 and 2 indicate the different substrate specificity of these two enzymes. One is active upon tyramine, and 5-hydroxytryptamine; the other upon tyramine, and benzylamine. Thus, 5-hydroxytryptamine and benzylamine are specific substrates for each component of the binary enzyme system. When these two substrates are mixed in equimolecular proportions, a plateau-shaped inhibition curve is obtained, Figs. 3 and 4, indicating that both substrates are acted upon independently and the oxidations are inhibited independently. The elevated plateau, indicating about three times as much activity on 5-hydroxytryptamine as on benzylamine can be predicted from Table 1 where it is seen that 5-hydroxytryptamine is a much better substrate than benzylamine. Evidence of the delicate nature of the MAO complex comes from the tryptamine curves. With tryptamine a plateau-shaped curve is obtained; whilst 5-hydroxytryptamine affords a simple sigmoid curve. The action on these two biogenic amines by MAO is very interesting in that a single hydroxyl group changes the specificity towards the two enzymes.

Variation of MAO between species

Analysis of the inhibition curves obtained from the MAO from various species yields information on whether or not a homogenous enzyme exists. Table 3 shows that rat and human brain and liver are very similar in showing the double enzyme system; however with cat, dog, rabbit and ox only the brain enzyme appears to be a binary system, the liver enzymes giving a simple sigmoid curve indicative of homogeneity. The reason for this may be that M & B 9302 is not differentiating between two enzymes or it may be that only one enzyme is present. The latter hypothesis seems to be more acceptable. It is unlikely that these single enzymes are similar to either the A or the B enzymes from rat brain, since, although the position of the sigmoid curves obtained indicates the presence of only the B enzyme, the enzyme is nevertheless active upon 5-hydroxytryptamine which has already been described as a specific substrate for the A enzyme. The hypothesis would be that there is a single enzyme present in these tissues suited to metabolise all substrates as necessary. Gorkin and Tatyanenko[11] also found that rat and human liver MAO differ from ox liver MAO. In the case of the pig, both brain and liver appear to contain a single enzyme. The pig contrasts with all other animals studied. Recent evidence of Tipton and Spires[12] also indicates that pig brain MAO is homogeneous.

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