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

COMPARISON OF M & B 9302 INHIBITION WITH THAT OF IPRONIAZID

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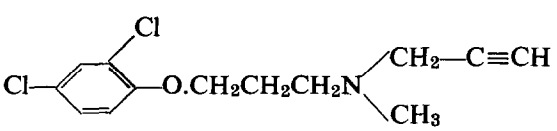
Abstract—The mode of action of two monoamine oxidase (MAO) inhibitors, M & B 9302, *N*-methyl-*N*-propargyl-3(2,4-dichlorophenoxy)propylamine hydrochloride, and iproniazid (*N*-isonicotinyl-*N'*-isopropylhydrazine), is compared. Unlike inhibition by iproniazid, there was no lag phase before the onset of inhibition by M & B 9302 nor was the inhibition affected by cyanide or copper ions.

JOHNSTON[1] and Hall, Logan and Parsons[2] have described the abnormal inhibitory kinetics obtained with M & B 9302. 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. These curves are thought to indicate the binary nature of the rat brain MAO. Johnston[1] found normal inhibitory kinetics with iproniazid; the plot of percentage inhibition against concentration of inhibitor giving a simple sigmoid curve.

MATERIALS AND METHODS

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

M & B 9302  , HCl

(I)

Iproniazid, *N*-isonicotinyl-*N'*-isopropylhydrazine, was obtained from Roche Products Ltd., Welwyn Garden City. 8-Hydroxyquinoline (May & Baker Ltd.), was recrystallized twice from aqueous ethanol.

Preparations of MAO were carried out as described previously[2] and MAO estimations were made by the Warburg method described by Johnston[1] and the radiochemical method described by Hall, Logan and Parsons.[2]

RESULTS

Effect of preincubation on the inhibition of MAO produced by M & B 9302 and iproniazid

Figure 1 shows the effect of preincubation of enzyme and inhibitor on the inhibition of tyramine oxidation by 10^{-7} M-M & B 9302 and 10^{-5} M-iproniazid. The radiochemical method of estimation of MAO activity was used. M & B 9302 needed no preincubation with the enzyme for the inhibition to develop; however, iproniazid inhibition increased with up to 20 min preincubation with the enzyme.

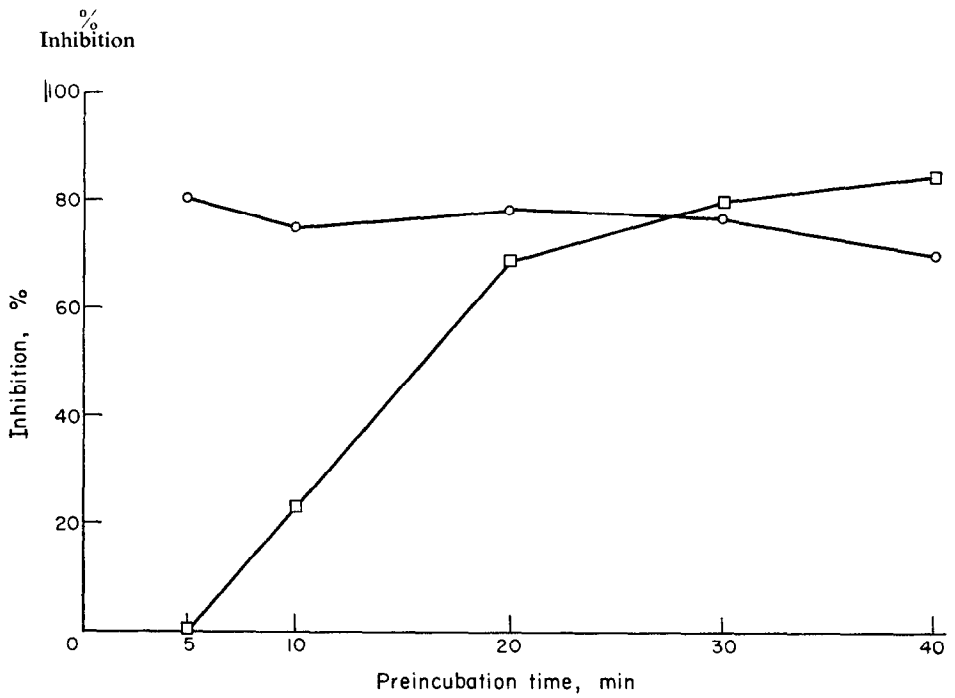


FIG. 1. Effect of preincubation on iproniazid and M & B 9302 with rat brain MAO. Activity of MAO was measured by radiochemical estimation of the products of reaction using 'total particulate' preparations. Substrate used—1-^{14}C-Tyramine hydrochloride.

Key:
○ M & B 9302 10^{-7} M
□ Iproniazid 10^{-5} M
See text for further details of method.

Effect of cyanide ions on the inhibition of MAO by M & B 9302 and iproniazid

Figures 2 and 3 show the effect of preincubation of the inhibitors with 10^{-3} M KCN instead of with enzyme. Iproniazid incubated alone for 40 min was an inefficient inhibitor. When preincubated for 40 min with enzyme a sigmoid curve with an I_{50} of about 5×10^{-6}M was obtained. A similar curve and I_{50} were obtained when iproniazid was preincubated instead with cyanide ions. Cyanide ions had no effect on the inhibition of tyramine oxidation by M & B 9302.

FIG. 2 and 3. Effect of preincubation with potassium cyanide on iproniazid and M & B 9302. Activity of MAO was measured by oxygen consumption using 'total particulate' preparations. Substrate was tyramine. Potassium cyanide was present at 10^{-3} M.
Key:
○ Preincubation, for 40 min, of the inhibitor with 10^{-3} M KCN.
□ Preincubation, for 40 min, of the inhibitor alone.
△ Preincubation, for 40 min, of the inhibitor with the enzyme.
For further experimental details see text.

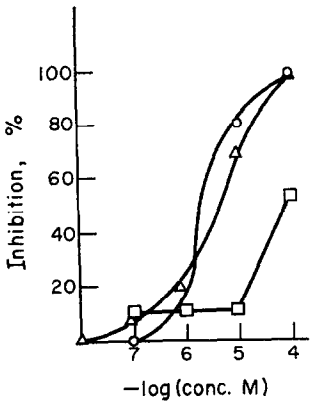


FIG. 2. Iproniazid.

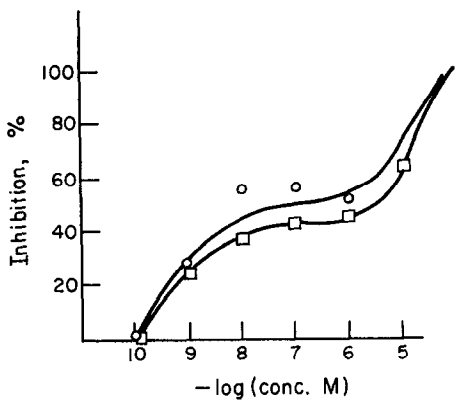


FIG. 3. M & B 9302.

Effect of cupric ions on the inhibition of MAO by M & B 9302, iproniazid and 8-hydroxyquinoline

Severina and Gorkin[3] reported that MAO inhibition by 8-hydroxyquinoline can be relieved by divalent ions. This property of this inhibitor was compared with inhibition by M & B 9302 and iproniazid. Inhibitors and cupric chloride were present in a concentration of 10^{-3} M. 5×10^{-2} M tyramine was used as substrate. Table 1 shows the effect of cupric ions on the inhibition.

The inhibition due to both 8-hydroxyquinoline and iproniazid was to some extent relieved by cupric ions. Inhibition by M & B 9302 was unaffected by cupric ions.

DISCUSSION

Previous papers have demonstrated the mode of inhibition of hydrazine inhibitors

TABLE 1. EFFECT OF CUPRIC CHLORIDE ON THE INHIBITION PRODUCED BY VARIOUS SUBSTANCES*

Substance	% Inhibition
8-Hydroxyquinoline	74·3
8-Hydroxyquinoline + Cu^{++}	24·5
Iproniazid	100
Iproniazid + Cu^{++}	65·8
M & B 9302	100
M & B 9302 + Cu^{++}	100
Cu^{++}	88·3

* Activity of MAO was measured by oxygen consumption using particulate rat brain MAO. Tyramine (5×10^{-2}M) was substrate. 8-Hydroxyquinoline, iproniazid, M & B 9302, and cupric chloride (Cu^{++}) were present in a concentration of 10^{-3}M.

of MAO in rat brain.[4–6] The present work was to compare this type of inhibition with that of M & B 9302. Initial work[1] with M & B 9302 demonstrated that it is very different in its effect on MAO, and evidence is now presented which confirms these differences.

This study has confirmed that for maximal inhibition of MAO by iproniazid preincubation is necessary.[7, 8] This can be adequately explained if it is assumed that iproniazid must first be converted into an 'active principle'.[4, 5, 9–11] Various suggestions as to the nature of this 'active principle' have been made. On the other hand M & B 9302 needs no such preincubation and by analogy is thought to inhibit in its original state.

It has been reported that cyanide ions affect hydrazine type inhibitors.[5, 6] The present work confirms that cyanide has a catalytic action on the conversion of iproniazid into its 'active principle'. Again, M & B 9302 shows no effect when preincubated with cyanide. Cyanide appears to be analogous to MAO in that the 'active principle' is produced when iproniazid is preincubated in their presence.

8-Hydroxyquinoline is a chelating agent of divalent ions. It is also the most powerful quinoline derivative known to date to inhibit MAO. It has been reported that Co^{++}, Ni^{++}, Zn^{++}, and Fe^{++} all relieve the inhibition produced by 8-hydroxyquinoline.[3] However, no evidence has been produced for cupric ions. The present results clearly show that Cu^{++} relieves the inhibition produced by 8-hydroxyquinoline. It is of interest that the inhibition produced by iproniazid is also relieved, although to a lesser extent. Therefore, iproniazid, or its 'active principle', has the ability to form non-inhibitory chelate complexes. Further evidence for this comes from Table 1 where it is seen that cupric ions alone strongly inhibit MAO, and one would expect at least as much inhibition from Cu^{++} and iproniazid together. This is not the case, indicating that at least some of the Cu^{++} and some of the iproniazid are used up in the formation of the chelate complex. Green[6] suggests that cupric ions can catalyse the conversion of iproniazid into its inhibitory products, and he suggests that copper present in the MAO complex may cause the conversion of iproniazid into its 'active principle'. We have shown, however, that excess of copper relieves this type of inhibition. In the case of M & B 9302 there is no such relief and it is unlikely that a chelating inhibitory mechanism is involved.

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