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Inhibition of rabbit mitochondrial monoamine oxidase by iprindole*

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The action of tricyclic antidepressant drugs has been attributed to inhibition of neuronal reuptake of the biogenic amines, norepinephrine (NE) and/or 5-hydroxytryptamine (5-HT), in brain [1–3]. This pharmacological property is common to the majority of the known antidepressant drugs in clinical use today. However, it has recently been shown that the antidepressant agent, iprindole [5-(3-dimethylaminopropyl)-6,7,8,9,10,11-hexahydro-5-H-cyclooct [*b*] indole–HCl; WY-3263], is much less effective than other tricyclic antidepressant drugs in preventing the reuptake of catechol or indole amines in rat and mouse brain and heart tissue [4–7]. Also, unlike the action of other tricyclic antidepressants, iprindole failed to alter the concentration of 5-HT in human platelets [8]. Thus, the mechanism of the clinical mode of action of this tricyclic drug is not consistent with the hypothesis described above.

It has been shown in several laboratories, including our own, that tricyclic antidepressant drugs inhibit mitochondrial monoamine oxidase (MAO) [9–11]. We recently reported that the antidepressant, imipramine, reversibly inhibited both type A and B forms of rabbit mitochondrial MAO. The type B form of the oxidase was further shown to be more susceptible to inhibition by this drug than was the type A form. Since inhibition of MAO by tricyclic antidepressant drugs may contribute to the clinical action of these substances, it was of interest to examine the effect of iprindole on the activity of both forms of the oxidase.

Male albino rabbits weighing approximately 2 kg were used in all experiments. Preparation of brain mitochondria and the assay used for MAO activity have been described previously [11]. In brief, reaction mixtures containing 1·8 μM ^{14}C-β-phenylethylamine or ^{14}C-5-hydroxytryptamine and varying amounts of inhibitor were incubated with rabbit brain mitochondrial MAO at 37° for varying lengths of time. The ^{14}C-deaminated products formed were separated from the amine starting material by cation-exchange (Bio Rex-70) chromatography and the radioactivity of effluents containing the deaminated products was measured in a liquid scintillation spectrometer (Packard TriCarb model 3320). All experiments were repeated at least twice.

5-Hydroxytryptamine-2-^{14}C creatinine sulfate (sp. act., 58 mCi/m-mole) was purchased from Amersham Searle Co., Arlington Heights, Ill. and β-phenylethylamine-2-^{14}C–HCl (sp. act., 7 mCi/m-mole) was purchased from New England Nuclear Corp., Boston, Mass. Iprindole was a gift from Dr. Michael R. Maise of Wyeth Laboratories, Philadelphia, Pa.

The effect of iprindole on the deamination of phenylethylamine (PEA), a specific type B MAO substrate [12], and 5-

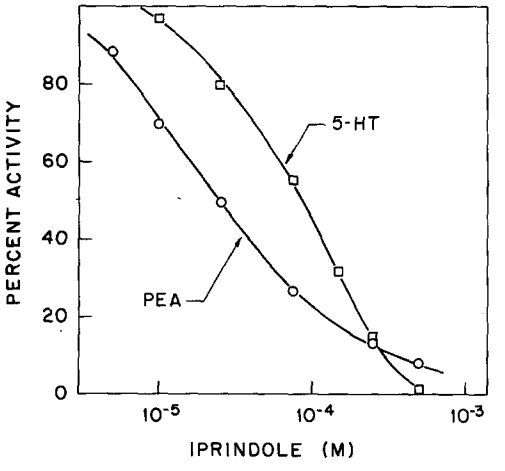

Fig. 1. Reaction mixtures containing 3·6 nmoles ^{14}C-PEA or ^{14}C-5-HT, 0·45 mg protein and varying concentrations of iprindole in a total of 2 ml of 0·05 M potassium phosphate buffer, pH 7·4, were incubated at 37° for 5 and 60 min respectively. The amounts of phenylacetic acid formed in the absence of drug were 1·10 and 0·32 nmoles respectively.

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Table 1. Drug concentration producing 50 per cent inhibition of PEA and 5-HT deamination*

Inhibitor	PEA (M)	5-HT (M)
Iprindole	$2{\cdot}5 \times 10^{-5}$	9×10^{-5}
Imipramine[11]	$2{\cdot}5 \times 10^{-5}$	10×10^{-5}
Amitriptyline	$3{\cdot}0 \times 10^{-6}$	9×10^{-5}

* Reaction mixtures contained 3·6 nmoles PEA or 5-HT and varying amounts of drug and mitochondrial protein in 2 ml of 0·05 M potassium phosphate buffer, pH 7·4. The value for drug concentration producing 50 per cent inhibition was obtained from graphs for each inhibitor as presented in Fig. 1.

HT, a specific type A MAO substrate [13], is illustrated in Fig. 1. Under the conditions used, 50 per cent inhibition of PEA and 5-HT deamination is achieved at iprindole concentrations of approximately $2{\cdot}5 \times 10^{-5}$ M and 9×10^{-5} M respectively. These data indicate that, at concentrations less than 2×10^{-4} M of this tricyclic antidepressant drug, the type B form of the oxidase is more susceptible to inhibition than is the type A form of MAO.

A comparison of the concentrations of iprindole and two widely used antidepressant drugs, imipramine and amitriptyline, required to inhibit PEA and 5-HT deamination by 50 per cent is presented in Table 1. These results indicate that the ability of iprindole to inhibit the type A and B forms of rabbit MAO is similar to that of imipramine. Amitriptyline, however, is considerably more potent as an inhibitor of the type B form of the oxidase than either of the other two antidepressant agents, although the ability of amitriptyline to inhibit the A form of MAO is similar to that of the other tricyclic drugs.

It has been shown previously that imipramine reversibly binds to MAO and that inhibition of PEA and 5-HT deamination could only be detected at substrate concentrations near or less than that of the inhibitor [11]. This may partly explain why Gluckman and Baum [4] had previously failed to notice any decrease of tyramine deamination by iprindole. In their experiments they used a 50-fold excess of substrate (0·05 M tyramine) compared to that of iprindole (0·001 M). Results of experiments in this paper reveal that a 15- and 50-fold excess of the drug concentration is required to inhibit 1·8 μM PEA and 5-HT by 50 per cent respectively.

Mosnaim *et al.* [14] and Fischer *et al.* [15] have suggested that a depletion of endogenous PEA may be one of the biochemical lesions in depression. Mosnaim *et al.* [16] have shown that the mean concentration of PEA in rabbit brain increases after acute or chronic treatment with either imipramine or iprindole. Whether or not an increase in brain PEA is actually involved in alleviating the symptoms of depression, the data obtained by Mosnaim *et al.* [16] are consistent with the view that imipramine and iprindole can also inhibit the type B form of MAO *in vivo*.

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Competitive inhibition of glucuronidation by *p*-hydroxyphenyl hydantoin

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5-(*p*-hydroxyphenyl)-5-phenylhydantoin (HPPH), the major diphenylhydantoin metabolite, as well as diphenylhydantoin itself, is present in hepatic tissues [1] and homogenates [2] of animals treated with diphenylhydantoin. It is