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Letter to the Editors

Demethylation Studies—II. The Competitive Inhibition of Microsomal *N*-Demethylating Enzyme by a Primary Amine, 2,4-Dichloro-6-phenylphenoxyethylamine (DPEA)

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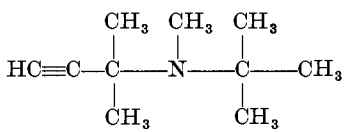
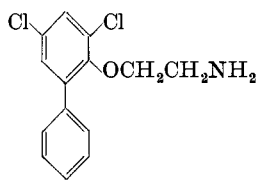
Sir:

Enzymes present in the submicroscopic particles of mammalian liver oxidatively *N*-demethylate a wide variety of lipid-soluble substrates.[1] These enzymes are inhibited by certain lipid-soluble tertiary amines, notably SKF 525A (β-diethylaminoethyl diphenylpropylacetate)[2,3] and Lilly 18947 (2,4-dichloro-6-phenyl-phenoxyethyl-diethylamine).[4]

Recently we have been studying the demethylation of a series of tertiary aliphatic amines[5,6] that possess hypotensive activity.[7] One of these, butynamine [3-methyl-3-(*N*-methyl-*t*-butylamino)-butyne-1] (I) has been selected for further study as a substrate for oxidative demethylation.



(I)



(II)

Since butynamine is readily demethylated in whole animals as well as in the isolated enzyme system, we investigated the effect of inhibitors on this enzymatic reaction. We found that 2,4-dichloro-6-phenylphenoxyethylamine (DPEA) (II), the primary amine analogue of Lilly 18947, is a more potent inhibitor than either Lilly 18947 or SKF 525A. For example, when butynamine

was present at an initial concentration of 1×10^{-3}M, it was found that the following concentrations of inhibitors produced a 50 per cent reduction in the rate of demethylation by a rat microsome preparation:[8] DPEA, $1 \cdot 4 \times 10^{-6}$M; SKF 525A, 5×10^{-6}M; Lilly 18947, 6×10^{-6}M; and iproniazid, $2 \cdot 5 \times 10^{-4}$M. Further work has shown that DPEA is also more active as an inhibitor of the *in vitro* demethylation of many other substrates. It is also more potent than Lilly 18947 or SKF 525A in prolonging the depressant effects of hexobarbital in mice.

The nature of the inhibition produced by compounds of this type is still in question although Brodie[1] has suggested that they are probably 'true enzyme inhibitors', and do not act through a

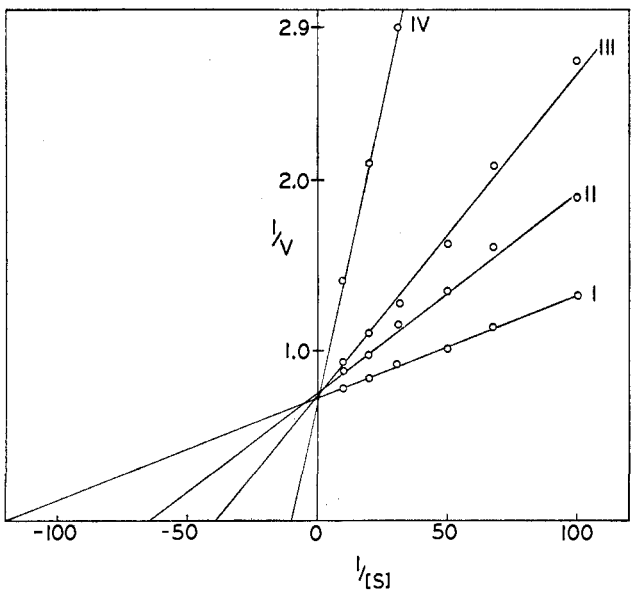


FIG. 1. Conditions: Incubation flasks contained 25 μM nicotinamide, 25 μM $MgCl_2$, soluble plus microsome fraction from 200 mg rat liver, 0·25 μM TPN+, 11 μM glucose 6-phosphate, 45 μM semicarbazide, 0·5 ml of 0·25M phosphate buffer, pH 7·4, substrate and sufficient water to bring the volume to 3 ml. [S] is the concentration of butynamine in moles/litre. V is the rate of demethylation measured in micromoles of formaldehyde formed in 20 min at 37° in air. The concentrations of inhibitor were as follows: curve I, none; curve II, 5×10^{-6}M; curve III, 1×10^{-5}M; and curve IV, 5×10^{-5}M.

physical effect upon microsomes, but probably interact directly with the enzyme within the microsome. By an analysis of the kinetics of substrate–inhibitor interaction, it is clear that DPEA is a competitive inhibitor of the oxidative demethylation of butynamine as shown in the Langweaver–Burke[9] plot (Fig. 1). It is of considerable interest that a primary amine should be so successful as a competitive inhibitor of an enzyme system which acts predominantly upon tertiary amines. In this connection it was observed that DPEA at a concentration of 1×10^{-5}M produces a 45 per cent inhibition in the rate of demethylation of its own *N*,*N*-dimethyl analogue (2,4-dichloro-6-phenylphenoxyethyl-*N*,*N*-dimethylamine) present at an initial concentration of 1×10^{-3}M.

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