PHENETHYLAMINE ISOSTERES AS INHIBITORS OF DOPAMINE β-OXIDASE

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The enzyme, dopamine β-oxidase, which catalyzes the conversion of dopamine to norepinephrine, has recently been shown to catalyze the hydroxylation of many phenethylamine compounds to form the corresponding β alcohol derivatives (Pisano et al., 1960; Levin and Kaufman, 1961; Bridgers and Kaufman, 1962; and Creveling et al., 1962). The relative nonspecificity of the enzyme suggested that isosteres of phenethylamine of the type, ϕ-C-X-NH_2, may also have an affinity for the enzyme and serve either as substrates or inhibitors. It was found that benzylhydrazine (ϕ-CH_2-NH-NH_2) was indeed a potent inhibitor of dopamine β-oxidase, far more potent than non-isosteric hydrazine derivatives (Creveling et al., 1962). This report presents evidence that benzylhydrazine, benzyloxyamine (ϕ-CH_2-O-NH_2) and related compounds, are inhibitory by virtue of their isostere relationship with phenethylamine and discusses the mechanism of the inhibition.

Dopamine β-oxidase was purified from beef adrenal glands according to the procedure of Kaufman et al. (1960). In these studies the enzyme obtained at the gel eluate stage was used. Enzyme activity was assayed by following conversion of tyramine

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to norsynephrine according to the procedure of Creveling et al., (1962).

In Table I are summarized data on the inhibition of dopamine β-oxidase by a variety of benzylhydrazines and benzyloxyamines. The relative activities of comparable phenethylamine substrates are shown for comparison. It can be seen that substitutions on the ring and side chain influence the inhibitory action of the isosteres in the same general way that they influence the substrate activity of the phenethylamines. Thus, substitution of a hydroxy group in the para position of the ring increases both activities. O-Methylation of the para hydroxy group produces a marked reduction in both activities. Substitution of a hydroxy group in the ortho position abolishes all activity. Substitution of electropositive substituents elsewhere on the ring has little effect on the activities. Substitution of a single methyl group on the terminal nitrogen lowers both activities but only to a limited extent. However, substitution of two methyl groups on the terminal nitrogen abolishes both activities. Lengthening the side chain to form non-isosteric compounds lowers both activities to a marked extent. Hydrazine and hydroxylamine substituents on several other ring systems (naphthalene, furane, pyrane, and pyrazine) were found to be without effect as inhibitors. In like manner, ethylamine substituents on other ring systems have been found to be inactive as substrates (Creveling et al., 1962).

The inhibitory action of these benzyloxyamines and benzylhydrazines is apparently associated with a direct interaction with the enzyme rather than indirectly through reaction with a cofactor. It is apparent from the data in Table I that this is not a simple case of enzyme inactivation due to hydrazine or hydroxylamine compounds. The possibility of interaction with

TABLE I

A Comparison of Substrates and Inhibitors of Dopamine β-Oxidase

ϕ-$\overset{\beta}{C}H_2$-$\overset{\alpha}{C}H_2$-NH_2	Relative Activity	ϕ-$\overset{\beta}{C}H_2$-N'N-NH_2	% Inhibition at 10^{-5} M	ϕ-$\overset{\beta}{C}H_2$-O-NH_2	% Inhibition at 10^{-5} M
Unsubstituted	50	Benzylhydrazine	63	Benzyloxyamine	60
p-OH (tyramine)	100	-	-	p-OH	79
p-OCH_3	2-5	-	-	p-OCH_3*	0.2
m-OH	75	-	-	m-OH**	64
m-OH,α-CH_3	50	m-OH,N'-CH_3**	64	-	-
o-OH	0	o-OH,N'-CH_3**	0	-	-
p-OH,m-OCH_3	60	p-OH,m-OCH_3**	47	-	-
p-OCH_3,m-OH	2-5	p-OCH_3,m-OH**	0	-	-
p-OH,α-CH_3	65	-	-	-	-
p-OH,β-CH_3	10	-	-	β-CH_3	17
3,4,5-tri-OCH_3	2-5	-	-	3,4,5-tri-OCH_3	3
p-OH,N-CH_3	25	-	-	N-CH_3	18
p-OH,N(CH_3)$_2$	0	-	-	N(CH_3)$_2$*	0
γ-phenylpropylamine	2	-C-C-N-N	0	-C-C-O-N	7
		-C-C(-C)-N-N*	4	-C-C(-C)-O-N	0

Substrate activities are those reported in a previous communication (Creveling et al., 1962). Activity with tyramine is arbitrarily taken as 100.

Inhibition was determined as previously described (Creveling et al., 1962). Enzyme was preincubated with the inhibitor for 15 min., following which tyramine was added and incubation continued for an additional 10 min.

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the ascorbic acid was also ruled out by showing that the inhibition was unaffected over wide ranges of ascorbic acid concentration.

In order to obtain maximal inhibition it was necessary to preincubate the inhibitors with the enzyme for about 15 minutes. With no preincubation, the inhibition produced by these compounds was prevented by substrate and over the short intervals of time used in these studies gave the appearance of a competitive inhibition (figure I). Following preincubation the inhibition could not be reversed by the addition of substrate. Furthermore, the combination of benzyloxyamine with dopamine β-oxidase did not dissociate on dilution and activity could not be restored on prolonged dialysis. These results indicate that initially benzyloxyamine and the substrate were in competition for the same enzyme site but that once the benzyloxyamine was bound to the enzyme it reacted in some manner so that the inhibition then became characteristic of a noncompetitive, irreversible inhibitor. Consistent with the idea that benzyloxyamine and tyramine combine with the same enzyme site was the finding that the inhibition is affected by pH in the same manner as tyramine oxidation, the optimum for both being between pH 5.5 and 6.0. These findings are similar to those reported by Davison (1957) for the inhibition of monoamine oxidase by isopropylisonicotinyl hydrazine (iproniazid, Marsilid).

It thus appears that benzyloxyamine and benzylhydrazine analogues, by virtue of their isostere relationship with phenethylamine, are uniquely structured to inhibit dopamine β-oxidase. This property should make them valuable in studies on dopamine β-oxidase *in vitro* and *in vivo*. Studies by Kuntzman *et al.* (1962) suggest that benzylhydrazine analogues can inhibit norepinephrine synthesis in animals *in vivo*. Preliminary studies in this laboratory have corroborated their findings and have also demonstrated *in vivo* effects with benzyloxyamines. The irreversible nature of the inhibition should make it possible to employ these compounds

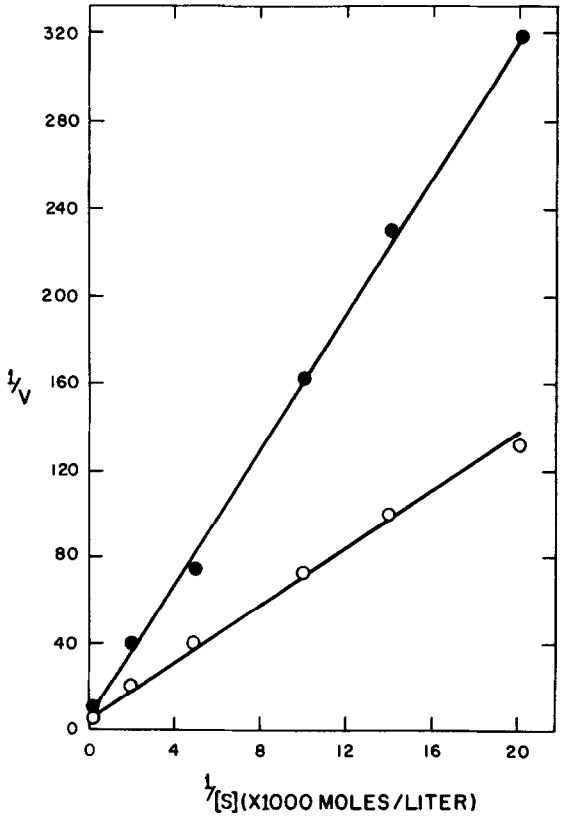

Fig. 1 - Tyramine was used as substrate with, ●——● and without benzyloxyamine (10^{-5} M) o——o; substrate and inhibitor were added simultaneously and incubation allowed to proceed for 10 minutes.

as pharmacologic agents in studies on the biochemistry and pharmacology of the sympathetic nervous system.

The nature of this enzyme inhibition is being investigated in greater detail.

REFERENCES

Bridgers, W. F., and Kaufman, S., J. Biol. Chem., 237, 526 (1962).
Creveling, C. R., Daly, J. W., Witkop, B., and Udenfriend, S., Biochim. Biophys. Acta, in press (1962).
Davison, A. N., Biochem. J., 67, 316 (1957).
Kuntzman, R., Costa, E., Creveling, C. R., Hirsch, C., and Brodie, B. B., Life Sciences 1, 85 (1962).
Levin, E. Y., Levenberg, B., and Kaufman, S., J. Biol. Chem., 235, 2080 (1960).
Levin, E. Y., and Kaufman, S., J. Biol. Chem., 236, 2043 (1961).
Pisano, J. J., Creveling, C. R., and Udenfriend, S., Biochim. Biophys. Acta, 43, 566 (1960).