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Inhibition of histidine decarboxylase by benzyl and aliphatic aminooxyamines

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THE DECARBOXYLATION of L-histidine by the specific enzyme, histidine decarboxylase (L-histidine-carboxy-lyase, EC 4.1.1.22), results in the formation of histamine. Studies on histidine decarboxylase and on the physiological importance of histamine have been facilitated by the recent development of more sensitive techniques for measuring histidine decarboxylase activity and by the discovery of inhibitors of this enzyme.[1–4] In particular, the potent inhibitor, 4-bromo-3-hydroxybenzyloxyamine dihydrogen phosphate (NSD-1055), has been studied recently and shown to inhibit histidine decarboxylase *in vitro*[5] and histamine synthesis in man.[6] The present report describes the inhibition *in vitro* of rat fetal histidine decarboxylase by a series of aromatic and aliphatic aminooxyamines.

The specific histidine decarboxylase was prepared from whole fetal rats (20–21 days of gestation) by Levine's modification[1] of Hakanson's method,[7] using acetate buffer at pH 5·5 instead of pH 4·5. The supernatant II fraction was used.

The incubation mixture consisted of 0·5 ml of the enzyme preparation (3–6 mg protein), 0·2 ml of $3{\cdot}7 \times 10^{-4}$ M pyridoxal-5-phosphate (PLP), 0·2 ml of $1{\cdot}0 \times 10^{-3}$ M streptomycin sulfate, 0·2 ml of 1·0 M phosphate buffer, pH 6·8, and 0·05 ml of $1{\cdot}25 \times 10^{-3}$ M L-histidine-^{14}C (New England Nuclear or Calbiochem), inhibitor, and water to a total volume of 2 ml. The substrate was added last and the entire mixture was incubated for 90 min in a rotary shaker at 37°. The decarboxylation was found to be linear for 90 min; therefore this length of time was used in order to increase the sensitivity of the assay.

The reaction vessel was similar to that described for measuring 3,4-dihydroxyphenylalanine decarboxylase activity[8] and is shown in Fig. 1. The enzymatic reaction was stopped by tipping in 0·3

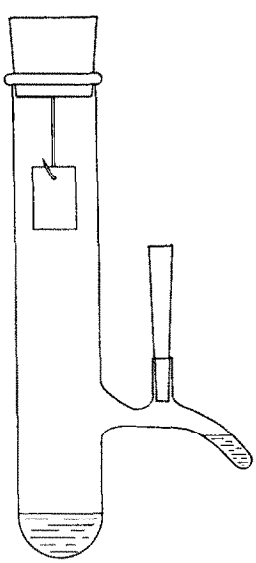


FIG. 1. Reaction vessel.

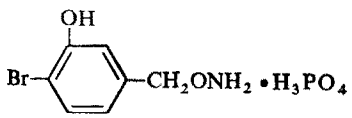
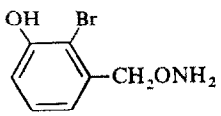
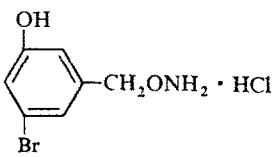
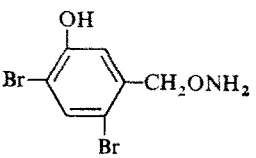
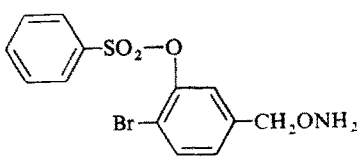
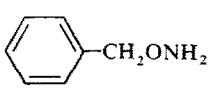
ml of 18% perchloric acid or 0·2 ml of 6 NHCl contained in the side-arm and the $^{14}CO_2$ was collected for 30 min. The $^{14}CO_2$ was quantitatively absorbed on to a 20 × 17 mm piece of filter paper moistened with NCS reagent (Nuclear-Chicago) and attached to a metal hook inserted in a rubber stopper. A 1½ in. hypodermic needle, bent to form the hook, was found to be more convenient than the glass hook previously described.[8] The radioactivity was determined by liquid scintillation counting after

placing the filter paper directly into Bray's solution.[9] The molar I_{50} values were obtained from a plot of the points obtained from the mean of duplicate analyses of at least three inhibitor concentrations.

The benzyloxyamines were obtained from Smith & Nephew Research Ltd. and the aliphatic oxyamines were a gift of Dr. J. A. Brockman of these laboratories.

The effects of various benzyloxyamines as well as of two aliphatic aminooxy compounds on rat fetal histidine decarboxylase activity *in vitro* are listed in Table 1. NSD-1055 was the most potent inhibitor, giving 50 per cent inhibition at 1×10^{-9} M. The 2-bromo and 5-bromo compounds (II and III) were about 100-fold less potent, inhibiting at 10^{-7} M. The 2,4-dibromo compound (IV) was also less potent than the NSD-1055. In addition, substitution of a benzenesulfonyl group on the 3-hydroxyl (V) or the removal of the 4-bromo and 3-hydroxyl groups (VI) markedly reduced potency.

TABLE 1. STRUCTURAL FORMULAE OF VARIOUS BENZYLOXYAMINES AND ALIPHATIC OXYAMINES AND THEIR MOLAR I_{50} VALUES FOR FETAL HISTIDINE DECARBOXYLASE

	Compound	Molar I_{50}	Relative potency
I.	 (NSD-1055)	1×10^{-9}	100·0
II.		1×10^{-7}	1·0
III.		2×10^{-7}	0·5
IV.		8×10^{-8}	1·2
V.		1×10^{-7}	1·0
VI.		5×10^{-7}	0·2
VII.	$NH_2OCH_2CH_2COOH$	8×10^{-7}	0·1
VIII.	NH_2OCH_2COOH	4×10^{-6}	<0·1

The fact that the 4-bromo-3-hydroxyl analog is most active may indicate certain steric requirements for binding of the inhibitor to the enzyme. The relatively poorer inhibition of the benzenesulfonyl group may reflect the inability of the bulkier group to enter the binding site of the enzyme.

The two aliphatic aminooxy compounds (VII and VIII) were less inhibitory than the aromatic aminooxy compounds. These aliphatic compounds cause elevation in the γ-aminobutyric acid content of the brain of animals.[10] Aminooxyacetic acid (VIII) inhibits the γ-aminobutyric acid-α-ketoglutaric acid transaminase activity *in vivo*, but does not affect the activity of the glutamic acid decarboxylase.[11] Both enzymes, found exclusively in the central nervous system, however, are inhibited *in vitro* by these compounds and, like histidine decarboxylase, are also PLP-dependent enzymes. The inhibition of the benzyloxyamines is, however, not simply due to a competition with PLP. NSD-1055 also inhibits dopamine-β-hydroxylase, which does not require PLP.[12] Preliminary kinetic studies indicate that the inhibition by NSD-1055 is competitive with substrate as well as with PLP. In addition, Dr. E. E. Snell (personal communication) has found that the molar I_{50} for NSD-1055 with the crystalline histidine decarboxylase from *Lactobacillus* 30*a* is about 10^{-3} M. This bacterial enzyme has been shown to be devoid of PLP by microbiological, enzymatic and spectrophotometric tests.[13] Further studies on the detailed mechanism of inhibition of the various aminooxy compounds and studies of theiri nhibitions *in vivo* are in progress.

Finally, the reaction vessel and procedure used for the assay of histidine decarboxylase, which was also used to assay nonspecific decarboxylase activity, is a useful, simple and rapid procedure for the measurement of the enzyme by the release of $^{14}CO_2$.

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Oxidative degradation of diazinon by rat liver microsomes*

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