adenosine-5′-monophosphate (AMP). The fact that this latter enzyme also is involved in purine synthesis in the formation of IMP from 5′-phosphoribosyl-5-formamidoimidazole-4-carboxamide should not necessarily predicate against such a hypothesis, since synthesis of the postulated intermediate (lethal synthesis?) at the immediate proximity of the enzymic site may predispose toward preferential inhibition of the enzyme in its conversion of AS to AMP.

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Mechanism of inhibition of histidine decarboxylase (*Clostridium welchii*) by 4-bromo-3-hydroxybenzyloxamine and amino-oxyacetic acid

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SINCE kinetic studies with fetal rat histidine decarboxylase and 4-bromo-3-hydroxybenzyloxyamine (NSD-1055) yielded uninterpretable data,[1] it was decided to ascertain if the bacterial enzyme (*Clostridium welchii*) would lend itself more favorably to such studies. This enzyme was found to be very useful in this respect and the results of these studies comprise the subject of this communication. Also included herein are kinetic studies utilizing amino-oxyacetic acid (AOAA) and 2-hydrazinopyridine (2-HP) as enzyme inhibitors. Since NSD-1055 and AOAA contain the amino-oxy moiety, it was of interest to determine whether the mechanism of inhibition was the same for the two compounds. The third inhibitor, 2-HP, was included for comparison because it lacked the amino-oxy group.

Results obtained with the bacterial enzyme were supported by those obtained with an assay of a specific histidine decarboxylase isolated from rat peritoneal mast cells.

An acetone powder of specific histidine decarboxylase was obtained from Worthington Biochemicals, Inc., and assayed manometrically according to the method of Gale.[2] Specificity of this enzyme for L-histidine di-HCl (Calbiochem) was verified, since 5-hydroxytryptophan (5-HTP) (General Biochemicals, Inc.) and 3,4-dihydroxyphenylalanine (Calbiochem) failed to serve as substrates. An atmosphere of 100% nitrogen was found to improve assay conditions. Addition of pyridoxal phosphate was not required due to tight binding of the coenzyme by the bacterial enzyme.

Histidine decarboxylase was isolated from rat peritoneal mast cells according to published methods[3,4] with certain modifications. Female Sprague–Dawley (Dublin) rats (120–150 g) were injected with 10 ml of heparinized saline. After stunning the animals with a blow on the head, the abdomen was massaged for 2 min to dislodge cells, then opened and the cell suspension removed. Suspensions from all rats were pooled and then centrifuged in 6 ml aliquots at 500 rpm for 3·5 min. The lowest 0·1 ml was resuspended in 1·0 ml of cold, isotonic phosphate buffer, pH 7·4, with 0·2% sucrose. The supernatant was recentrifuged and the lowest 0·1 ml resuspended in 1·0 ml phosphate buffer. Cell suspensions were

rapidly frozen and thawed 5–6 times and then frozen until just prior to assay. The enzyme preparation was then thawed and diluted 1:1 with phosphate buffer.

The enzyme was assayed manometrically at room temperature (22°). Warburg flasks contained 0·5 ml pyridoxal phosphate (1 μg/ml) (General Biochemicals, Inc.); 1·0 ml of 0·1 M phosphate buffer, pH 7·4; 1·0 ml enzyme preparation; 0·7 ml of 0·25 M histidine HCl; and sufficient distilled water to bring the final volume to 3·5 ml. (Final pH is 6·9.) Each run included flasks without substrate to correct for possible decarboxylation due to any endogenous substrate present in the enzyme preparation. After 5 min of equilibration, initial readings were made and substrate was tipped in from the side-arm. The enzyme assay was found to be extremely rapid, with the greatest activity occurring in the initial 5 min of the assay thus necessitating readings every 30 sec. Results are expressed in μM of histamine produced/flask/min based on the μl of CO_2 released.

Since addition of the coenzyme, pyridoxal phosphate, was found to increase enzyme activity[5] (see Fig. 1), an excess was included in all assays unless otherwise noted. Activity was absent when either 5-HTP or 3,4-dihydroxyphenylalanine was substituted for L-histidine as substrate. Loss of activity also occurred when the enzyme preparation was heated for 15 min at 60°. Bioassay for histamine with the guinea pig ileum indicated an appreciable increase in histamine content following the enzyme reaction.

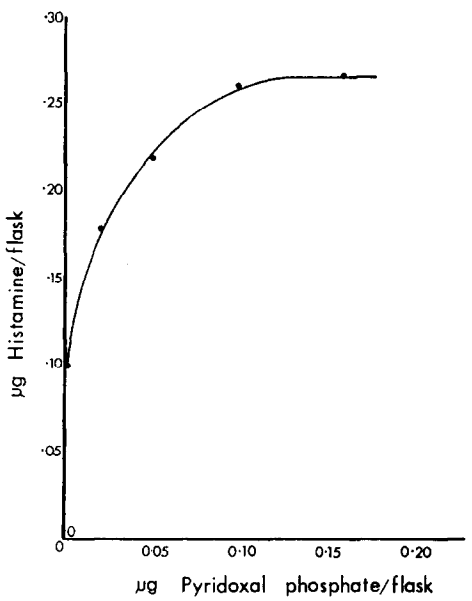

FIG. 1. Effect of increasing concentrations of pyridoxal phosphate on histidine decarboxylase (mast cell) activity. Decarboxylation was measured in ·067 M phosphate buffer, pH 7·4, at 22°.

Due to the ready availability of and ease of working with the bacterial enzyme, this enzyme was utilized for inhibition studies. For comparative purposes, experiments were conducted with the mast cell enzyme. Both enzyme systems were similarly inhibited by a number of reported inhibitors of specific histidine decarboxylase.

The primary differences between the two enzymes concern the coenzyme and the pH optimums. Histidine decarboxylase (*Cl. welchii*) binds the coenzyme tightly, and additional amounts result in no increase in activity. The mast cell enzyme is not saturated with coenzyme.[3] However, addition of sufficient pyridoxal phosphate to saturate the enzyme minimizes this difference. The pH optimum of the bacterial enzyme is 4·5, whereas that of the mast cell enzyme is about 7·4.[3]

Drugs utilized and their sources were as follows: amino-oxyacetic acid was kindly provided by Dr. O'Connell, The Upjohn Co.; 4-bromo-3-hydroxy benzyloxyamine was a gift from Mr. David J. Drain, Smith and Nephew Research, Ltd.; 2-hydrazinopyridine was purchased from the Aldrich Chemical Co.

NSD-1055, AOAA and 2-HP were found to inhibit histidine decarboxylase (*Cl. welchii*) 50 per cent at concentrations of 5×10^{-6} M, 2×10^{-5} M and 10^{-4} M respectively. They were also found to inhibit the specific enzyme isolated from rat peritoneal mast cells. However, the latter studies revealed a variation in the inhibition of the enzyme, apparently dependent upon the excess pyridoxal phosphate included in this assay. Results of these experiments are summarized in Table 1. Without excess

TABLE 1. INHIBITION OF HISTIDINE DECARBOXYLASE FROM TWO SOURCES

Inhibitor (10^{-3} M)	Mast cell (%)	*Cl. welchii* (%)
NSD-1055	90 (45)*	100
AOAA	88 (47)*	100
2-HP	60 (59)*	80

* With excess pyridoxal phosphate.

coenzyme, the inhibitory effect of both NSD-1055 and AOAA was increased, suggesting the involvement of the coenzyme in the mechanism of inhibition. This increased inhibition with decreasing amounts of pyridoxal phosphate was previously reported for NSD-1055,[1] but not for AOAA. However, AOAA has been reported to be an inhibitor of several other pyridoxal-dependent enzymes.[6-9] These observations and the structural similarity of these two compounds suggested the possibility

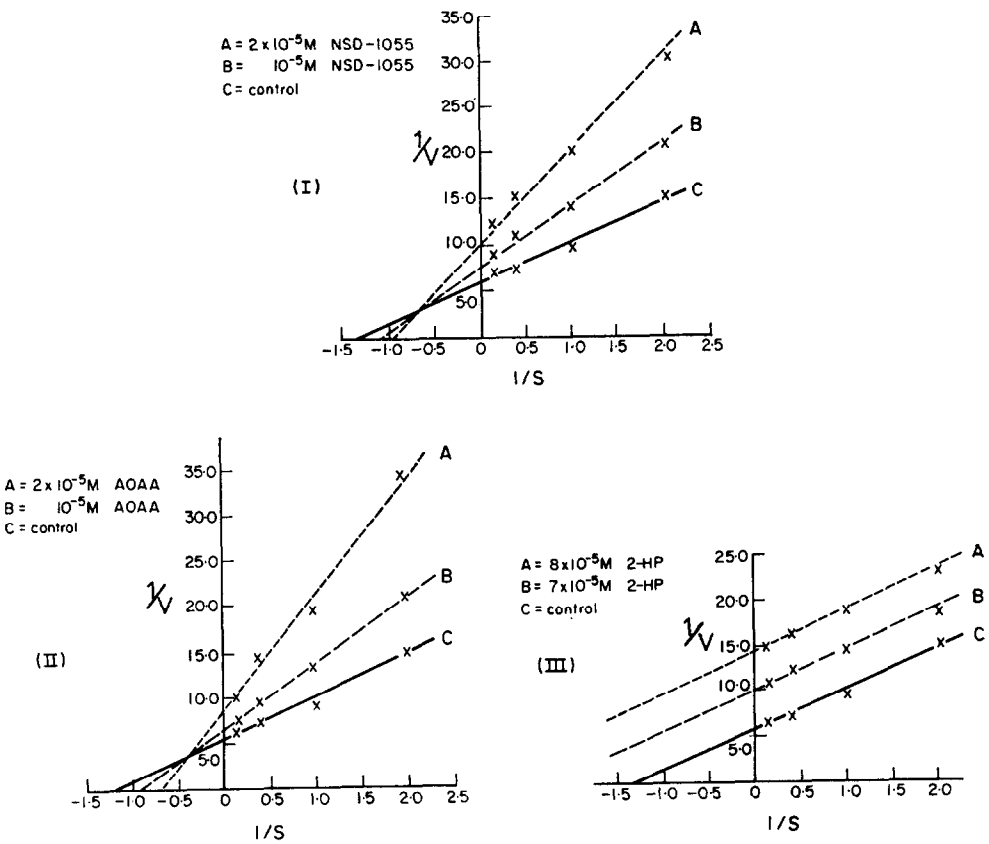

FIG. 2. Lineweaver–Burk plots of inhibition of histidine decarboxylase (*Cl. welchii*) by NSD-1055 (I), AOAA (II) and 2-HP (III). Velocity (V) is taken as micromoles of histamine produced per flask per minute. Substrate concentration (S) is mM of L-histidine per ml reaction solution. Reaction was carried out in Warburg flasks with 0·2 M acetate buffer, pH 4·5, at 37°.

that NSD-1055 and AOAA had a common mode of action. This possibility seemed even more plausible when it was found that inhibition of the mast cell enzyme by the structurally unrelated compound, 2-HP, was not altered by variation in the amount of coenzyme present in the assay. This indicated a difference between the inhibitory action of 2-HP and the amino-oxy derivatives.

In view of this difference, kinetic studies utilizing the bacterial enzyme were made to determine the type of inhibition involved for each compound. The results of these studies are expressed in Lineweaver–Burk plots (Fig. 2) and support the observations made with the mast cell enzyme relating the activities of NSD-1055 and AOAA. They are also in partial agreement with the findings of Levine *et al.*[1] in that an interpretation of the involvement of a specific type of inhibition (competitive, non-competitive or uncompetitive) cannot be made. However, it is evident that both compounds exhibit a mixed type of inhibition. Since AOAA is known to inhibit several other pyridoxal-dependent enzymes and increased levels of coenzyme have been observed to decrease the potency of both AOAA and NSD-1055 as inhibitors, it seems probable that their inhibition involves the coenzyme in some way. Levine *et al.*[1] have, in fact, suggested that inhibition by NSD-1055 may be dependent upon interaction with the coenzyme. Furthermore, the common amino-oxy portion of these two inhibitors suggests that this group, in particular, may be the active portion of these molecules, reacting with pyridoxal phosphate. It is this portion of the AOAA molecule which is known to chemically react with the aldehyde group of pyridoxal phosphate to yield an oxime.[10] On this basis, it could be postulated that NSD-1055 may also react with the coenzyme in this manner.

Inhibition of histidine decarboxylase by 2-HP was found to be of the uncompetitive type, implying that this compound affects the enzyme–substrate complex.[11] The present concept of the interaction of hydrazine derivatives with pyridoxal phosphate is not contradicted by these results. The co-enzyme might be involved as an integral part of the complex without being directly affected by 2-HP. This distinguishes 2-HP from the α-hydrazino analog of histidine, reported as an inhibitor of specific histidine decarboxylase,[1] which is directly affected by coenzyme concentration.

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