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In vitro inhibition of rat hypothalamus histidine decarboxylase by a specific inhibitor—4-imidazolyl-3-amino-2-butanone (McN-A-1293)

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4-Imidazolyl-3-amino-2-butanone (McN-A-1293) was shown to be an effective inhibitor *in vitro* of hypothalamic rat histidine decarboxylase. McN-A-1293 at two concentrations acted by increasing the effective K_m which was found to be 2×10^{-4} M. The changes of K_m and the low K_i value (0.021 mM) suggest that this inhibitor competes with the substrate for the enzyme and should therefore be a useful compound for further studies on histamine.

Specific histidine decarboxylase (EC4.1.1.22) plays an important role in the synthesis of histamine in several tissues [1, 4, 5, 9, 10, 12]. Decarboxylase inhibiting properties and structure–activity relationship have been studied with a number of synthetized compounds, especially with brocresine (NSD-1055) [3, 5, 12, 13]. The benzyloxyamines, however, are powerful inhibitors not only of histidine decarboxylase (HD) but also a nonspecific decarboxylase and other pyridoxal-5-phosphate (PLP) requiring enzymes, e.g. diamine oxidase (DAO) [5, 13]. Recently, McN-A-1293 was shown to be an effective inhibitor of fetal and gastric rat HD *in vitro* and *in vivo* [13]. In the present study this compound has been found to inhibit *in vitro* activity of hypothalamic HD in the rat brain.

Chemicals. 4-Imidazolyl-3-amino-2-butanone was obtained from McNeil Labs. L-Histidine [ring-2-^{14}C] was purchased from Amersham-Searle. Pyridoxal-5-phosphate, L-histidine mono-hydrochloride, histamine dihydrochloride was obtained from Sigma Chemical Co.

Methods. Male albino rats were used in these studies. Following decapitation, the brain was rapidly removed, washed with physiological saline and the hypothalamus dissected.

For determination of HD activity the dissected tissue was homogenized in 1/15 M phosphate buffer (pH 6.8), centrifuged at 10,000 *g* for 10 min at 0° and the supernatant assayed for [^{14}C]histamine forming ability by the procedure of Bielkiewicz [6] with slight modification [7].

The inhibiting properties of the new compound (McN-A-1293) on specific HD in hypothalamus of rat brain *in vitro* are shown in Fig. 1. The effectiveness of the inhibitor is expressed by the inhibitor constant K_i which is reciprocal to the enzyme–inhibitor affinity. The reaction velocities were measured at different concentrations of McN-A-1293 (4.9 μM, 10 μM, 20.3 μM, 31.3 μM, 50 μM) and at two concentrations of histidine 26 μM and 53 μM).

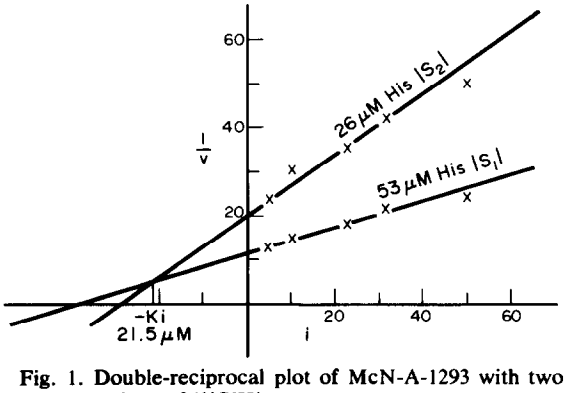

Fig. 1. Double-reciprocal plot of McN-A-1293 with two concentrations of [^{14}C]His.

Results in Fig. 1 show the K_i to be 0.021 mM when calculated according to Lineweaver and Burk [2]. This value correlates closely with the K_i for fetal and gastric HD [13].

The K_m of hypothalamic HD was found to be approximately 2×10^{-4} M (Fig. 2) at pH 6.8, a value very close to that in rat brain [10] and to that in rat and mice fetal tissue and gastric mucosa [4, 8, 13].

McN-A-1293 at two concentrations acted by increasing the effective K_m; hence the inhibitor competes with the substrate for the enzyme.

The characterization and properties of HD in the rat brain have been previously reported [10]; it showed a high specificity like fetal and gastric HD and is effectively inhibited by brocresine (NSD-1055) and α-hydrazino histidine (α-HH) [3, 8, 10, 12, 13]. Other studies on the inhibition of rat hypothalamic HD by McN-A-1293 show it to be a specific inhibitor of this enzyme, but weaker than brocresine or α-HH. However, it appears to be more selective than benzyloxyamines like NSD-1055 and hydrazines [10, 12]. Benzyloxyamines were found to inhibit other PLP-requiring enzymes as aromatic aminoacids decarboxylase and diamine oxidase [3, 5, 13]. Although α-HH was reported to inhibit HD specifically *in vitro*, thus suggesting high affinity to the enzyme, hydrazines are known to form hydrazones with PLP and reversal of α-HH inhibition by PLP indicates that inhibition results from coenzyme elimination [8]. Another type of inhibitor, α-methyl-histidine, is competitive with histidine but as this inhibitor is decarboxylated by HD, very high doses were required to inhibit enzyme activity [11]. McN-A-1293 possesses an imidazole ring for the specific binding site, an amino group for binding to the coenzyme and a carbonyl group incapable of decarboxylation [13]. Substances, especially those related in structure to the substrate, which combine with the enzyme at the same site as the substrate, produce a competitive type of inhibition. The effect of the competitive inhibitor is thus to produce an increase of K_m of the enzyme as inhibitor concentrations are increased.

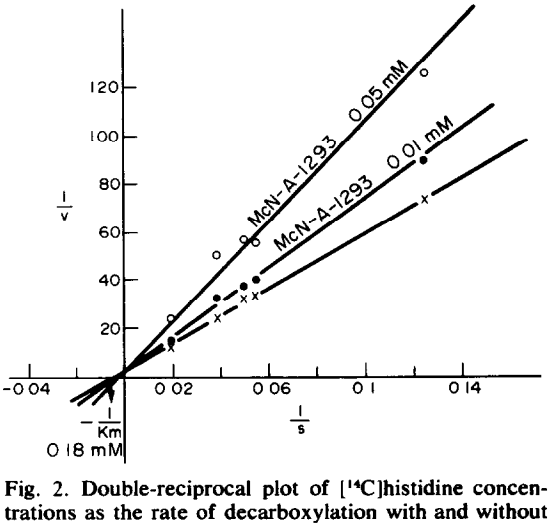

Fig. 2. Double-reciprocal plot of [^{14}C]histidine concentrations as the rate of decarboxylation with and without McN-A-1293.

Such changes of K_m found in our studies, and the low K_i value, suggest a competitive type of inhibition of hypothalamic HD and the usefulness of McN-A-1293 in further studies on histamine.

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