Biochemical Pharmacology, Vol. 22, pp. 2299–2310. Pergamon Press, 1973. Printed in Great Britain.

4-IMIDAZOLYL-3-AMINO-2-BUTANONE (McN-A-1293), A NEW SPECIFIC INHIBITOR OF HISTIDINE DECARBOXYLASE*

RUSSELL J. TAYLOR, JR., FRANZ J. LEINWEBER† and GEORGE A. BRAUN‡

Biochemistry Department, McNeil Laboratories, Inc., Fort Washington, Penn. 19034, U.S.A.

(*Received* 15 *January* 1973; *accepted* 9 *March* 1973)

Abstract—4-Imidazolyl-3-amino-2-butanone (McN-A-1293) was shown to be an effective inhibitor of fetal rat histidine decarboxylase, but not guinea pig kidney aromatic L-amino acid decarboxylase *in vitro*. McN-A-1293 was a more potent inhibitor than α-methylhistidine, but less potent than brocresine (NSD-1055) and other aminooxyamines. Kinetic studies using fetal rat histidine decarboxylase indicated the inhibition to be reversible and competitive with histidine. McN-A-1293 *in vitro* was a less potent inhibitor of diamine oxidase than brocresine and was only a weak inhibitor of imidazole-*N*-methyltransferase. Administration of McN-A-1293 (200 mg/kg, i.p.) to fed rats resulted in 55 per cent inhibition of gastric histidine decarboxylase, while gastric aromatic L-amino acid decarboxylase was only slightly inhibited (17 per cent). Brocresine (200 mg/kg, i.p.) inhibited both gastric decarboxylases > 60 per cent. Doses of McN-A-1293 as low as 50 mg/kg, i.p., inhibited gastric histidine decarboxylase activity. In fasted rats, McN-A-1293 (200 mg/kg, i.p.) was found to inhibit the elevation in gastric histidine decarboxylase induced by gastrin or insulin. 4-Imidazolyl-3-amino-2-butanone (McN-A-1293) is an effective and specific inhibitor of histidine decarboxylase both *in vitro* and *in vivo* and should be a useful agent for studies on the role of histidine decarboxylase in histamine physiology.

HISTAMINE is synthesized in mammalian tissues by the decarboxylation of histidine.[1,2] The decarboxylation may be catalysed by a specific histidine decarboxylase[3,4] or a non-specific aromatic L-amino acid decarboxylase.[5] In recent years, considerable evidence has accrued which indicates that the specific, inducible histidine decarboxylase is the more important enzyme for synthesis of histamine *in vivo*.

With the development of more sensitive methods for measuring histidine decarboxylase activity,[6,7] several inhibitors of the enzyme have been reported.[8] The most potent inhibitors *in vitro* found were a series of benzyloxyamines,[8,9] among which 4-bromo-3-hydroxy-benzyloxyamine (NSD-1055, brocresine) has been the most studied.[5,10–12] The benzyloxyamines, however, appear to react with pyridoxal phosphate[11] and as a consequence also inhibit the non-specific decarboxylase[8] as well as other pyridoxal phosphate-requiring enzymes such as diamine oxidase.[13]

A more specific inhibitor of histidine decarboxylase would be of greater value in elucidating the role of histamine in mammalian physiology, and Shepherd and Mackay[8] have reviewed the attempts to find such a compound. α-Methylhistidine and

* A preliminary report of this investigation was presented at the annual meeting of the American Society of Pharmacology and Experimental Therapeutics, Chicago, Ill., April, 1971.

† Present address: Warner–Lambert Research Institute, Morris Plains, N.J. 07950, U.S.A.

‡ Present address: Ortho Pharmaceutical Corp., Raritan, N.J. 08869, U.S.A.

α-hydrazino-histidine (MK-785) were the only compounds reported to possess significant and specific inhibiting activity for histidine decarboxylase *in vitro*.[2,12] Recently, Smissman and Weis[14] proposed 4-imidazolyl-3-amino-2-butanone (McN-A-1293) as a potential specific inhibitor. The present communication describes studies *in vitro* and *in vivo* with this compound.

EXPERIMENTAL

Materials. 4-(4-Imidazolyl)-3-amino-2-butanone and 4-(4-imidazolyl)-3-acetamido-2-butanone were synthesized by Drs. J. A. Weis and E. E. Smissman, Department of Medicinal Chemistry, University of Kansas;[14] and 1-imidazolyl-2-amino-3-hexanone was prepared by an analogous synthesis by Dr. C. R. Rasmussen and B. Twardzik in our laboratories. These compounds were prepared as their hydrochloride salts.

DL-α-Methylhistidine was obtained from Regis Chemical Company; DL-α-methyldihydroxyphenylalanine from Merck, Sharp & Dohme; 4-bromo-3-hydroxy-benzyloxyamine dihydrogen phosphate (brocresine) from Lederle Laboratories; 3-hydroxybenzyloxyamine from Smith–Nephews Ltd.; and amino-oxyacetic acid from Eastman Organic Chemicals. Gastrin (mixtures I and II) was purchased from Nutritional Biochemicals, and insulin from Eli Lilly & Company.

L-Histidine-carboxyl-^{14}C and DL-dihydroxyphenylalanine-carboxyl-^{14}C were obtained from New England Nuclear; and histamine (ring-2-^{14}C) from Amersham–Searle. Pyridoxal-5-phosphate, L-histidine, DL-dihydroxyphenylalanine and histamine dihydrochloride were purchased from Sigma; and *S*-adenosylmethionine from Mann.

Histidine decarboxylase (EC 4.1.1.22) was prepared by a modification of methods described by Håkanson[4] and Levine and Watts.[15] Whole rat fetuses (19–20 days gestation) obtained from Sprague–Dawley rats (Carworth Farms) were homogenized in 2 volumes of 0·1 M sodium acetate, pH 5·5. After centrifugation for 45 min at 90,000 g, the supernatant was fractionated using ammonium sulfate. The protein precipitating between 25 and 45 per cent saturation was dissolved in 0·1 M potassium phosphate, pH 7·0, and dialysed overnight at 5° against water. The dialysed fraction was diluted with 0·05 M potassium phosphate, pH 7·0, to a concentration of 40 mg of protein/ml, and could be stored at −15° for several months with no loss of activity.

Aromatic L-amino acid decarboxylase (EC 4.1.1.26) was prepared from guinea-pig kidneys by the method of Clark *et al.*[16] The kidneys were homogenized in 4 volumes of water and were centrifuged at 20,000 g for 30 min. The protein fraction precipitated from the supernatant by ammonium sulfate between 37 and 55 per cent saturation was dissolved in 0·05 M potassium phosphate, pH 7·0, and dialysed overnight against water at 5°. The volume after dialysis was adjusted to a concentration of 65 mg of protein/ml, and the enzyme was stored at −15°.

Pyloric histidine decarboxylase and pyloric aromatic L-amino acid decarboxylase (dopa decarboxylase) were partially purified from the glandular portions of rat stomachs and assayed according to the method previously described.[17] The histidine decarboxylase was purified through the 0–45 per cent ammonium sulfate step, and the dialysed 160,000 *g* fraction was used for the aromatic L-amino acid decarboxylase activity.

Imidazole-*N*-methyltransferase (EC 2.1.1.c) was partially purified from guinea-pig brains as reported by Snyder *et al.*[18] The fraction which precipitated between 45 and

70 per cent saturation with ammonium sulfate was used in these studies. Hog kidney diamine oxidase (EC 1.4.3.6) was purchased from Sigma Chemical Company.

Enzyme assays in vitro. Histidine decarboxylase activity was determined by measuring the $^{14}CO_2$ produced from L-histidine-carboxyl-^{14}C as previously described.[7] The standard reaction mixture contained 100 μmoles of potassium phosphate buffer (pH 6·8), 0·25 μmole of L-histidine containing 0·25 μCi of L-histidine-carboxyl-^{14}C, 0·01 μmol of pyridoxal-5-phosphate and 2 mg (0·05 ml) of the histidine decarboxylase preparation. Inhibitors were added at various concentrations in 0·1 ml of water. The final volume was made to 1·0 ml with water. The mixture was incubated for 90 min at 37°. Controls were included to correct for non-enzymatic decarboxylation. Molar I_{50} values were obtained graphically from duplicate analyses using at least three inhibitor concentrations.

Aromatic L-amino acid decarboxylase activity was determined using DL-dihydroxyphenylalanine-carboxyl-^{14}C as the substrate and measuring the $^{14}CO_2$ in the same manner as with histidine decarboxylase.[7] The standard reaction mixture contained 50 μmoles of potassium phosphate buffer (pH 6·8), 0·3 μmole of DL-dihydroxyphenylalanine containing 0·11 μCi of DL-dihydroxyphenylalanine-carboxyl-^{14}C, 0·035 μmole of pyridoxal-5-phosphate and 0·13 mg of the decarboxylase preparation. Inhibitors were added in 0·1 ml of water, and the final volume was made to 0·5 ml with water. The mixture was incubated for 5 min at 37°. Controls were included to correct for non-enzymatic decarboxylation, and molar I_{50} values were calculated as described above.

Histamine-*N*-methyltransferase activity was determined by measuring the *N*-methylhistamine-^{14}C formed from histamine-^{14}C as described by Brown *et al.*[19] The reaction mixture contained 200 μmoles of sodium phosphate buffer (pH 7·4), 0·5 μmole of *S*-adenosylmethionine, 0·066 μmole of histamine containing histamine-ring-2-^{14}C, 0·2 ml of enzyme preparation and 0·6 ml of inhibitor solution (aqueous), and made up to a final volume of 3 ml with water. The mixtures were incubated for 60 min at 37°, then 1·0 ml of 1 N sodium hydroxide and 1·0 g of sodium sulfate were added. The *N*-methylhistamine was extracted into 10 ml of chloroform. A 5-ml aliquot of the chloroform extract was evaporated to dryness and the residue was dissolved in 10 ml of an ethanol–toluene (20:80) scintillator solution (0·02% POPOP, 0·4% PPO)* for determination of radioactivity. Corrections were made for the radioactivity produced by non-enzymatic controls. The amount of methylhistamine formed was calculated from the extracted radioactivity and the radioactivity initially present as histamine.

Diamine oxidase activity was determined using the radioactive method reported by Kobayashi *et al.*[13] The incubation mixture contained 200 μmoles of potassium phosphate buffer (pH 6·9), 0·17 μmole of putrescine containing 0·1 μCi of putrescine-1,4-^{14}C, and 1·0 mg of diamine oxidase in a final volume of 2·0 ml. Inhibitors were added in 0·1 ml of water. Incubation was for 2 hr at 37° in air, and the reaction was terminated by addition of 0·23 mg of aminoguanidine and 200 mg of sodium bicarbonate. The reaction product, Δ′-pyrroline-^{14}C, was extracted in 15 ml of toluene containing 0·4% PPO and counted in a liquid scintillation counter. Corrections were made for the radioactivity produced by non-enzymatic controls.

Estimation of decarboxylases in vivo. The compounds to be tested were dissolved in

* POPOP = 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)] benzene; PPO = 2,5-diphenyloxazole.

saline and administered intraperitoneally to male Sprague–Dawley rats (175–200 g) which had been either fed or fasted. At various times after administration of the compounds, the rats were sacrificed by decapitation and the whole stomachs were immediately excised. The thin forestomach was separated from the glandular portion by cutting along the line of demarcation. The glandular portion was washed free of stomach contents in cold saline, blotted, weighed and homogenized in glass homogenizers with 3 volumes of cold saline. The resulting homogenates were centrifuged at 25,000 *g* for 30 min at 4° and the supernatants decanted.

Histidine decarboxylase activity was determined in 0·25-ml aliquots of the supernatants by a modification of the *in vitro* method described above. The incubation mixtures contained 100 μmoles of potassium phosphate buffer (pH 6·8), 0·5 μmole of L-histidine containing 0·5 μCi of L-histidine-carboxyl-^{14}C, 0·25 ml of the stomach supernatant, and water in the final volume of 1·0 ml. No pyridoxal-5-phosphate was added. The incubation was for 1 hr at 37°. The histidine decarboxylase activity of each rat stomach was determined by duplicate assays of each supernatant. A boiled sample of each supernatant served as a control for correction for non-enzymatic decarboxylation.

Aromatic L-amino acid decarboxylase activity was similarly determined in 0·25-ml aliquots of the same supernatants used for histidine decarboxylase, but using DL-dihydroxyphenylalanine-carboxyl-^{14}C as the substrate. The incubation mixture contained 100 μmoles of potassium phosphate buffer (pH 7·0), 0·3 μmole of DL-dihydroxyphenylalanine containing 0·11 μCi of DL-dihydroxyphenylalanine-carboxyl-^{14}C, 0·25 ml of supernatant, and water in a final volume of 1·0 ml. The incubation was for 30 min at 37° and $^{14}CO_2$ was determined as described above. Decarboxylase activity was determined by duplicate assay of the homogenates from each rat stomach. Boiled samples were assayed to allow for correction for non-enzymatic decarboxylation.

In the studies with insulin or gastrin, the rats were allowed only water for 48 hr prior to treatment. The rats were injected intraperitoneally with saline or McN-A-1293 in saline, followed 30 min later by administration of insulin (10 units/kg, i.m.), gastrin (5 μg/kg, i.p.) or saline. After 90 additional min, the rats were sacrificed by decapitation, and histidine decarboxylase and aromatic L-amino acid decarboxylase activities were measured in extracts of the glandular stomachs as described above.

RESULTS

Comparison with known inhibitors. A comparison of the inhibition of histidine decarboxylase and aromatic L-amino acid decarboxylase *in vitro* by McN-A-1293 and other decarboxylase inhibitors is shown in Table 1. McN-A-1293 (Fig. 1) was found to be a potent inhibitor of histidine decarboxylase, with an I_{50} of 0·03 mM, and thus was 33 times more potent than α-methylhistidine, which had an I_{50} of 1·0 mM in our assay system. The compound was less potent than 4-bromo-3-hydroxy-benzyloxyamine (brocresine, NSD-1055) and 3-hydroxy-benzyloxyamine (NSD-1024) which had I_{50} values of 0·001 mM and 0·006 mM. Amino-oxyacetic acid inhibited with an I_{50} of 0·008 mM. No inhibition of the specific decarboxylase was observed with α-methyl-dopa at concentrations as high as 1·0 mM.

Neither McN-A-1293 nor α-methylhistidine inhibited the aromatic L-amino acid decarboxylase at concentrations as high as 2·0 mM. This enzyme was inhibited,

however, by α-methyl-dopa, and the aromatic and aliphatic oxyamines. The I_{50} values for 4-bromo-3-hydroxy-benzyloxyamine, 3-hydroxy-benzyloxyamine and amino-oxyacetic acid were 0·008 mM, 0·01 mM and 0·1 mM, respectively, and 0·2 mM for α-methyl-dopa.

TABLE 1. INHIBITION OF HISTIDINE DECARBOXYLASE AND AROMATIC L-AMINO ACID DECARBOXYLASE BY 4-IMIDAZOLYL-3-AMINO-2-BUTANONE AND OTHER COMPOUNDS

Compound	Molar $I_{50} \times 10^3$*	
	HD	AD
4-Imidazolyl-3-amino-2-butanone (McN-A-1293)	0·03	>2·0
1-Imidazolyl-2-amino-3-hexanone	0·10	>2·0
4-Imidazolyl-3-acetamido-2-butanone	>1·0	>2·0
DL-α-Methylhistidine	1·0	>2·0
DL-α-Methyldihydroxyphenylalanine	>1·0	0·2
4-Bromo-3-hydroxybenzyloxyamine (Brocresine, NSD-1055)	0·001	0·008
3-Hydroxy-benzyloxyamine (NSD-1024)	0·006	0·01
Amino-oxyacetic acid	0·008	0·1

* Molar I_{50} values were obtained graphically from duplicate analyses using at least three inhibitor concentrations. HD = fetal histidine decarboxylase; and AD = kidney aromatic L-amino acid decarboxylase.

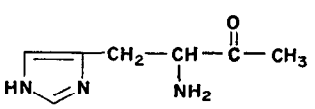

FIG. 1. Structure of McN-A-1293.

The effect of structural modification of McN-A-1293 on the potency of inhibition of fetal histidine decarboxylase and kidney aromatic L-amino acid decarboxylase is also shown in Table 1. Lengthening the alkyl chain on the ketone moiety resulted in a slight decrease in inhibition of the fetal enzyme. 1-Imidazolyl-2-amino-3-hexanone exhibited a molar I_{50} of 0.1 mM compared to 0·03 mM for the parent compound. Acetylation of the 3-amino group to give 4-imidazolyl-3-acetamido-2-butanone also decreased inhibition of the fetal enzyme, the compound having the molar I_{50} of > 1·0 mM.

Neither structural modification was effective in altering the specificity of enzyme inhibition. Like the parent compound, both analogs had no effect on the aromatic L-amino acid decarboxylase at concentrations as high as 2·0 mM.

Studies on the mechanism of inhibition. The degree of inhibition of fetal histidine decarboxylase *in vitro* by two concentrations of McN-A-1293 was not dependent on the amount of enzyme present in the incubation mixture indicating that the inhibition was essentially reversible.

In the standard histidine decarboxylase assay, the reaction was initiated by addition of the enzyme. However, it was observed that preincubation of McN-A-1293 with the enzyme in the presence or absence of pyridoxal phosphate did not increase the degree

of inhibition. In addition, no change in inhibition was observed when the inhibitor was allowed to react with pyridoxal phosphate prior to addition of enzyme and histidine.

Double-reciprocal plots of histidine concentration vs the rate of CO_2 formation indicated that the inhibition of fetal histidine decarboxylase by McN-A-1293 was competitive with histidine over a range of histidine concentrations from 0·050 mM to 0·375 mM (Fig. 2). These concentrations were in the range of the K_m for histidine, which was found to be 0·25 mM. The K_i was calculated from the double-reciprocal plots and was found to be 0·017 mM.

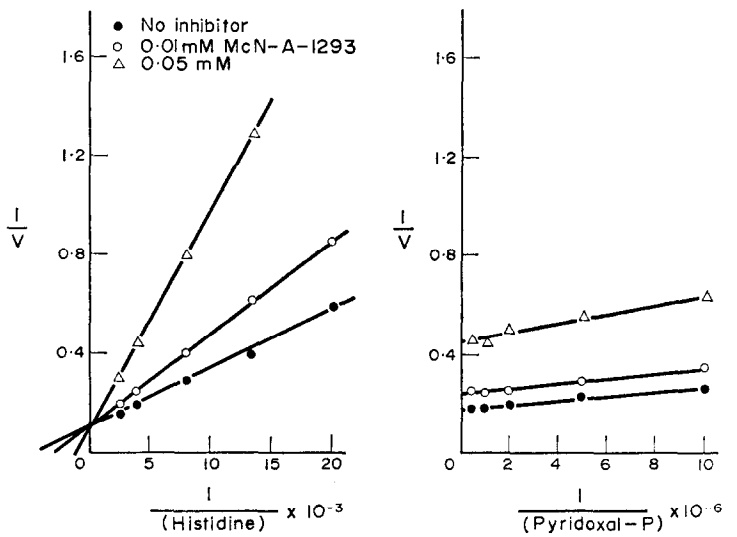

FIG. 2. Double-reciprocal plots of histidine or pyridoxal phosphate concentrations vs the rate of decarboxylation with and without McN-A-1293. The concentrations of other constituents were as described in the text.

Although the double-reciprocal plots of decarboxylase activity vs pyridoxal phosphate concentration appeared parallel using 0·01 mM McN-A-1293 (Fig. 2), they were found to diverge when using the higher concentration of 0·05 mM McN-A-1293. These data suggest the inhibition by McN-A-1293 to be non-competitive with pyridoxal phosphate.

During experiments with McN-A-1293, it was observed that solutions of the compound turned dark yellow in neutral or alkaline solutions at room temperature. This has been reported to be due to the formation of a dimer of the compound, which is then oxidized in air and light to 2,5-di(4-imidazolylmethyl)-3,6-dimethylpyrazine.[20] This pyrazine was prepared in our laboratories and found to be ineffective as an inhibitor of both fetal histidine decarboxylase and the non-specific amino acid decarboxylase, with I_{50} values of $>1·0$ mM and $>2·0$ mM respectively.

Inhibition of rat stomach decarboxylases in vitro. The effect of McN-A-1293 on the specific histidine decarboxylase and the non-specific L-amino acid decarboxylase partially purified from rat glandular stomach was also tested *in vitro*. As was seen with the fetal and kidney enzymes, the inhibitor had a specificity for histidine decarboxylase,

with an I_{50} of 0·05 mM, compared to an I_{50} of 12·5 mM for inhibition of stomach aromatic L-amino acid decarboxylase. The K_i of 0·016 mM for the histidine decarboxylase from stomach was similar to that obtained for the fetal enzyme, giving further evidence to the previously reported[17] identity of fetal and stomach histidine carboxylases.

Effect on catabolizing enzymes. The effect of McN-A-1293 on diamine oxidase *in vitro* is shown in Table 2, and is compared to brocresine. Brocresine inhibited diamine oxidase more effectively than did McN-A-1293. Brocresine had an I_{50} value of approximately 0·04 mM, while McN-A-1293 had an I_{50} of > 0·1 mM.

TABLE 2. EFFECT OF MCN-A-1293 AND BROCRESINE ON DIAMINE OXIDASE AND IMIDAZOLE-*N*-METHYL-TRANSFERASE ACTIVITY*

Compound	Concn (mM)	Inhibition (%)	
		DAO	ImNMT
McN-A-1293	1·0	87	48
	0·1	0	32
	0·01	0	17
	0·001	0	12
Brocresine	1·0	100	0
	0·1	90	0
	0·01	28	0
	0·001	0	0

* Diamine oxidase (DAO) and imidazole-*N*-methyltransferase (ImNMT) were assayed as described in text.

The effect of these two inhibitors on imidazole-*N*-methyltransferase *in vitro* is also shown in Table 2. McN-A-1293 inhibited about 50 per cent of the activity of imidazole-*N*-methyltransferase at 1·0 mM, the highest concentration tested. A similar concentration of brocesine had no effect. It appears that McN-A-1293 inhibited the *N*-methylation of histamine more effectively than brocresine, but that both are relatively weak inhibitors of this enzyme *in vitro*.

Effect on decarboxylases in vivo. One hr after administration to fed rats, McN-A-1293 was found to be as effective as brocresine in inhibiting gastric histidine decarboxylase activity. Intraperitoneal administration of McN-A-1293 produced a significant dose-dependent inhibition of gastric histidine decarboxylase activity over the range of doses from 50 to 400 mg/kg (Table 3). A similar degree of inhibition was obtained with brocresine over the same dose range. No significant inhibition was observed with McN-A-1293 at 25 mg/kg.

The histidine decarboxylase inhibition produced by a single 200 mg/kg, i.p., dose of McN-A-1293 was found to occur within 30 min after dosing, and to remain effective for at least 3 hr (Table 4). At all time intervals studied, the inhibition was found to be between 51 and 68 per cent of the controls at the same time period. The decline in histidine decarboxylase activity of the control rats likely reflects the decline in activity after termination of nocturnal feeding, since the test period was during the daylight hours of 10 a.m. to 12.30 p.m.

TABLE 3. EFFECT OF ADMINISTRATION OF MCN-A-1293 OR BROCRESINE ON GASTRIC HISTIDINE DECARBOXYLASE ACTIVITY OF FED RATS*

Treatment	Dose (mg/kg, i.p.)	Histidine decarboxylase (nmoles $CO_2 \pm$ S.E.)	Inhibition (%)
Saline	—	4·11 ± 0·34	—
McN-A-1293	400	1·10 ± 0·30	73†
	200	1·55 ± 0·33	62†
	100	2·18 ± 0·19	47†
	50	2·68 ± 0·50	35†
Brocresine	400	0·98 ± 0·27	76†
	200	1·46 ± 0·22	64†
	100	2·77 ± 0·34	33†
	50	3·03 ± 0·47	26

* Compound or saline was administered 60 min prior to sacrifice. Histidine decarboxylase activity was determined in gastric extracts as described in the text. Enzyme activity is expressed as nmoles CO_2 formed in 60 min by 0·25 ml of extract ± S. E. Six rats were used per group.

† $P < 0·05$.

TABLE 4. DURATION OF INHIBITION OF GASTRIC HISTIDINE DECARBOXYLASE ACTIVITY OF FED RATS BY MCN-A-1293 (200 mg/kg, i.p.)*

Time after administration (hr)	Histidine decarboxylase (nmoles CO_2 + S. E.)		Inhibition (%)
	Saline	McN-A-1293	
0·5	4·77 ± 0·89	1·94 ± 0·26	59†
1·0	4·98 ± 0·56	1·61 ± 0·31	68†
1·5	3·59 ± 0·56	1·58 ± 0·23	56†
2·0	3·34 ± 0·58	1·51 ± 0·29	55†
3·0	2·55 ± 0·58	1·24 ± 0·16	51†

* Histidine decarboxylase activity was measured in 0·25 ml of gastric extracts as described in text. Five rats were used per group.

† $P < 0·05$.

In view of the specificity of McN-A-1293 as an inhibitor of histidine decarboxylase and its lack of action upon aromatic L-amino acid decarboxylase *in vitro*, a study was made of its inhibitory specificity in the intact rat. McN-A-1293 or brocresine was administered intraperitoneally to fed rats at doses of 200 and 400 mg/kg, and both histidine decarboxylase (HD) and aromatic L-amino acid decarboxylase (AD) activities were measured in the same gastric extracts (Table 5). Both McN-A-1293 and brocresine markedly inhibited gastric histidine decarboxylase activity at 200 and 400 mg/kg, i.p. At 200 mg/kg, 55 and 60 per cent inhibition was observed for McN-A-1293 and brocresine, respectively, and 72 and 73 per cent at 400 mg/kg. In addition, brocresine completely inhibited the aromatic L-amino acid decarboxylase activity at both doses. However, McN-A-1293 at 200 and 400 mg/kg caused only slight and insignificant inhibition of the aromatic L-amino acid decarboxylase activity.

The effects of McN-A-1293 on gastrin-stimulated and insulin-stimulated histidine decarboxylase activity in fasted rats are shown in Table 6. McN-A-1293 (200 mg/kg)

TABLE 5. GASTRIC DECARBOXYLASE ACTIVITIES OF FED RATS TREATED WITH McN-A-1293 OR BROCRESINE*

Treatment	Dose (mg/kg, i.p.)	HD (nmoles $CO_2 \pm$ S.E.)	HD Inhibition (%)	AD (nmoles $CO_2 \pm$ S.E.)	AD Inhibition (%)
Saline	—	4·38 ± 0·94	—	8·21 ± 0·57	—
McN-A-1293	400	1·22 ± 0·21	72†	6·46 ± 1·11	21
	200	1·97 ± 0·36	55†	6·81 ± 0·92	17
Brocresine	400	1·20 ± 0·42	73†	0·13 ± 0·01	98†
	200	1·75 ± 0·35	60†	0·08 ± 0·05	99†

* Compound or saline was administered 60 min prior to sacrifice. Histidine decarboxylase (HD) and aromatic L-amino acid decarboxylase (AD) activities were determined in gastric extracts as described in the text. Enzyme activity is expressed as nmoles of CO_2 formed in 60 min (HD) or 30 min (AD) by 0·25 ml of gastric extract ± S.E. Six rats were used per group.
† $P < 0·05$.

TABLE 6. EFFECT OF McN-A-1293 ON THE STIMULATION OF GASTRIC HISTIDINE DECARBOXYLASE IN FASTED RATS BY GASTRIN OR INSULIN

Treatment	Histidine decarboxylase (nmoles $CO_2 \pm$ S. E.)
Fasted controls	0·42 ± 0·08
Gastrin (5 μg/kg, i.p.)	1·02 ± 0·10
Gastrin + McN-A-1293 (200 mg/kg, i.p.)	0·42 ± 0·05
Fasted controls	0·27 ± 0·08
Insulin (10 units/kg, i.m.)	1·30 ± 0·13
Insulin + McN-A-1293 (200 mg/kg, i.p.)	0·48 ± 0·09

* Significant from controls ($P < 0·05$).

was administered intraperitoneally to fasted rats, followed 30 min later by insulin or gastrin. The rats were sacrificed 90 min later for determination of gastric histidine decarboxylase activity. The administration of gastrin (5 μg/kg, i.p.) caused a 2·4-fold increase in histidine decarboxylase activity, which was completely inhibited by pretreatment with McN-A-1293. Similarly, administration of insulin (10 units/kg, i.m.) produced a 4·8-fold elevation in histidine decarboxylase activity, which was also inhibited by pretreatment with McN-A-1293.

DISCUSSION

An agent which inhibits the specific, inducible histidine decarboxylase and not the non-specific aromatic L-amino acid decarboxylase would facilitate studies involved with elucidating the role of endogenously synthesized histamine in mammalian physiology, and may also be of considerable therapeutic value. Several benzyloxyamines, especially brocresine (NSD-1055), were earlier found to be very potent inhibitors of histidine decarboxylase *in vitro*,[8,9] but were also found to inhibit the non-specific decarboxylase,[8] diamine oxidase,[13] and, indeed, probably other pyridoxal

phosphate-requiring enzymes. It has been reported that benzyloxyamines readily form oximes with pyridoxal phosphate.[11] Although it was a very potent inhibitor *in vitro*, brocresine (NSD-1055) has been shown to require substantial doses to produce inhibition of histidine decarboxylase *in vivo*.[13]

Substrate analogs such as the hydrazine derivative of histidine (MK-785) and α-methylhistidine have been reported to inhibit histidine decarboxylase more effectively than the non-specific decarboxylase *in vitro*.[2,12] Although it does not appear to have been reported, it has been suggested[8] that the hydrazino group of MK-785 may also combine with pyridoxal phosphate, and thereby decrease the specificity of inhibition. α-Methylhistidine has been reported[2] to be competitive with histidine and not dependent on pyridoxal phosphate concentration. α-Methylhistidine has also been shown to be decarboxylated itself by histidine decarboxylase,[2] and this may explain why substantially high doses were required to produce inhibition of histamine synthesis *in vivo*.

In an attempt to design a specific and reversible inhibitor of histidine decarboxylase, Smissman and Weis[14] proposed a compound which would possess an imidazole ring for the specific binding site, an amino group for binding to the coenzyme, and a carbonyl group incapable of decarboxylation. 4-Imidazolyl-3-amino-2-butanone (McN-A-1293) was found to meet these requirements, and was readily synthesized in good yields.[14] While our enzyme studies were in progress, Mardashev *et al.*[20] reported the inhibition of a bacterial histidine decarboxylase *in vitro* by 4-imidazolyl-3-amino-2-butanone.

In our studies, McN-A-1293 was found to be a potent inhibitor of fetal rat histidine decarboxylase *in vitro* but not of kidney aromatic L-amino acid decarboxylase (Table 1). Concentrations of greater than 65 times that which inhibited the fetal enzyme were required to inhibit the kidney enzyme. The compound was a more effective inhibitor of the specific enzyme than α-methylhistidine, which also did not inhibit the non-specific enzyme. The compound was less potent than brocresine and certain other oxyamines in inhibiting the specific fetal decarboxylase, but the oxyamines were also potent inhibitors of the non-specific kidney decarboxylase.

McN-A-1293 was also found to be a less effective inhibitor of diamine oxidase *in vitro* than was brocresine, requiring about 10 times greater concentration than brocresine for comparable inhibition (Table 2). No studies were done to determine if the inhibitor was a substrate for diamine oxidase. McN-A-1293 appears to be a more potent inhibitor of imidazole-*N*-methyltransferase *in vitro* than brocresine, but required a high concentration (1·0 mM) to give 50 per cent inhibition.

Structural modification (Table 2) of McN-A-1293 resulted in changes in potency of inhibition *in vitro* consistent with the proposals of Smissman and Weis.[14] Acetylation of the amino group prevented inhibition, presumably by blocking the binding of the amine to pyridoxal phosphate. Lengthening the alkyl chain of the ketone moiety maintained the specificity, but reduced the potency. This is probably due to increased steric hindrance around the carbonyl binding site.

Kinetic studies with the specific histidine decarboxylase indicated that the inhibition by McN-A-1293 was reversible and competitive with histidine, but not dependent on pyridoxal phosphate concentration.

On the basis of these studies *in vitro*, McN-A-1293 is an effective inhibitor of specific histidine decarboxylase and not the non-specific aromatic amino acid decar-

boxylase. In addition, McN-A-1293 is a more potent inhibitor than α-methylhistidine and can be readily synthesized in good yields.[14]

The large difference between the potency *in vitro* of McN-A-1293 and brocresine suggested that even larger doses than required for brocresine[13] would be required of McN-A-1293 inhibition *in vivo*. However, McN-A-1293 and brocresine were found to be equipotent in inhibiting histidine decarboxylase activity of the gastric mucosa of fed rats (Table 3). This lack of correlation between potency for brocresine *in vitro* and *in vivo* may be due to its recently reported rapid metabolism to less inhibitory metabolites.[21] The inhibition of gastric histidine decarboxylase by McN-A-1293 (200 mg/kg, i.p.) was found to occur within 30 min of administration and was equally effective after 3 hr (Table 4). Although brocresine (approximately 150 mg/kg, i.p.) has been reported to inhibit gastric histidine decarboxylase within 30 min, considerable recovery of activity was seen after 3 hr, and by 4–8 hr the activity was back to normal.[13]

The specificity of inhibition observed with McN-A-1293 *in vitro* was also found to occur *in vivo*. In the mucosal extracts from McN-A-1293-treated rats, there was marked inhibition of histidine decarboxylase, but no significant inhibition of aromatic L-amino acid decarboxylase (Table 5). Equivalent doses of brocresine, however, effectively inhibited both decarboxylases in the mucosal extracts.

The administration of insulin or gastrin has been reported to increase histidine decarboxylase activity in the glandular stomach of fasted rats.[13,22] In our studies, McN-A-1293 successfully blocked the stimulation of histidine decarboxylase activity resulting from administration of both gastrin and insulin (Table 6).

Although McN-A-1293 was less potent than brocresine *in vitro* in inhibiting histidine decarboxylase, it was equally effective in inhibiting the histidine decarboxylase activity of rat glandular stomach *in vivo*. However, McN-A-1293 did not inhibit the aromatic L-amino acid decarboxylases of the stomach, indicating its specificity is also effective *in vivo*. In addition, administration of McN-A-1293 to fasted rats effectively blocked the stimulation of histidine decarboxylase activity by gastrin and insulin.

The results of these studies indicate that McN-A-1293 is an effective inhibitor of histidine decarboxylase and not aromatic L-amino acid decarboxylase both *in vitro* and *in vivo*. Because of its effectiveness and specificity *in vivo*, McN-A-1293 may prove useful in elucidating the role of the inducible histidine decarboxylase in histamine physiology.

Acknowledgements—The authors wish to acknowledge the valuable technical assistance of Ms. Louise Walker and Ms. Cheryl Lethemon.

REFERENCES

1. R. W. SCHAYER, *J. biol. Chem.* **199,** 245 (1952).
2. G. KAHLSON, E. ROSENGREN and R. THUNBERG, *J. Physiol., Lond.* **169,** 467 (1963).
3. H. WEISSBACH, W. LOVENBERG and S. UDENFRIEND, *Biochem. biophys. Acta* **50,** 177 (1961).
4. R. HÅKANSON, *Biochem. Pharmac.* **12,** 1289 (1963).
5. W. LOVENBERG, H. WEISSBACH and S. UDENFRIEND, *J. biol. Chem.* **237,** 89 (1962).
6. R. W. SCHAYER, *Meth. biochem. Analysis* **16,** 273 (1968).
7. F.-J. LEINWEBER and L. A. WALKER, *Analyt. Biochem.* **21,** 131 (1967).
8. D. M. SHEPHERD and D. MACKAY, in *Progress in Medicinal Chemistry* (Eds. G. P. ELLIS and G. B. WEST), Vol. 5, p. 199. Plenum Press, N.Y. (1967).
9. L. ELLENBOGEN, E. MARKLEY and R. J. TAYLOR, JR., *Biochem. Pharmac.* **18,** 683 (1969).
10. J. D. REID and D. M. SHEPHERD, *Life Sci.* **1,** 5 (1963).
11. F.-J. LEINWEBER, *Molec. Pharmac.* **4,** 337 (1968).
12. R. J. LEVINE, T. L. SATO and A. SJOERDSMA, *Biochem. Pharmac.* **14,** 139 (1965).

13. Y. KOBAYASHI, J. KUPELIAN and D. V. MAUDSLEY, *Biochem. Pharmac.* **19,** 1761 (1970).
14. E. E. SMISSMAN and J. A. WEIS, *J. med. chem.* **14,** 945 (1971).
15. R. J. LEVINE and D. E. WATTS, *Biochem. Pharmac.* **15,** 841 (1966).
16. C. T. CLARK, H. WEISSBACH and S. UDENFRIEND, *J. biol. Chem.* **210,** 139 (1954).
17. F.-J. LEINWEBER and G. A. BRAUN, *Molec. Pharmac.* **6,** 146 (1970).
18. S. H. SNYDER, R. J. BALDESSARINI and J. AXELROD, *J. Pharmac. exp. Ther.* **153,** 544 (1966).
19. D. D. BROWN, J. AXELROD and R. TOMCHIK, *Nature, Lond.* **183,** 680 (1959).
20. S. R. MARDASHEV, N. A. GONCHAR and N. S. DABAGOV, *Dokl. Acad. Nauk SSR* **189,** 895 (1969).
21. L. ELLENBOGEN, R. G. KELLY, C. S. STUBBS and R. H. ENGEL, *Fedn Proc.* **30,** 1146 (1971).
22. D. AURES, R. HÅKANSON and A. SCHAUER, *Eur. J. Pharmac.* **3,** 217 (1968).