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INHIBITION OF HISTIDINE DECARBOXYLASE ACTIVITY *IN VITRO* BY NOREPINEPHRINE AND RELATED COMPOUNDS

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Abstract—Norepinephrine and several structurally related compounds were found to inhibit histidine decarboxylase (prepared either from fetal rat tissue or adult rat gastric mucosa) activity *in vitro*. This inhibition was reversible and had the characteristics of competition with the cofactor, pyridoxal-phosphate, and also with substrate. Substances that possessed inhibitory activity had the following structural features in common. They were *m*-hydroxy derivatives of phenylethylamine with unsubstituted primary amine endings in the side chain. Substitution of either a methyl or a carboxyl group on the α-carbon altered inhibitory potency only slightly; substitution of both inactivated the compound completely. β-Hydroxylation increased slightly the potency of an inhibitor. Although histidine decarboxylase is stereospecific for L-histidine, both D-dopa and L-dopa were inhibitors. Identical structural requirements must also be met for phenylethylamine derivatives to form tetrahydroisoquinoline condensation products with pyridoxal-phosphate. It is suggested that formation of such substance may be an essential step in the inhibition of histidine decarboxylase activity by norepinephrine and related substances.

IT HAS been reported that when histamine is released from skin[1] or stomach[2] there follows within a short period of time an increase in the capacity of that tissue to synthesize histamine. This information prompted Kahlson *et al.* to suggest a negative feedback relationship in the biosynthesis of histamine.[2] The most simple of the possible explanations for such a relationship would be inhibition by histamine of the enzyme that catalyzes its synthesis, histidine decarboxylase. This possibility was tested recently; histamine in high concentrations did not inhibit histidine decarboxylase activity *in vitro*.[3]

Evidence has been reported previously from this laboratory that naturally occurring inhibitors of histidine decarboxylase activity may exist in gastric tissue of the rat.[3] The nature of these inhibitors has not been determined. When histamine is released from tissue, other amines may also be released.[4] Some pharmacologic effects of several naturally occurring aromatic amines are antagonistic to those of histamine; e.g. norepinephrine, epinephrine, and serotonin.[5, 6] Therefore, it seemed possible that the increased histidine decarboxylase activity associated with histamine release could be mediated by release of some other pharmacologically antagonistic amine that inhibited histidine decarboxylase activity.

The present report describes studies of the effects of some naturally occurring aromatic amines on histidine decarboxylase activity *in vitro*. Norepinephrine and

dopamine were found to be potent inhibitors. Inhibition was reversible and had the characteristics of competition with the cofactor, pyridoxal-phosphate, as well as with substrate. The effects of several structural analogs of norepinephrine were studied for the purpose of defining structure–activity relationships.

MATERIALS AND METHODS

The compounds used, their abbreviations, and their sources are listed in Table 1.

Animals from which enzymes were prepared were female CD rats obtained from Charles River Laboratories, North Wilmington, Mass. Fetal histidine decarboxylase

TABLE 1. COMPOUNDS USED AND THEIR SOURCES

Compound	Abbreviated designation	Source
L-Norepinephrine bitartrate		Sigma*
3,4-Dihydroxyphenylethylamine·HCl	dopamine	Sigma
3,4-Dihydroxy-L-phenylalanine	L-dopa	Sigma
3,4-Dihydroxy-D-phenylalanine	D-dopa	Sigma
Tyramine·HCl		Sigma
Tryptamine·HCl		Sigma
5-Hydroxytryptamine-creatinine sulfate	serotonin	Sigma
L-Epinephrine bitartrate		Sigma
Pyridoxal-5′-phosphate	pyridoxal-phosphate	Sigma
DL-*m*-Tyrosine		Sigma
L-*p*-Tyrosine		Calbiochem†
DL-Metanephrine·HCl		Calbiochem
DL-Normetanephrine·HCl		Calbiochem
Histamine·2HCl		NBC‡
L-Histidine·HCl		NBC
α-Methyl-3,4-dihydroxy-L-phenylalanine	α-methyldopa	Merck§
α-Methyl-3,4-dihydroxy-L-phenylethylamine·HCl	α-methyldopamine	Merck
L-Metaraminol bitartrate		Merck
4-Bromo-3-hydroxybenzyloxyamine dihydrogen phosphate	NSD-1055	NSD‖

* Sigma Chemical Co.
† California Corp. for Biochemical Research.
‡ Nutritional Biochemicals Corp.
§ Merck Sharp and Dohme Laboratories.
‖ Smith and Nephew Research, Ltd.

was partially purified from whole fetal rats (19–20-day gestation) by a modification[3] of the method of Håkanson.[7] Gastric histidine decarboxylase was prepared[3] from the glandular portion of the stomach of fed rats. Histidine decarboxylase activity was assayed by a method described previously.[3] This method is based upon trapping in hydroxide of Hyamine for subsequent counting in a Packard Tri-Carb scintillation spectrometer the $^{14}CO_2$ evolved during incubation of enzyme with substrate labeled in the carboxyl carbon. Carboxyl-labeled ^{14}C-DL-histidine, specific activity 11·9 mc/m-mole, was obtained from Calbiochem. For most experiments, substrate was prepared by mixing isotopically labeled histidine with carrier L-histidine. Since these enzymes are stereospecific for L-histidine,[3] in calculating the final specific activity and molarity of substrate the D-isomer is disregarded as inert.

All incubation mixtures contained 4–9 mg protein and were buffered to pH 6·8 with sodium phosphate buffer. Other components of incubations varied and will be defined in relation to results of experiments in which they were used. Blanks were prepared by adding a potent, irreversible inhibitor of histidine decarboxylase activity, NSD-1055,[3] to incubation mixtures that were duplicates of experimental incubations. We found that this provides more reliable estimates of background radioactivity than blanks from which enzyme is omitted.

Protein determinations were done by a modification of the phenol reagent method.[8]

RESULTS

Preliminary studies, designed to examine naturally occurring aromatic amines as possible inhibitors of fetal histidine decarboxylase activity, were performed under usual conditions of incubation. The concentration of L-histidine was $2{\cdot}5 \times 10^{-4}$ M and of pyridoxal-phosphate, $3{\cdot}7 \times 10^{-5}$ M. All components of the incubation mixture were preincubated for 10 min with the exception of substrate which was added at 0-time to begin the reaction. Under these conditions norepinephrine and dopamine in concentrations of 5×10^{-3} M produced 95 and 83 per cent inhibition respectively.

Experiments were done next to define optimum conditions for demonstrating inhibition of histidine decarboxylase activity by norepinephrine. Enzyme, cofactor, and norepinephrine were added to incubation mixtures in various combinations at either 0-time or at the beginning of the 10-min preincubation (Table 2). Maximal inhibition was demonstrated only when norepinephrine was allowed to react with

TABLE 2. INHIBITION OF HISTIDINE DECARBOXYLASE ACTIVITY AS A FUNCTION OF PREINCUBATION CONDITIONS

Time of addition to incubation mixture (min)			Inhibition (%)
Enzyme	Norepinephrine 10^{-3} M	Pyridoxal-phosphate $3{\cdot}7 \times 10^{-5}$ M	
−10	−10	0	37
−10	0	−10	34
−10	−10	−10	56
0	−10	−10	100

Incubation medium contained fetal enzyme (4·2 mg protein). Reaction was begun by addition of L-histidine $2{\cdot}5 \times 10^{-4}$ M (0·5 mc/m-mole) at 0-time and ended at 30 min. Results represent average of two incubations. See text for additional details.

pyridoxal-phosphate under incubation conditions in the absence of enzyme. In other experiments it was found that preincubation of substrate with enzyme either in the presence or absence of cofactor did not reduce the degree of inhibition by norepinephrine as long as norepinephrine was first allowed to react with pyridoxal-phosphate in a separate incubation vessel in the absence of enzyme.

Similar findings were obtained when dopamine was substituted for norepinephrine. It was further observed that the degree of inhibition produced by either norepinephrine

or dopamine varied inversely with pyridocal-phosphate concentration (see also below). Accordingly, in all subsequent experiments, all components of the incubation mixture were preincubated for 10 min with the exception of enzyme and substrate. Enzyme was added 1 min before substrate, which was added at 0-time. Furthermore, unless specified otherwise, the concentration of cofactor used was $3{\cdot}7 \times 10^{-6}$ M; this is approximately five times the amount required to support maximal histidine decarboxylase activity under these conditions.

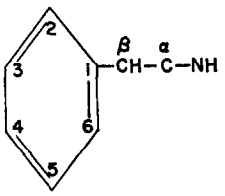

FIG. 1. Basic phenylethylamine structure labeled to illustrate convention used in Table 3 to identify positions of ring and side-chain substituents.

Various aromatic amines were re-examined for inhibitory properties under these optimum conditions. Serontonin produced 19 per cent inhibition of activity at a concentration of 5×10^{-3} M but less than 5 per cent at 10^{-3} M. At concentrations of 5×10^{-3} M, histamine and tryptamine produced no significant inhibition. The effects of norepinephrine and some structurally related compounds are listed in Table 3. Of the compounds that were found to inhibit histidine decarboxylase activity, those that occur naturally—L-dopa, norepinephrine, and dopamine—were selected for further study.

TABLE 3. INHIBITION OF HISTIDINE DECARBOXYLASE ACTIVITY BY ANALOGS OF CATECHOLAMINES

Compound	Substituents						Inhibition	
	3	4	β	α_1	α_2	N	5×10^{-3} M (%)	50% M
Norepinephrine	OH	OH	OH	H	H	H	100	4×10^{-4}
Dopamine	OH	OH	H	H	H	H	100	7×10^{-4}
L-α-Methyldopamine	OH	OH	H	CH_3	H	H	100	1×10^{-3}
L-Metaraminol	OH	H	OH	CH_3	H	H	100	2×10^{-4}
L-dopa	OH	OH	H	H	COOH	H	100	6×10^{-4}
D-dopa	OH	OH	H	H	COOH	H	100	1×10^{-4}
DL-*m*-Tyrosine	OH	H	H	H	COOH	H	*	1×10^{-3}
Metanephrine	CH_3O	OH	OH	H	H	CH_3	<5	
Normetanephrine	CH_3O	OH	OH	H	H	H	<5	
L-*p*-Tyrosine	H	OH	H	H	COOH	H	<5	
Tyramine	H	OH	H	H	H	H	<5	
Epinephrine	OH	OH	OH	H	H	CH_3	<5	
L-α-Methyldopa	OH	OH	H	CH_3	COOH	H	<5	

Results represent average of at least two 1-hr incubations under conditions described in text. The numbering and lettering convention used to identify ring and side-chain substituents is illustrated in Fig. 1. Inhibition is expressed as per cent inhibition by a compound at a concentration of 5×10^{-3}M and concentration required to produce 50 per cent inhibition.

* Insoluble.

Studies of the mechanism of inhibition by L-dopa were complicated by the fact that this amino acid is decarboxylated actively by preparations of enzyme derived by present methods from either fetal rat tissue or from adult rat gastric mucosa. D-dopa was not decarboxylated by either of these preparations; it inhibits the decarboxylation of histidine by the same mechanism as norepinephrine.

The remainder of these studies are concerned with the mechanism of inhibition of histidine decarboxylase activity by norepinephrine and dopamine. Although all studies were done with both amines, for the sake of brevity only those results obtained with norepinephrine are reported. The effects of dopamine differed only in that the degree of inhibition was, in each case, slightly smaller.

The degree of inhibition of histidine decarboxylase activity by norepinephrine was not related to the amount of enzyme added to the incubation mixture (Fig. 2). This indicates that the inhibition is freely reversible.

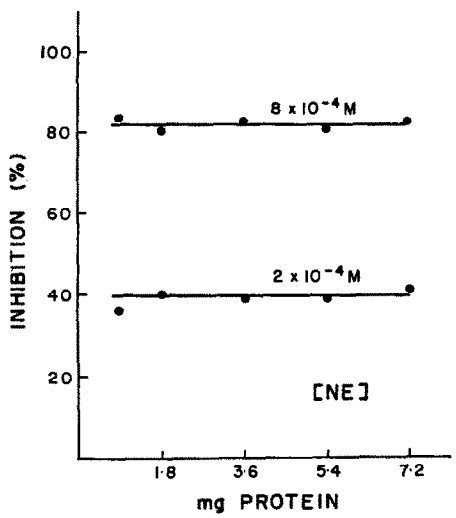

FIG. 2. Inhibition of histidine decarboxylase activity by two different concentrations of norepinephrine plotted as a function of enzyme concentration. Each point represents the average result of two 1-hr incubations.

Histidine decarboxylase activity was found to be a linear function of pyridoxalphosphate concentration in a range from $1{\cdot}48 \times 10^{-7}$ M to $7{\cdot}4 \times 10^{-7}$ M. Incubations were carried out with cofactor added at these concentrations in the presence and absence of norepinephrine. Double-reciprocal plots were constructed as described by Lineweaver and Burk[9] (Fig. 3). Inhibition had the characteristics of competition with cofactor.

At pH 6·8, enzyme activity was found to be a linear function of histidine concentration between $2{\cdot}5 \times 10^{-6}$ M and $10{\cdot}5 \times 10^{-6}$ M. Incubations were carried out with substrate added at these concentrations in the presence and absence of norepinephrine (Fig. 4). Inhibition also had the characteristics of competition with substrate.

Of the observations reported above, the following were repeated with enzyme prepared from gastric mucosa. The enzyme was inhibited markedly by norepinephrine,

dopamine, α-methyldopamine, L-dopa, and D-dopa; weakly by serotonin; and insignificantly by histamine, tryptamine, tyramine, epinephrine, and α-methyldopa. Inhibition by norepinephrine had the characteristics of competition with both substrate and cofactor. The results obtained with gastric enzyme did not differ quantitatively from

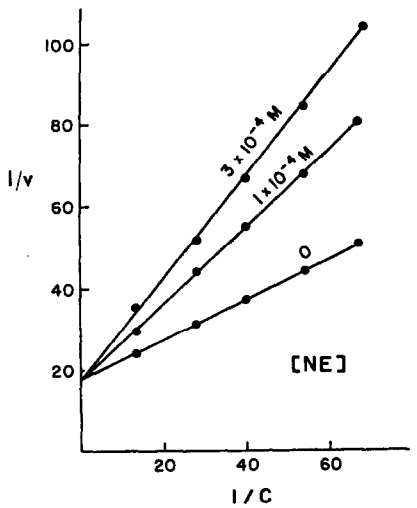

FIG. 3. Double-reciprocal plot of cofactor concentration (1/C = reciprocal of pyridoxal-phosphate concentration × 10^{-5} M) against rate of enzyme reaction (1/V = reciprocal of velocity × 10^6 cpm /hr) in the presence and absence of norepinephrine. The initial concentration of L-histidine was 2·5 × 10^{-4} M; specific activity was 0·5 mc/m-mole. Each point represents the average of two 1-hr incubations.

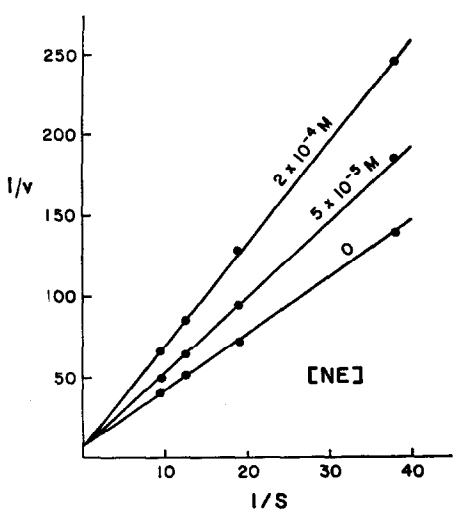

FIG. 4. Double-reciprocal plot of substrate concentration (1/S = reciprocal of L-histidine concentration × 10^{-4} M) against rate of enzyme reaction (1/V = velocity × 10^6 cpm/hr) in the presence and absence of norepinephrine. Pyridoxal-phosphate was added to a concentration of 3·7 × 10^{-6} M. The specific activity of histidine was 11·9 mc/m-mole. Each point represents the average of four 30-min incubations.

those obtained with fetal enzyme, within the bounds of expected experimental variation.

DISCUSSION

These experiments indicate that norepinephrine and certain related compounds inhibit histidine decarboxylase activity; this inhibition is reversible and has the characteristics of competition with not only the cofactor but also the substrate.

The data in Table 3 indicate the following about structural requirements for compounds in this series for activity as inhibitors of histidine decarboxylase activity. All inhibitors were *m*-phenols with a free primary amine group in the side chain; substitution in either of these positions caused complete loss of inhibitory potency. β-Hydroxylation, at least in the case of norepinephrine as compared with dopamine, increased potency slightly. Single substitution of a methyl or carboxyl group on the α-carbon alters inhibitory potency only slightly. Substitution of both methyl and carboxyl groups on the α-carbon is associated with complete loss of activity as an inhibitor.

Schott and Clark reported in 1952 that certain phenylalanine and phenylethylamine derivatives that contain a *m*-hydroxy substituent react at pH 6·8 with pyridoxal-phosphate to form tetrahydroisoquinoline condensation products.[10] In their studies, all compounds that underwent this reaction to a significant extent also had in common unsubstituted amine groups in the side chain. β-Hydroxylation seemed to enhance the affinity of a compound for pyridoxal-phosphate. Of the compounds they tested, norepinephrine had the greatest affinity for pyridoxal-phosphate. We have confirmed the findings of Schott and Clark and extended them to include some compounds used in the present experiments that were not studied by them. Metaraminol, D-dopa, dopamine, and α-methyldopamine were also found to react with pyridoxal phosphate at pH 6·8, a reaction characterized by loss of the light absorption peak at 385 mμ. To their list of compounds that by this criterion did not react to any significant extent we have added tyramine, metanephrine, and normetanephrine.

The structural requirements for inhibition of histidine decarboxylase activity are virtually identical with those for formation of the condensation product described by Schott and Clark. In view of the relationships of inhibition to cofactor found in the present studies, it seems reasonable to suggest that formation of a tetrahydroisoquinoline compound is an essential step in the inhibition of histidine decarboxylase activity by these compounds.

We have reported previously that, in all respects thus far examined, no important differences have been detected between the properties of histidine decarboxylase prepared from fetal rat tissue and that prepared from gastric tissue of the adult rat.[3] However, since it cannot be proved that these enzymes are identical, and since we wish to speculate on the possible physiologic significance of the present findings, we thought it appropriate to confirm the more important findings by using the gastric enzyme. Changes in histidine decarboxylase activity in gastric mucosa have been implicated as playing an important role in the mediation of gastric acid secretion in the rat.[2, 11]

Most of the known pharmacologic properties of norepinephrine are antagonistic to those of histamine.[5, 6] It is generally accepted that norepinephrine is the major postganglionic neurotransmitter in the sympathetic nervous system. Recent evidence suggests that histamine may function as the postganglionic neurotransmitter for certain functions of the parasympathetic nervous system, e.g. gastric acid secretion[11]

and active reflex vasodilatation.[12] It is tempting to speculate that the known antagonism of parasympathetic effects by the sympathetic nervous system may be mediated by secretion by the latter of a substance that inhibits the biosynthesis of the neurotransmitter for the former. This speculation must be supported by evidence that norepinephrine inhibits histidine decarboxylase activity *in vivo*. Experiments designed to explore this possibility are in progress in this laboratory.

The concentrations of norepinephrine required to produce significant inhibition of histidine decarboxylase activity *in vitro* seem higher than those one may expect to occur *in vivo*. However, since it is well established that many substances, including norepinephrine, tend to concentrate within various subcellular compartments, it is difficult to estimate the concentration of norepinephrine to which histidine decarboxylase may be exposed *in vivo*. Similarly, subcellular concentrations of histidine and pyridoxal-phosphate are unknown. If concentrations of cofactor and substrate are lower *in vivo* than those chosen arbitrarily for purposes of the present experiments *in vitro*, enzyme activity would be inhibited by proportionately lower concentrations of norepinephrine. Additional encouragement for pursuing studies *in vivo* is provided by the results of recent studies on the effects of norepinephrine on its own synthesis. Although, *in vitro*, norepinephrine inhibits tyrosine hydroxylase activity[13] less potently than it inhibits histidine decarboxylase activity, recent evidence indicates that norepinephrine, in physiologic concentrations, inhibits the hydroxylation of tyrosine *in vivo*.[14]

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