Structure–Activity Relationship Studies on Inhibition of Histamine Uptake by Rabbit Blood Platelets

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Abstract □ The correlation of histamine uptake inhibition with small changes in inhibitor structure was studied to determine the chemical requirements for the inhibitor. ^{14}C-Histamine was added to platelet-rich rabbit plasma *in vitro* with or without inhibitor, and the total accumulation of radioactivity into the platelets was counted. Several amino acids, amines, and their derivatives possessing an imidazole ring, an indole nucleus, or an aliphatic chain were studied. The results indicate that the most important function for the inhibitory effect is a protonated amino group. If the positive charge is decreased or if a negatively charged group is in the vicinity of the amino function, the activity is decreased. The results are the same with indole, imidazole, and aliphatic derivatives. The secondary binding, which evidently takes place through the heterocyclic ring or aliphatic chain, appears to be quantitatively less important, although it contributes to the relative potency of different amines. It is suggested that the primary binding is due to ionic bond formation between the positively charged nitrogen and some negative group in the carrier and the secondary binding to some weak forces such as a π-bond or hydrophobic forces. A few amines, such as butylamine and β-phenethylamine, increase rather than inhibit histamine uptake in long incubations, although they too are inhibitory in short incubations. This effect appears to be due in part to histamine catabolism inhibition, which prevents the fall in histamine concentration in the plasma, which then overcomes the uptake inhibiting effect of the substances.

Keyphrases □ Histamine uptake by rabbit blood platelets, inhibition—correlation between inhibitor activity and structure □ Inhibition of histamine uptake by rabbit blood platelets—structure–activity relationships □ Blood platelet uptake of histamine, inhibition—structure–activity relationships □ Amines, as inhibitors of histamine uptake by rabbit blood platelets—correlation between activity and structure

Histamine is taken up by blood platelets by what appears to be an active process (1). It is probable that a common mechanism operates for several amines such as 5-hydroxytryptamine, tryptamine, and phenethylamines, although the affinity of 5-hydroxytryptamine appears to be by far the highest (1, 2). Other amines inhibit the uptake of histamine (1). Because their structures differ from each other, it was decided to compare the chemistry and activity of some compounds related to the inhibitory amines to determine which parts of the molecule are essential to the uptake inhibition. This finding could then be correlated with the affinity of the compound to the carrier operating at the cellular membrane (3–6). Both imidazole and indole derivatives as well as some aliphatic compounds were studied.

EXPERIMENTAL

Male albino rabbits, weighing 2.0–2.5 kg, were bled, and platelet-rich plasma was prepared as previously described (7). Radioactive histamine was added to ice-cold plasma to give the final concentration of 10^{-6} *M*. Two-milliliter portions of plasma were immediately added to incubation tubes containing 0.2-ml saline solutions of the substances to be tested. All concentrations refer to the final concentration in 2.2-ml samples. After incubation for 80 min at 37° under an atmosphere of oxygen containing 5% carbon dioxide, the platelets were sedimented by centrifugation for either 30 min at 2000×*g* or 5 min at 20,000×*g* and were washed once with 2 ml of saline to remove the extracellular radioactivity. Washed platelets were solubilized[1] and the radioactivity was determined in a scintillation counter[2]. Results are given as percent inhibition calculated compared to a control sample incubated with the solvent at the same time and under the same conditions. All experiments were performed in duplicate, and the difference between duplicates was usually 0–2%.

Chemicals—The following were used: *n*-propylamine[3], *n*-butylamine[3], isobutylamine[3], *n*-pentylamine[3], isopentylamine[3], *n*-hexylamine[3], phenylacetic acid[3], *dl*-α-aminobutyric acid[4], *dl*-β-aminobutyric acid[4], γ-aminobutyric acid[4], α-aminoisobutyric acid[4], β-aminoisobutyric acid[4], *l*-histidine hydrochloride[5], melatonin[5], *N*-methyltryptamine hydrogen oxalate[5], tryptamine hydrochloride[6], 5-hydroxytryptamine creatinine sulfate[6], β-phenethylamine hydrochloride[7], 2-mercaptoethylamine[7], bufotenine[7],

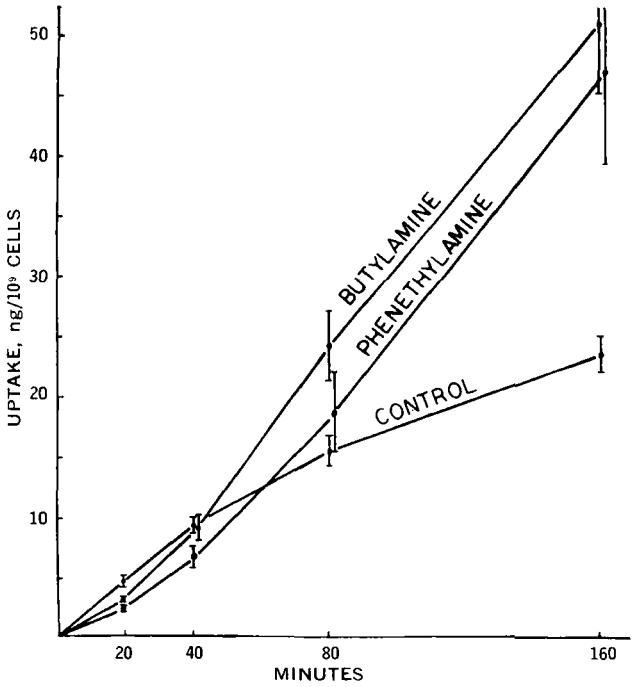
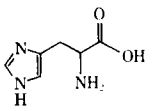
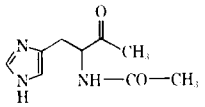
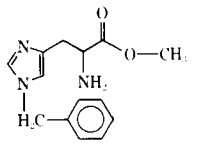
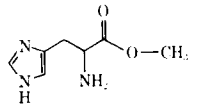
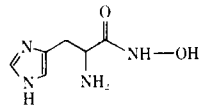
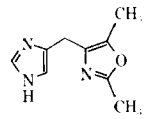
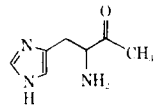
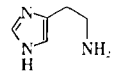


Figure 1—*Time course of the inhibition and increase of histamine uptake by 10^{-3} M n-butylamine and 10^{-3} M β-phenethylamine. Mean ± SE of six experiments.*

[1] Soluene-100.
[2] Packard Tri-Carb 3320.
[3] Matheson, Coleman and Bell.
[4] Nutritional Biochemicals Corp.
[5] California Corporation for Biochemical Research.
[6] Sigma Chemical Co.
[7] Calbiochem.

Table I—Inhibition of ^{14}C-Histamine Uptake (in Percent) by Some Histamine Analogs[a]

Inhibitor	10^{-5} *M*	10^{-4} *M*	10^{-3} *M*
I. Histidine 	—	—	−0.5 ± 2.2 (6)
II. 4-Imidazolyl-3-(*N*-acetyl)-amino-2-butanone 	−2.8 ± 1.7 (5)	7.3 ± 2.1 (5)	8.5 ± 2.8 (6)
III. 3-(1′-*N*-Benzyl-4′-imidazolyl)-2-aminopropionic acid methyl ester 	—	8.3 (3)	31.4 ± 3.2 (6)
IV. Histidine methyl ester 	—	20.9 ± 2.3 (5)	37.3 ± 3.6 (8)
V. Histidine hydroxamic acid 	—	25.7 ± 3.3 (7)	35.4 ± 3.5 (7)
VI. 4-(4-Imidazolylmethyl)-2,5-dimethyloxazole 	−0.5 ± 2.5 (5)	12.7 ± 2.9 (7)	44.8 ± 4.1 (8)
VII. 4-Imidazolyl-3-amino-2-butanone 	6.4 ± 2.6 (6)	21.8 ± 2.7 (8)	58.9 ± 2.2 (8)
VIII. Histamine (nonradioactive) 	—	27.4 (6) —	58.8 (2) 49.1 ± 3.7 (7)[b]

[a] Platelets were incubated in plasma for 80 min at 37° under oxygen with 5% carbon dioxide. Values are the means ± *SE*. Number of experiments is given in parentheses. [b] The latter is an earlier result using 160 min incubation.

methylamine hydrochloride[8], ethylamine hydrochloride[8], ethanolamine[9], histamine-(ring-2-^{14}C) hydrochloride[10], and scintillation chemicals[11]. The other substances were synthesized according to Smissman and Weis (8).

RESULTS AND DISCUSSION

Inhibition by Histamine Analogs (Table I)—Nonradioactive histamine, 10^{-3} *M*, inhibited ^{14}C-histamine uptake about 50%, and two of the other imidazoles (VI and VII) were comparable to histamine. Histidine was totally ineffective, and the effect of Compound II was negligible. Other compounds were intermediate in their ability to block histamine uptake. Thus, compounds that have an amino group capable of being protonated and that do not have a negative ion directly adjacent to the protonated nitrogen were effective. If the ability of the side-chain amino function to protonate was abolished by amide formation, no inhibition of histamine uptake was observed (compare Compounds II and VII). If the ability of an adjacent carboxyl to form an anion was removed through ester formation, the compound became effective again (compare Compounds I and IV).

Inhibition by Tryptamine Analogs (Table II)—Both tryptamine and 5-hydroxytryptamine were very potent histamine uptake inhibitors. As in the case of histidine, the amino acid 5-hydroxytryptophan, IX, was completely devoid of effect. The *N*-acetylated derivative, melatonin, was also ineffective, as was Compound XI, in which the amino group is also acylated. Although *N*-methyltryptamine and bufotenine were active compounds, *N*-monomethylation and *N,N*-dimethylation of 5-hydroxytryptamine decreased the activity compared to that of the parent compound.

Inhibition by Aliphatic Compounds (Table III)—An aromatic or heterocyclic ring was not an absolute necessity for uptake inhibition, since simple aliphatic amines had some effect. In the aliphatic series, the potency increased with an increase in chain length, with the exception of *n*-butylamine. Methylamine was ineffective and *n*-hexylamine was the most effective of the aliphatic amines studied.

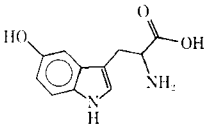
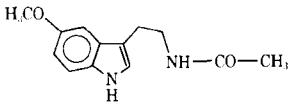
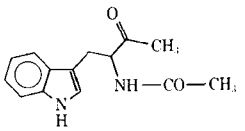
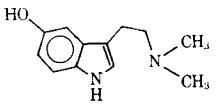
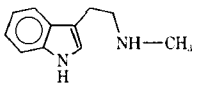
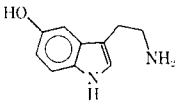
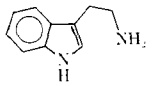
When a hydroxyl or thiol group is introduced two carbons from the amino function in the aliphatic amines, there is an increase in potency over that of the parent amine.

The aliphatic amino acids were ineffective in their ability to inhibit histamine uptake (Table III).

Effect of Butylamines and β-Phenethylamine—*n*-Butylamine and 3-methylbutylamine (isopentylamine) in high concentrations significantly increased histamine uptake. Since β-phenethylamine has a similar effect and also inhibits histamine metabolism (1, 9), the time course of their effects was studied (Fig. 1). Both *n*-butylamine and β-phenethylamine inhibited histamine uptake during short incubation periods ($p < 0.001$). After 1 hr, the rate of histamine uptake in control samples decreased, but it continued lin-

[8] Eastman Organic Chemicals.
[9] K & K Laboratories.
[10] Amersham/Searle Corp.
[11] Packard Instrument Co.

Table II—Inhibition of ^{14}C-Histamine Uptake (in Percent) by Some Tryptamine Analogs[a]

Inhibitor		10^{-5} *M*	10^{-4} *M*	10^{-3} *M*
IX. 5-Hydroxy-tryptophan		—	—	0.1 ± 0.5 (5)
X. Melatonin		—	−1 ± 0.8 (5)	−6.0 ± 3.8 (5)
XI. 4-Indolyl-3-(*N*-acetyl)-amino-2-butanone		—	−1.5 ± 1.1 (4)	1.6 ± 2.3 (3)
XII. Bufotenine		14.7 ± 2.1 (4)	35.0 ± 2.1 (4)	—
XIII. *N*-Methyl-tryptamine		18.8 ± 1.8 (4)	46.4 ± 1.8 (4)	—
XIV. 5-Hydroxy-tryptamine		15.8 ± 3.7 (6)	58.0 ± 1.1 (6)	86.0 ± 0.6 (3)
XV. Tryptamine		23.8 ± 1.0 (6)	62.2 ± 1.1 (6)	70.6 ± 3.7 (2)

[a] Platelets were incubated in plasma for 80 min at 37° under oxygen with 5% carbon dioxide. Values are the means ± *SE*. Number of experiments is given in parentheses.

early or increased in samples containing *n*-butylamine or β-phenethylamine.

To determine if the uptake increasing activity of *n*-butylamine and β-phenethylamine could be due to acidic metabolites, butyric acid, phenylacetic acid, and other aliphatic and aromatic carboxylic acids were tested. They were found to be inactive in concentrations that could result from oxidation of the amine to the corresponding carboxylic acid in plasma ($<10^{-3}$ *M*). However, at a concentration of 10^{-2} *M*, all tested acids showed some activity with the exception of acetic acid, which may be rapidly removed by metabolic processes. At a concentration of 3×10^{-2} *M*, phenylacetic and butyric acids more than doubled histamine uptake during 80 min of incubation.

It can be concluded from the results that the most important group for histamine uptake inhibition is a protonated amino function. The amount of substitution on the amino nitrogen does not appear to be critical since amines other than primary amines are also active.

Since 5-hydroxytryptamine and tryptamine are by far the most effective of the compounds studied, it can be assumed that the indole ring is a favorable function for a fit to the carrier-receptor. This is in agreement with the fact that 5-hydroxytryptamine is taken up far more effectively by platelets than either histamine (1) or catecholamines (10–12). Since tryptamine and 5-hydroxytryptamine show only a minor difference in uptake inhibition, the 5-hydroxyl group evidently is not critical. Both in this laboratory and in earlier work (1), tryptamine was found to be more effective in low concentrations as an uptake inhibitor whereas 5-hydroxytryptamine became relatively more potent with increasing concentrations. A possible explanation is that in low concentrations 5-hydroxytryptamine is rapidly and effectively taken up itself and stored in the amine granules (13). Therefore, during the prolonged incubation, there is no longer an effective concentration of 5-hydroxytryptamine in the incubation medium unless the capacity of the storage mechanism is exceeded. Tryptamine, however, is stored much less effectively (14). Thus the molecules taken up by the platelets could leak out again, giving inhibition even with lower concentrations.

On the basis of these results, the following type of attachment is suggested for the amine carrier operating on the outer membrane of the platelet. The amine molecule is attached primarily by ionic binding through its positively charged nitrogen to some negatively charged group in the carrier. This type of binding is suggested to be the most powerful because various amines having no other active groups cause some inhibition, whereas molecules having the preferred binding function except for the charged nitrogen are ineffective. Secondary binding seems to occur through the aromatic ring. The indole ring appears preferable to others, but the imidazole ring and possibly even an aliphatic carbon chain seem to replace it to some extent. Amphetamine was reported earlier to inhibit histamine uptake very effectively (1); thus the phenyl ring can also replace the indole ring. Because of these relatively flexible requirements and because no substance lacking an amino nitrogen was an inhibitor, it is suggested that this secondary binding is relatively weak and might be due to π-binding, charge transfer binding, or hydrophobic binding.

Because of the small difference observed between 5-hydroxytryptamine and tryptamine, the 5-hydroxyl group evidently has no special role in binding, although it evidently is important for binding in storage granules (14). The role of the heterocyclic ring is not excluded and binding through the indole ring could explain the increased activity of the tryptamine compounds. The reasons for the time course effect noted with butylamine and β-phenethylamine are under study. It is known that histamine is partly metabolized during the incubation (1, 9) and that phenethylamine inhibits this metabolism, which increases its availability in plas-

Table III—Inhibition of ^{14}C-Histamine Uptake (in Percent) by Some Aliphatic Compounds[a]

Inhibitor		10^{-4} *M*	10^{-3} *M*
XVI. Methylamine	$CH_3—NH_2$	−0.6 ± 1.1 (4)	−21.3 ± 4.4 (4)[b]
XVII. Ethylamine	$CH_3—CH_2—NH_2$	0.6 ± 1.0 (5)	17.0 ± 3.9 (5)
XVIII. *n*-Propylamine	$CH_3—(CH_2)_2—NH_2$	18.6 ± 3.5 (4)	30.8 ± 5.8 (4)
XIX. Isobutylamine	$CH_3—CH_2—CH(CH_3)—NH_2$	20.9 ± 1.0 (3)	42.8 ± 1.5 (3)
XX. *n*-Butylamine	$CH_3—(CH_2)_3—NH_2$	15.6 ± 6.1 (4)	−53.8 ± 13.7(4)[b]
XXI Isopentylamine	$(CH_3)_2—CH(CH_2)_2—NH_2$	25.7 ± 2.6 (3)	−31.8 ± 3.1 (3)[b]
XXII. *n*-Pentylamine	$CH_3—(CH_2)_4—NH_2$	26.1 ± 1.6 (4)	14.0 ± 5.1 (4)
XXIII. *n*-Hexylamine	$CH_3—(CH_2)_5—NH_2$	37.4 ± 1.6 (8)	39.7 ± 7.5 (8)
XXIV. Ethanolamine	$HO—CH_2—CH_2—NH_2$	6.6 ± 1.5 (4)	29.6 ± 4.6 (3)
XXV. 2-Mercapto-ethylamine	$HS—CH_2—CH_2—NH_2$	32.9 ± 8.1 (4)	76.5 ± 6.3 (3)
XXVI. α-Aminobutyric acid	$CH_3—CH_2—CH(COOH)—NH_2$	—	1.6 ± 1.0 (5)
XXVII. β-Aminobutyric acid	$CH_3—CH(CH_2—COOH)—NH_2$	–	3.4 ± 2.0 (3)
XXVIII. γ-Aminobutyric acid	$HOOC—(CH_2)_3—NH_2$	—	−0.5 ± 1.0 (3)[b]

[a] Platelets were incubated in plasma for 80 min at 37° under oxygen with 5% carbon dioxide. Values are the means ± *SE*. Number of experiments is given in parentheses. [b] Negative number shows an increase in uptake.

Table IV—Effect of Some Carboxylic Acids on Histamine Uptake by Platelets[a]

Acid	10^{-3} *M*	10^{-2} *M*	3×10^{-2} *M*
Control	100	100	100
Aliphatic			
Acetic acid	99.6 ± 1.2	103.8 ± 0.7	105.5 ± 2.2
Propionic acid	101.9 ± 1.4	116.0 ± 4.9	39.6 ± 4.6
Butyric acid	113.9 ± 2.1	174.0 ± 8.6	223.5 ± 11.0
Aromatic			
Phenylacetic acid	102.2 ± 0.3	174.2 ± 4.7	212.3 ± 8.8
Indoleacetic acid	101.6 ± 2.9	129.9 ± 3.3	142.7 ± 16.7

[a] Incubation conditions were as in Table I. Acids were dissolved in 0.9% saline and neutralized with solid sodium bicarbonate to pH 7. Uptake is given as percent of control incubated with saline only. Values are the means ± *SE* of three to four duplicate experiments.

ma during the incubation. High concentrations of monocarboxylic acids increase 5-hydroxytryptamine uptake (15), and a similar increase in histamine uptake was found in this study (Table IV). Although the concentrations required were high, these findings may be relevant to the increase of histamine uptake by the corresponding amines. This interpretation is supported by the preliminary finding in these laboratories that monoamine oxidase inhibitions reversed the effect of butylamine and phenethylamine on histamine uptake.

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