P-388 lymphoid leukemia cells in tissue culture[21] was determined using varying concentrations of peptides 3–7.

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Supplementary Material Available. Figure 1 will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.00 for photocopy or $2.50 for microfiche, referring to code number JMED-75-826.

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Notes

Potential Histamine H_2-Receptor Antagonists.[1] 2. N^{α}-Guanylhistamine

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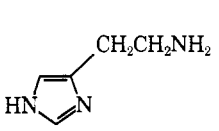
The agonist molecule, histamine, has been used as a starting point for the design of potential H_2-receptor antagonists. Converting the side-chain amino group into a guanidine yielded the first histamine H_2-receptor antagonist, N^{α}-guanylhistamine. Antagonism of H_2 receptors was demonstrated by the inhibition of histamine-stimulated gastric acid secretion in the rat at high dose levels (approximate ID_{50} 800 μmol/kg, iv) and by the inhibition of histamine-stimulated tachycardia of guinea-pig right atrium (pA_2 = 3.9). Guanylhistamine behaves as a partial agonist at histamine H_2 receptors.

In previous publications from these laboratories[1,2] we have described some preliminary investigations in a program aimed at the discovery of compounds that could specifically antagonize certain pharmacological actions of histamine that are not blocked by conventional antihistamine drugs such as pyrilamine. This work led to the eventual synthesis and characterization of the histamine H_2-receptor antagonists, burimamide,[3] metiamide,[4] and cimetidine.[5] The purpose of this series of publications is to describe various approaches that have been used in our quest for the

desired H_2-receptor antagonist. Our starting point throughout has been the agonist molecule, histamine (I), and we have attempted to identify those structural features and associated physicochemical properties which we thought likely to be important for interactions with the putative H_2 receptor and, by modifying these properties, to influence biological activity in the direction of the desired antagonism of histamine.



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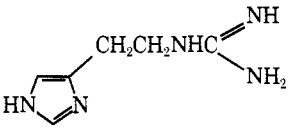
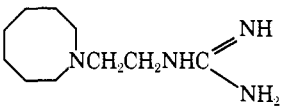


II

Agonist–antagonist relationships among various receptor systems were considered for possible extrapolation to the histamine H_2 receptor. Thus a possible analogy between histamine H_2 receptors and adrenergic β receptors led to the synthesis of aminoethylimidazopyridines as potential histamine H_2-receptor antagonists. However, these ring-modified derivatives of histamine did not antagonize histamine.[1] Furthermore, no other ring-modified histamine analogs could be found that were able to antagonize histamine at H_2 receptors.[2,6,7]

As part of our general approach toward histamine H_2-receptor antagonists by means of structural changes within the agonist molecule, we have also explored modifications of the side-chain amino group of histamine. This led to the consideration of the guanidine, N^{α}-guanylhistamine (II), which like histamine at physiological pH will be protonated and possess a positively charged group linked by a chain of two carbon atoms to an imidazole ring.

Guanidine derivatives in general are known to possess a diverse range of biological properties. Thus guanidine and simple alkyl derivatives stimulate muscle contraction and cause peripheral vasoconstriction; longer chain guanidines and bis(guanidines) can lower blood pressure by various mechanisms and guanidine derivatives are also known as hypoglycemic, trypanocidal, and antibacterial agents. Much of the early work on the pharmacology of guanidines has been compiled by Fastier and pharmacological effects of guanidine and related amidine derivatives have been compared.[8] Some of the antimicrobial actions of guanidine derivatives[9] are also possessed to a varying degree by analogous amines and it seems likely that the presence in a structure of either type of basic group can impart this biological property by providing a potential cationic head (or heads) with the remainder of the molecule affording supplementary binding. In fact, Albert originally envisaged these basic chemotherapeutic agents acting through their cations competing with hydrogen ions for an anionic site on a vital enzyme.[10] However, many other biological and biochemical properties of guanidines and amidines are significantly different from those of analogous amines and, in some instances, the guanidine derivatives are able to inhibit the action of the amine. For example, primary amines such as tyramine and noradrenaline are typical substrates for the enzyme monoamine oxidase, while various alkylguanidines and arylalkylguanidines are known to be competitive inhibitors with a high affinity for the enzyme.[11,12] Similarly diguanidines are competitive inhibitors of the oxidation of diamines by the enzyme diamine oxidase (histaminase).[13] The high affinity of guanidines for these enzymes is suggestive of particularly strong binding between the positively charged guanidinium group and anionic sites on the enzyme surface. In recent years guanidine derivatives have acquired a special interest following the discovery of guanethidine (III),[14] a hypotensive guanidine derivative which acts by inhibiting neurotransmitter release from post-ganglionic sympathetic nerve endings.[14] A large number of adrenergic neurone blocking agents containing a guanidine group have since been studied;[12] many of them deplete catecholamines from nerve terminals and peripheral tissues and the guanidines are strongly bound to "specific" noradrenaline storage sites for which they have a much higher affinity than noradrenaline itself.[15,16] The apparently high affinity of guanidinium and other amidinium ions relative to ammonium ions for anionic sites in biological systems suggests that guanidine-containing structures should be capable of antagonizing amines at receptor sites. In fact, certain guanidines have been reported to antagonize norepinephrine and epinephrine[17] particularly at α receptors.[18]



III

Guanidine derivatives therefore appeared suitable candidates for consideration as antagonists of histamine and, in pursuance of our general approach of designing antagonists by modification of the agonist molecule, the guanidine II, N^{α}-guanylhistamine, was synthesized as a potential histamine H_2-receptor antagonist.

Pharmacology. N^{α}-Guanylhistamine (II), as its sulfate salt, was tested for interactions with histamine H_2 receptors in vivo on gastric acid secretion using the lumen perfused stomach of the anesthetized rat preparation.[19] Stimulant activity was found at doses in the range 16–512 μmol/kg given by rapid iv injection. The maximal response obtained with guanylhistamine was between 50 and 60% of that obtainable with histamine. Agonist potency, which was assessed by comparison of the doses of II and histamine required to produce equal acid secretory responses, was found to be less than 0.5% of histamine (n = 9). Guanylhistamine is therefore a weak agonist on gastric acid secretion.

To test for antagonist activity, II was injected iv after a plateau of secretion had been established to an iv infusion of histamine. When first tested the rate of histamine infusion used (0.05 μmol/kg/min) produced a secretory response which was less than 50% of maximal and guanylhistamine caused either no effect or a slight further increase in acid secretion. However, when at a later date II was tested against a near-maximal secretory response to histamine (infusion rate 0.25 μmol/kg/min), inhibition was detected at doses of 256 μmol/kg and greater. The approximate iv ID_{50} (that is the dose to produce 50% inhibition of near-maximal secretion) was calculated to be 800 μmol/kg (n = 15).[20] Guanylhistamine appears to be acting as a partial agonist[21] on gastric acid secretion and its antagonist activity went undetected initially because the secretory response to the histamine infusion did not exceed the maximal agonist activity of guanylhistamine. Antagonism could only be demonstrated when the rate of histamine infusion was increased so that the secretory response exceeded the maximum response to guanylhistamine. Although these studies indicate that guanylhistamine acts as a partial agonist on gastric acid secretion, further evidence is necessary to establish that this action is the result of interaction with histamine H_2 receptors. The compound was therefore investigated on isolated guinea-pig atrium in vitro where histamine causes an increased rate of beating by interaction with histamine H_2 receptors.[3] Using cumulative dose–response curves II was found to have agonist activity at high concentrations up to 5 μM but only achieved 60 ± 5% (n = 7) of the maximal response obtainable with histamine and

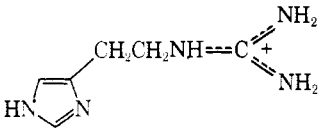
the dose–response curves were not parallel. This behavior is typical of a partial agonist and, because of nonparallelism, only an approximate activity relative to histamine of 0.7% could therefore be estimated. Antagonist activity was demonstrated by comparing control cumulative dose–response curves for histamine with those obtained after equilibration with a series of different concentrations of guanylhistamine. The apparent dissociation constant (K_b) was estimated to be 1.3×10^{-4} M (0.44–4.6, n = 8) which corresponds to an empirical pA_2 value of 3.9 (3.3–4.4).[22] Partial agonist activity could also be demonstrated by superimposing a cumulative dose–response curve to guanylhistamine on top of a sustained maximal tachycardia to histamine. As seen in the gastric secretion studies the maximal inhibitory effect of guanylhistamine (34.5 ± 8.3%, n = 6) did not exceed the maximal agonist activity seen with the compound alone.

Guanylhistamine was also tested for interactions with histamine H_1 receptors on the isolated guinea-pig ileum.[3] No agonist activity was detected at doses up to 1 μM (i.e., potency <0.01% histamine; n = 4). However, guanylhistamine had a weak antagonist action against histamine on the guinea-pig ileum giving a pA_2 value of approximately 3.8 (n = 5).

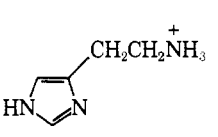
Discussion

The above results which indicate that N^{α}-guanylhistamine is a partial agonist at histamine H_2 receptors support the proposition outlined earlier that guanidines may be capable of acting as antagonists of analogous amines at receptor sites. Guanylhistamine contains a strongly basic guanidino group (cf. methylguanidine, pK_a = 13.6)[23] in place of the amino group of histamine and the guanidine should be protonated at any physiological pH that might be envisaged. The apparent pK_a value of the imidazole cation in guanylhistamine is 6.55 at 37°.[24] This value is higher than that for the imidazole cation in histamine (pK_a = 5.80)[25] and this may be a consequence of the positive charge being delocalized over three nitrogen atoms in the guanidinium ion. Although the population of N^{α}-guanylhistamine dication (n = 0.12) is higher than that of histamine dication (n = 0.03) at physiological pH (7.4), the preferred species should be the N^{τ}-H tautomer IV of the monocation analogous to the preferred tautomer V of histamine monocation.[26]

The properties of the ring and side chain of histamine and of guanylhistamine would therefore not be expected to differ to any significant extent and it is reasonable to assume that the antagonist activity of guanylhistamine derives from differences in chemical properties between the guanidinium group of IV and the ammonium group of V.



IV



V

Now the amino group is important for agonist activity at histamine receptors and a specific ionic interaction between the ammonium cation and an acidic receptor site may be envisaged. The acidic receptor site could contain a phosphate anion as proposed originally by Belleau[27] in his adrenergic receptor model and subsequently suggested by Bloom and Goldman[28] in their "dynamic receptor" for both noradrenaline and histamine. Differences in chemical properties between ammonium and guanidinium cations could then result in differences in the nature of ion pairs formed with anions such as phosphate. Unlike ammonium which is tetrahedral, guanidinium is a planar resonance-stabilized cation with a positive charge delocalized over all three nitrogen atoms. Walker[29] has suggested that resonance in an amidinium cation such as guanidinium and an anion such as carboxylate or phosphate is complementary and, as a result, ionic bonds could be formed simultaneously to give a rigid planar cyclic hydrogen-bonded configuration in the salt. Evidence in favor of this postulate has been provided by Kennard and Walker[30] from a crystallographic study of S-methylthiouronium p-chlorobenzoate; recent crystallographic studies by Cotton and coworkers[31] and Furberg and Solbakk[32] have shown that cyclic hydrogen-bonded structures of this kind also occur in methylguanidinium phosphates and propylguanidinium diethyl phosphate. The additional suggestion by Walker[29,30] that such doublet ion pairs might exist in solution has been refuted by Tanford[33] who found that association between guanidinium ions and acetate ions is weak in aqueous solution. However, it is known that ion-pair interactions between amidinium ions and anions occur in nonaqueous media.[34] Thus if one considers a hydrophobic receptor environment it seems quite possible that differences between amidinium and ammonium ions in their interactions with anions could underlie differences in biological properties between structures containing ammonium ions and "pharmacophoric" amidinium ions and might be relevant to the antagonism of histamine by guanylhistamine.

Many years ago Ackermann first noted that various guanidine derivatives inhibited some of the actions of histamine.[35] Even earlier, guanylhistamine had been synthesized by van der Merwe[36] and reported to be "devoid of interesting physiological activity". However, it transpired that N^{α}-guanylhistamine was the first compound found with detectable H_2-receptor antagonist activity and the structure of this weakly active antagonist provided a vital lead for the design of highly active and clinically effective histamine H_2-receptor antagonists.

Experimental Section

N-[2-[4(5)-Imidazolyl]ethyl]guanidine (N^{α}-Guanylhistamine). A solution of histamine base [(55.5 g, 0.5 mol), generated from the dihydrochloride (B.D.H. Ltd.) and sodium ethoxide in ethanol] and *S*-methylthiouronium sulfate (70.0 g, 0.25 mol) in H_2O (300 ml) was heated under reflux for 6 hr, cooled, and acidified with H_2SO_4. Concentration followed by the addition of EtOH afforded the guanidine sulfate (70.0 g, 56%), mp 278–279° (H_2O). Anal. ($C_6H_{11}N_5 \cdot H_2SO_4$) C, H, N, S.

Ion exchange with IRA-401(Cl^-) afforded the guanidine dihydrochloride, mp 222–223° (EtOH–*i*-PrOH–Et_2O) (lit.[37] mp 214–216°). Anal. ($C_6H_{11}N_5 \cdot 2HCl$) C, H, N, Cl.

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Hypolipidemic Imidazoles

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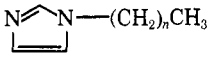
A series of analogs of *N*-benzylimidazole was prepared and tested for hypolipidemic activity. Both plasma cholesterol and triglyceride-lowering activity were found in several members of the series. The most active compounds were *N*-3-methoxy-, *N*-4-methoxy-, and *N*-4-methylbenzylimidazole. Structure–activity relationships are discussed.

In the course of a search for novel hypolipidemic agents, it was discovered that a number of simple imidazole derivatives, namely *N*-alkylimidazoles of type I, inhibited cholesterol biosynthesis at the 2,3-oxidosqualene cyclase step both in vitro and in vivo and also showed hypocholesterolemic activity in experimental animals.[1]



I, $n = 9$–13

Extension of this work revealed that certain other substituted imidazoles also showed both hypocholesterolemic and hypotriglyceridemic activity and we here report the results of these studies.

Chemistry. Compounds **1–27** and **29–33** (see Tables I and II and the Experimental Section) were prepared by the alkylation of imidazole or a substituted imidazole with the corresponding halide, usually the bromide (see the Experimental Section). The amino derivative **28** was obtained by $NaBH_4$–10% Pd/C reduction of the nitro analog **10**.[2] The known compounds **34–36** were synthesized by literature methods (see Table III).

Structure–Activity Relationships. It was observed that many benzylimidazoles (Table I) possessed hypolipidemic properties, the hypocholesterolemic effect being more general than the hypotriglyceridemic property. The derivatives bearing a free 4-hydroxy group **24**, esterified hydroxy group **23**, or 4-carbethoxy group **22** were inactive. However, related to the hypocholesterolemic activity, an associated increase in liver lipids and liver cholesterol was seen.

Various modifications to the substitution pattern in the benzene ring (Table I) and to the basic arylimidazolylmethane structure (Tables II and III) were investigated in an attempt to obtain a cholesterol-lowering effect without production of fatty liver. The performance of two known hypolipidemic agents and *N*-dodecylimidazole in our test system is shown in Table IV for comparison.

Substitution in the imidazole ring resulted in loss of activity (**29** and **30**) as did substitution on the methylene bridge (**33**). Removal of the methylene bridge as in compounds **34** and **35** preserved the hypocholesterolemic activity but caused an increase in liver lipid levels. Extension of the bridging link to 2 and 3 carbon atoms gave the active compounds **37** and **39**. The compounds which exhibited hypocholesterolemic properties yet gave the smallest rise in liver lipids, the nitro derivatives **10**, **12**, and **37** and the cinnamyl derivative **39**, were further evaluated in rats. How-