Molecular Determinants for the Agonist Activity of 2-Methylhistamine and 4-Methylhistamine at H_2-Receptors

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A model for drug action at the histamine H_2-receptor has been evaluated computationally for the agonists 2- and 4-methylhistamine. Based on molecular properties calculated for molecular structures optimized with ab initio quantum mechanical methods, the activities of these compounds and their potencies relative to histamine are found to be explained by the previously proposed model. Recognized in the N3-H tautomeric form of their monocations, both compounds exhibit a change in ring tautomeric preference when the cationic side chain is neutralized. This change makes possible their participation in a proposed proton relay event that was postulated to initiate the receptor response of H_2-agonists. The relative concentrations of the mono- and dication forms of the molecules in equimolar concentrations of histamine and the two derivatives are calculated from the values of the molecular electrostatic potentials at the ring protonation sites. Because the monocation is the species recognized at the H_2-receptor, the reduced potency of 2-methylhistamine relative to histamine and to the 4-methyl derivative is explained by the finding that 2-methylhistamine will have the lowest concentration of the recognized species. The rank order of potencies obtained from the ratio of monocationic species of the molecules is in agreement with experimental results.

The histamine congeners, 2-methylhistamine (Figure 1, **1**) and 4-methylhistamine (Figure 1, **2**), are known to exhibit selective agonist activity with respect to the H_1- and H_2-receptors that mediate the pharmacological actions of histamine.[1] On the H_1-receptor, 2-methylhistamine (2-MeHA) possesses approximately 20% of the potency of histamine, but on the H_2-receptor it exhibits only 2–4% of the potency of histamine (HA). In contrast, 4-methylhistamine (4-MeHA) exhibits 0.2% of the potency of histamine as an H_1-receptor agonist and 40% of histamine's potency as an H_2-receptor agonist.[1-3] Both derivatives are full agonists of histamine.

In solution, histamine and its ring-methylated analogues exist as mixtures of the neutral forms, the monocations, and the dications in which both the side-chain amine and the imidazole ring are protonated. The neutral molecules and the monocations have two tautomeric forms, designated here N3-H and N1-H. In aqueous solutions at pH 7.4, more than 96% of all histamine molecules are in the monocation form, protonated at the alkyl nitrogen.[3-5] This monocation is considered most likely to be the physiologically active form of histamine.[3,5,6] Both experimental[1-5,7,8] and theoretical studies[6,9,10] have established that the tautomeric preference in histamine changes with the protonation state of the side chain: N3-H is the more stable tautomer in the monocation, and the equilibrium is shifted toward N1-H in the neutral form of the molecule.

A mechanism describing a possible role for histamine tautomerism in the activation of the H_2-receptor has been proposed.[6] In this "charge-relay" mechanism histamine N3-H monocation is recognized at the receptor through the interaction of its cationic amine with a matching receptor site (site I), the hydrogen-bonding interaction of the N3-H group with a proton acceptor (site II), and the hydrogen-bonding interaction of the imine nitrogen with a putative proton donor (site III). Calculations showed that as the protonated amine side chain of histamine approaches site I, the interaction with this site causes a redistribution of the electron density in the imidazole ring, which increases the proton affinity of N1 and decreases that of N3.[6] The proposed activation mechanism is based on the hypothesis that the change in proton affinity of the ring nitrogen induced by neutralization of the side chain at site I leads to a proton relay process at the receptor in which a proton can be attracted from site III to N1, and possibly even released from N3 to site II. To be active at the H_2-receptor, histamine agonists must mimic both the recognition and the activation process. Here we examine if the molecular properties of 2-MeHA and 4-MeHA are compatible with the hypothesis that they are also recognized at the H_2-receptor according to the mechanism proposed for HA, and we probe this hypothesis and its predictive value by investigating if differences in the measured potencies of 2-MeHA and 4-MeHA can be explained on the basis of their ability to fit the proposed scheme. From the molecular properties and tautomeric preferences of the two agonists we also infer whether the proposed model for H_2-receptor activation by HA is also possible for 2-MeHA and 4-MeHA.

Methods

Complete geometry optimizations of 2-MeHA and 4-MeHA were done for the N1-H and N3-H tautomers of the neutral and monocationic species as described also for HA.[10] Calculations were done at the Hartree-Fock level

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Table I. Optimized Structures[a]

	2-methylhistamine				4-methylhistamine			
	N1-H tautomer		N3-H tautomer		N1-H tautomer		N3-H tautomer	
	neutral	cation	neutral	cation	neutral	cation	neutral	cation
				Angle, deg				
$C_4C_5N_1$	105.1	105.5	110.6	111.3	105.6	106.0	110.9	111.7
$C_5N_1C_2$	107.3	106.9	105.1	104.8	106.9	106.4	104.8	104.4
$N_1C_2N_3$	111.2	111.3	111.3	111.0	111.9	112.1	111.8	111.7
$C_2N_3C_4$	104.7	104.9	106.8	107.5	104.7	104.8	106.9	107.5
$N_3C_4C_5$	111.7	111.5	106.2	105.4	110.9	110.6	105.5	104.8
tau1	75.0	98.5	60.0	40.0	80.8	98.0	52.6	35.8
tau2	183.7	175.4	181.8	191.1	183.8	176.1	181.7	190.3
				Bond Length, Å				
C_5N_1	1.396	1.398	1.415	1.409	1.396	1.398	1.415	1.410
N_1C_2	1.388	1.387	1.318	1.321	1.383	1.381	1.313	1.315
C_2N_3	1.318	1.323	1.387	1.386	1.314	1.317	1.383	1.382
N_3C_4	1.410	1.404	1.394	1.386	1.415	1.411	1.396	1.392
C_4C_5	1.349	1.350	1.348	1.349	1.355	1.355	1.355	1.355

[a] Calculated ab initio with the STO-3G basis set.

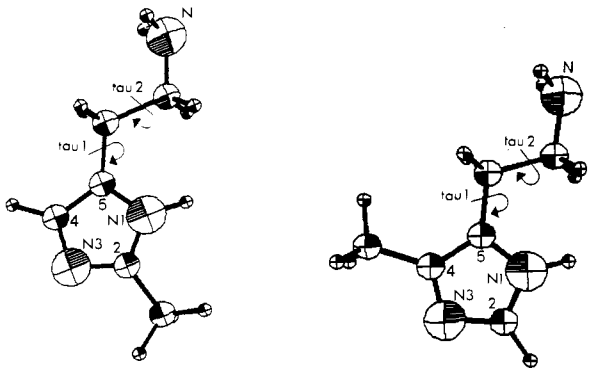

Figure 1. Molecular structure and numbering scheme of 2-methylhistamine (1) and 4-methylhistamine (2).

using the STO-3G basis set as implemented in the GAUSSIAN 80 system of programs.[11] All the parameters defining each molecular structure were optimized, but the imidazole ring was kept planar. There is a tendency in the N3-H cation for the end of the chain to twist toward the ring so as to form an internal hydrogen bond between the imine nitrogen and a proton of the protonated amine.[12] As described before,[10] this minimum was intentionally avoided in the calculations. Energies were stable to within 10^{-6} hartrees. The resultant total energy for each minimum-energy structure was used to draw conclusions as to relative tautomer stability in solution, since the hydration energies of the equivalent species of HA, 2-MeHA, and 4-MeHA can be assumed to be very similar.

At the geometries obtained from the ab initio optimization, the molecular orbitals were recalculated with the effective core potential method (ECP)[13,14] and the LP-3G basis set developed specifically for use with these ECP's.[15] For reasons described in detail,[15-17] these molecular orbitals were used to calculate the molecular electrostatic potentials (MEP) maps, as previously described.[18]

Molecular electrostatic potentials have been shown to provide reliable information on the interaction sites in molecules with point charges and on the comparative reactivities of these sites (for a review see ref 19). The molecular electrostatic potentials were used to deduce relative proton affinities and to calculate the abundance of the dicationic species of the molecules relative to the monocation concentration.

Results and Discussion

Molecular Structures. The results of the geometry optimizations on both the N1-H and the N3-H tautomers of the neutral and monocationic forms of 2-MeHA and 4-MeHA are summarized in Table I. The minimum-energy structures for all forms considered were found to be essentially in the trans conformation (fully extended chain, $\tau_2 = 180°$). This finding is in keeping with results from crystal structure studies of histamine monocation and its neutral form, which have thus far only been found in the trans conformation.[5,7,8] The crystal structure of 4-methylhistamine monohydrobromide has also been examined[20] and found to be isostructural with histamine monohydrobromide having $\tau_2 = 178.1°$.

The results of the conformational studies also indicate that the imidazole ring may take on a variety of orientations with respect to the side chain; these are represented by the values of τ_1 illustrated in Figure 1. Results from crystallographic studies also indicate a variety of orientations of imidazole in histamine congeners.[5] In general, the results obtained here (Table I) show that the N3-H tautomers of the monocations of both 2-MeHA and 4-MeHA have lower τ_1 values than the same tautomers of the neutral molecules. The N1-H tautomers of either molecule have an increased value of τ_1, reflecting a tendency of the imidazole ring to reside in a more out of plane position relative to the side chain. The conformation of the energetically optimized form of 4-MeHA falls within the central region of the conformational probability maps obtained by Farnell et al.[21] from EHT calculations of the

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Table II. Total Molecular Energies and Tautomer Stabilizations

species	total energy, amu	E_d,[a] kcal/mol	% N3-H
2-methylhistamine			
N3-H neutral	-392.052810		16.7
N1-H neutral	-392.054386	0.99	
N3-H monocation	-392.500014		99.9
N1-H monocation	-392.483628	-10.28	
4-methylhistamine			
N3-H neutral	-392.050664		35.6
N1-H neutral	-392.051247	0.37	
N3-H monocation	-392.498018		99.9
N1-H monocation	-392.480263	-11.14	

[a] $E_d = E(\text{N3-H}) - E(\text{N1-H})$.

most stable conformers of 4-methylhistamine monocation.

Analyses of crystal structures of numerous imidazole derivatives reveal that the five-membered ring is not symmetric. The endocyclic angle at the unprotonated nitrogen atoms (105.3° average) is smaller than the endocyclic angle at the protonated nitrogen atom (107.3° average), showing that a change in tautomeric form is accompanied by a rearrangment of the atoms of the imidazole skeleton.[4,22] The optimized geometries of 2-MeHA and 4-MeHA also exhibit such ring asymmetry (Table I) and indicate that ring geometries are dependent on the ionization of the side chain (i.e., free base vs. monocation) mostly through the change in stability of the tautomeric form (N1-H vs. N3-H, respectively). Similar results were obtained in previous ab initio studies on the structures of the tautomers of histamine.[10,22]

The optimized geometries of 2-MeHA and 4-MeHA exhibit tautomeric preferences similar to those calculated for histamine.[10] From the energies of all neutral and monocationic forms of both 2-MeHA and 4-MeHA (Table II), the N3-H tautomers appear more stable than the N1-H tautomers in the monocationic forms of the two molecules by 10.3 kcal/mol and 11.1 kcal/mol, respectively. In the neutral species forms, the tautomeric forms of the molecules achieve a more nearly equal probability in the dynamic equilibrium. The relative probability can be calculated from the ratio of the molar fractions (n) of the two tautomers, which has the form

$$n(\text{N3-H})/n(\text{N1-H}) = \exp(-E_d/RT) \quad (1)$$

where E_d is the difference in the energy of the two tautomers listed in Table II. The tautomeric ratios obtained from these calculations (Table II) agree with our previous findings on histamine,[3,10] which showed that neutralization of the side chain is likely to cause a change in the tautomeric preference of the imidazole and can therefore trigger a receptor activation mechanism based on proton transfer between the molecule and the recognition sites in the receptor.

Relative Abundance of the Protonated Species. Based on the assumption that the value of the minimum in the molecular electrostatic potential associated with the imine nitrogen (N1) in the N3-H monocation tautomers reflects the proton affinity of the cation at this ring nitrogen, as was shown for many systems,[19,23,24] our calculations of the MEP generated by histamine, 2-MeHA, and 4-MeHA indicate that methylation at the 2- or 4-position has a noticeable effect on the degree of protonation of the imine nitrogen of the imidazole ring. Thus, the N1 in 4-MeHA monocation seems to be more attractive to a proton than N1 of histamine monocation (MEP values of -18.3 vs. -14.8 kcal/mol, respectively). The minimum near N1 in 2-MeHa monocation is the lowest, -21.1 kcal/mol, indicating an even higher proton affinity. Consequently, the abundance of the dicationic species in which both the side chain and the imidazole ring are protonated should be greater for 2-MeHA, followed by 4-MeHA, and then histamine. In equimolar solutions of histamine, 2-MeHA, and 4-MeHA, histamine would have the highest concentration of monocation, followed by 4-MeHA and 2-MeHA. Since the monocation is considered to be the active form of histamine at the H_2-receptor,[5,6,10,23] the expected trend of the potencies would follow this same order if the affinities of the three molecules do not differ much. The ratio of potencies of histamine to 4-MeHA to 2-MeHA measured experimentally is of the order of 100:40:2.[5] This rank order is similar to that obtained from a calculation of the relative concentrations of the active monocationic species in equimolar solutions of histamine and its two congeners.

Table III. Relative Amounts of Monocation and Dication Present at pH 7.4

molecule	[M]/[D][a]	%M
histamine (HA)	40.25	96.6
4-methylhistamine (4-MeHA)	0.14	12.3[b]
2-methylhistamine (2-MeHA)	0.0015	0.15[b]

[a] Ratio of concentration of the monocation (M) and the dication (D) forms of the molecules, calculated from $\Delta\text{MEP}_{min} = -RT(\ln M_{HA}/D_{HA} - \ln M_{MeHA}/D_{MeHA})$. [b] Calculated using the experimental values $M_{HA} = 96.6\%$ and $D_{HA} = 2.4\%$ from ref 5 and assuming negligible concentrations of the neutral form.

To calculate the predicted ratio of monocation concentrations, we used the differences in the values of the electrostatic potential mimima near N1 in histamine and any of its methyl derivatives (ΔMEP_{min}) to evaluate the ratio of their monocation (M) to dication (D) fractions according to

$$\Delta\text{MEP}_{min} = -RT \ln (K_{HA}/K_{MeHA}) \quad (2)$$

where

$$K = [\text{D}]/([\text{M}][\text{H}^+]) \quad (3)$$

Equation 2 simplifies to

$$\Delta\text{MEP}_{min} = -RT (\ln M_{HA}/D_{HA} - \ln M_{MeHA}/D_{MeHA}) \quad (4)$$

where the square parentheses for concentration were droped to simplify the notation.

The ratio M_{HA}/D_{HA} is known from the experimental values (M = 96.6%, D = 2.4%) measured at pH 7.5[5] so that for T = 310 K and R = 1.987 cal/deg·mol, the M/D ratios can be calculated for the methyl derivatives from eq 4 (Table III). Assuming that at physiological pH the concentration of the neutral species will be negligibly small (e.g., 1% for HA), the ratio of concentration of the monocation in equimolar concentrations of HA, and its 4- and 2-Me derivatives (see Table III), will be 100:13:0.2, in qualitative agreement with the experimentally observed rank order of potencies.[5]

Conclusions

Our results demonstrate that 2-MeHA and 4-MeHA exhibit the same tautomeric preferences as histamine. In the monocation form, N3-H is the preponderant tautomer and the equilibrium is shifted considerably toward N1-H

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in the neutral molecules. Consequently, both methyl derivatives of histamine possess the ability to be recognized at the histamine H_2-receptor and to activate it according to the proposed mechansm.[6,10,23]

The reduced potencies exhibited by 4-MeHA and 2-MeHA on the H_2-receptor are consistent with predictions from the recognition hypothesis proposed earlier,[6,10] which defined the N3-H tautomer of the monocation as the only recognizable species in this class of histamine congeners. Accordingly, the lower potencies of the methyl derivatives are explained by the increased fraction of their dicationic species compared to equimolar solutions of histamine. Moreover, our calculations indicate that 4-MeHA should be a more potent agonist at the histamine H_2-receptor than 2-MeHA. The rank order of potencies predicted from these calculations is in agreement with experimental observations, thus providing additional support for the mechanistic hypothesis describing the interactions of agonists at the histamine H_2-receptor.

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Registry No. 2-MeHA, 34392-54-6; 4-MeHA, 36507-31-0.

2,3-Dialkyl(dimethylamino)indoles: Interaction with $5HT_1$, $5HT_2$, and Rat Stomach Fundal Serotonin Receptors

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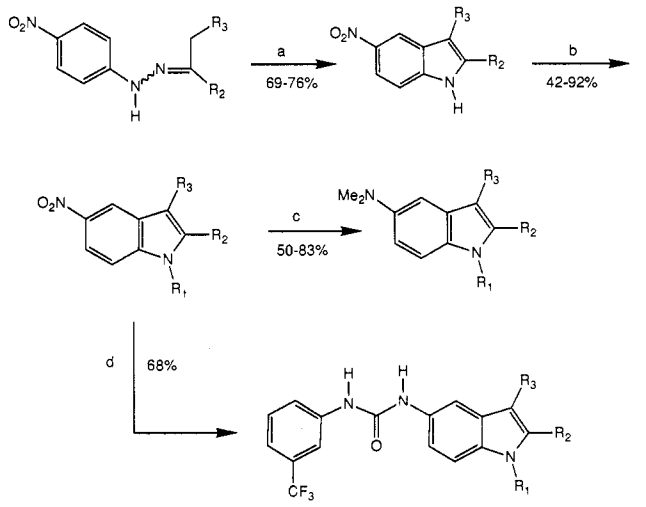
2,3-Dialkyl(dimethylamino)indoles, synthesized via the Fisher indole synthesis, were found to weakly bind to $5HT_1$ and $5HT_2$ sites in brain cortical membranes (IC_{50} greater than 1 μM at both sites for all compounds). These (dimethylamino)indoles were relatively potent antagonists of the serotonin receptor in the rat stomach fundus. At higher concentrations, several of the compounds were weak agonists at this receptor. For direct comparison with data obtained in the isolated rat fundus, antagonism of serotonin-induced contractions at $5HT_2$ receptors in the rat jugular vein was also examined. Several of the compounds showed good selectivity for the fundus receptor relative to the $5HT_2$ receptor; together with minimal affinity for $5HT_1$ and $5HT_2$ binding sites in brain cortical membranes, these results support the idea that the serotonin receptor in the stomach fundus is distinct from $5HT_1$ and $5HT_2$ binding sites.

In the mid-1950s, Shaw and Woolley reported that the indole derivative 2-methyl-3-ethyl-5-(dimethylamino)-indole (medmain) was a partial agonist at the serotonin receptor in certain isolated smooth muscle preparations such as sheep vascular tissue and the rat uterus.[1] In those tissues, now known to possess $5HT_2$ receptors,[2] the activity of medmain as both an agonist and antagonist was relatively weak. However, it has been our observation that medmain is a relatively potent antagonist of the serotonin receptor in the rat fundus. Serotonin receptors in the rat fundus preparation, originally described by Vane,[3] have recently been shown to be distinct from the serotonin receptor subtypes already described: $5HT_{1a}$, $5HT_{1b}$, or $5HT_2$.[4]

Encouraged by the antagonist activity of medmain at the serotonin receptor in the fundus, we made several derivatives to explore the nature of this receptor. Specifically, we examined both the agonist and antagonist activity of medmain at this receptor, as well as the specificity for the serotonin receptor in the fundus over the other serotonin receptors already described. In addition, we explored the effect of fundus activity/selectivity imparted by adding substituents at N-1, or by varying the alkyl groups at the 2- and 3-position, or finally, by moving the dimethylamino group from the 5-position to the 7-position. In this paper, we describe a series of 2,3-dialkyl(dimethylamino)indole derivatives and their agonist and antagonist activity at the fundus receptor, as well as their activity at other serotonergic receptors. Activity at $5HT_2$ receptors was examined in the rat jugular vein, a

Scheme I[a]



[a] (a) Concentrated HCl, reflux overnight; (b) NaH in 5/1 PhMe/DMF, R_1I, room temperature or 80 °C; (c) H_2, 5% Pd/C, formalin solution in EtOH; (d) H_2, 5% Pd/C in EtOH, followed by *m*-$CF_3C_6H_4NCO$ in EtOH, then chromatography.

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