Theoretical Studies on Molecular Determinants for Recognition at H_3 Histamine Receptors

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The determinants for recognition at H_3 histamine receptors are considered. Findings based on quantum-chemical calculations suggest that H_3 histamine receptor is less hydrophilic than the H_2. The form most likely to be recognized by the H_3 receptor is an intramolecularly hydrogen-bonded form of α-methylhistamine. Receptor environment and hydration effects of active form of histamine analogs are of crucial importance.

Introduction

The histamine receptors are diversified into three different classes. The classification of histamine H_1 and H_2 receptors (Ganellin and Parsons, 1982) has been derived on the basis of its pharmacological effects. Action of histamine such as contraction of some smooth muscles (*e.g.* guinea pig ileum), or relaxation, are assumed to be mediated by H_1 receptors. Other effects of histamine, like stimulation of gastric acid secretion and modulation of the atrial rate, are attributed to a different class of receptors, the H_2 receptors. This subclassification has brought about not only clarification of the observed tissue and drug specificities of histamine sensitive receptors, but has made possible to design an entirely new class of H_2 antagonists (Ganellin and Parsons, 1982; Black *et al.,* 1972). More recent studies on histamine function and metabolism performed with brain tissue revealed that histamine is able to inhibit its own synthesis and release (Arrang *et al.,* 1987). Based on results from tests with a variety of H_1 and H_2 antagonists it appeared that the observed inhibition is mediated by another subtype of histamine receptors, the H_3 receptors. The presence of H_3 receptors has also been discovered in the autonomic nervous system on perivascular nerve terminals (Ishikawa and Sperelakis, 1987). Highly potent and selective H_3 histamine agonists and antagonists have been identified by Arrang and coworkers (Arrang *et al.,* 1987). The (*R*)α-methylhistamine (α-MeHA) was found to be roughly 15 times more potent than the histamine (HA) as inhibitor of histamine release from brain slices. In the test on perivascular nerve stimulation of vascular smooth muscle, histamine appeared to differ significantly in its histamine agonistic effect on depression of an excitatory junction potential. The 2-methylhistamine (2-MeHA) and 4-methylhistamine (4-MeHA), as well as the selective H_2 agonist, dimaprit, were found to be inactive at H_3 receptors.

The high selectivity observed for these compounds towards particular subclasses of histamine receptors, *e.g.* H_2 *vs.* H_3, provides an opportunity to identify a structural properties responsible for different effects on particular receptors, and also to characterize the basic properties of receptor environment. One of the major physical-chemical properties that could discriminate this class of structurally related compounds, and therefore serve as useful probe for the observed biological activity, is their ability to form intramolecular hydrogen bonding. Anchoring of the ethylamine side chain at the N(1)-imidazole nitrogen by formation of such a hydrogen bond would lead to a conformation for which the H_3 receptor becomes accessible (Scheme I).

Methods

Computational details

Ab initio molecular orbital calculations were performed with GAUSSIAN 92 system of pro-

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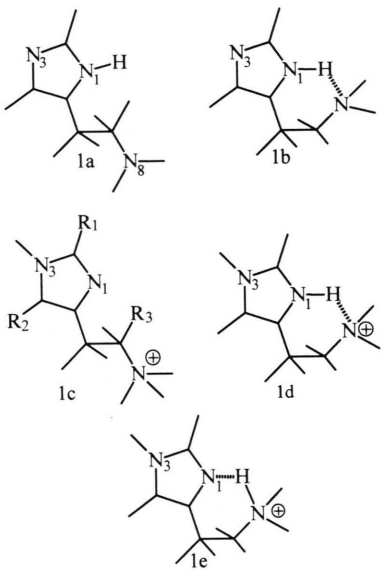
Scheme I



Histamine	$R_1, R_2, R_3 = H$
α-Methylhistamine	$R_1, R_2 = H$; $R_3 = -CH_3$
2-Methylhistamine	$R_2, R_3 = H$; $R_1 = -CH_3$
4-Methylhistamine	$R_1, R_3 = H$; $R_2 = -CH_3$

grams (Frish *et al.*, 1992) using minimal STO-3G (Hehre *et al.*, 1969, 1970) and 6-31G split valence basis set (Hehre *et al.*, 1972). Geometry optimizations were performed with optimization procedures in the GAUSSIAN 92 package, based on the analytical calculation of the first derivatives of the energy at the Hartree-Fock level. The hydration enthalpies were calculated using method proposed by Rashin (Rashin and Namboodiri, 1987) for arbitrary polar and charged molecules. Within this method the total enthalpy of hydration is expressed as sum of the enthalpy of electrostatic interactions of the solute molecule with the solvent, and of the hydration enthalpy of non-polar molecule forming the same cavity in the solvent. The electrostatic contribution to ΔH was calculated employing Mulliken 6-31G point charges.

Results and Discussion

At this stage we focus on recognition at the H_3 receptor site. The following step, *e.g.* activation of receptor is probably a result of proton-relay process. Therefore the proton transfer from the donor receptor site through intramolecular hydrogen bond is to be considered. The path of proton transfer, the transition states as well as stabilization energies defined by equilibrium geometry of the system will be discussed elsewhere. Here we focus on determinants responsible for recognition process yielding different potency at the particular receptor.

At physiological pH histamine and its derivatives exist in the cationic and neutral forms of N(3)–H and N(1)–H tautomer, respectively (Gannelin and Parsons, 1982). At the H_2 receptor site the most abundant N(3)–H protonated form is an active species. In the closed form also the N(3)–H tautomer (structure **1d**) appeared to be the most stable (Karpińska and Mazurek, 1994). However, the strong interaction with negatively charged ionic site is unlikely because it would lead to the opening of the side chain. Therefore the nature of the H_3 histamine receptor seems to be different from the H_2 interaction site. Now the N(1)–H tautomer closed by intramolecular hydrogen bonding cannot be neglected. Total energies for fully optimized structures of neutral forms of N(1)–H tautomer in extended and hydrogen-bonded conformations (structure **1b**) are presented in Table I. The total vacuum stabilization energies calculated with a 6-31G basis set following full optimization with that basis predict the hydrogen bond to be the most stable in α-MeHA. In 2-MeHA the hydrogen bond stabilization energy is reduced by 0.68 kcal/mol when compared to α-MeHA, while in HA and 4-MeHA hydrogen bonds are weaker by 0.37 and 0.56 kcal/mol, respectively. The hydration enthalpies calculated for

Table I. Total molecular energies (in hartrees[a]) and hydrogen bond stabilization energies (in kcal/mol) calculated for N(1)–H tautomeric forms of histamine analogs.

Structure		Total energy	ΔE_{hb}
HA:	extended	−357.749160[b]	–
	H-bonded	−357.75539[c]	3.88
α-MeHA:	extended	−396.770127	–
	H-bonded	−396.776906	4.25
2-MeHA:	extended	−396.777310	–
	H-bonded	−396.783000	3.57
4-MeHA:	extended	−396.773787	–
	H-bonded	−396.779675	3.69

[a] 1 hartree = 627.5095 kcal/mol; [b] structure **1a**; [c] structure **1b**.

each compound in extended and hydrogen-bonded form (when the solvent molecule is represented by Mulliken point charge positioned at the nuclei) are given in column 1 of Table III. In each case, the hydrogen-bonded form is hydrated better than corresponding opened form. The enthalpic contribution enhances the hydrogen bond strength calculated in vacuum. Thus for neutral species stabilization energy due to intramolecular hydrogen bonding is increased in aqueous medium. However, the order of the total stabilization energy is different for unhydrated molecules. The α-MeHA appeared to gain relatively less stabilization due to hydration when changing from opened to hydrogen-bonded conformation. As reported by Rashin (Rashin and Namboodiri, 1987) for some systems a discrepancy was found between the calculated and experimental values of hydration enthalpy, probably due to failure of the Mulliken population analysis to yield a charge distribution that correctly reproduces the dipole moment and other electrostatic properties of molecular systems. At this point it became important to verify if the method itself, regardless of representation of charge distribution, can properly describe side chain structures of the studied compounds, especially HA *vs.* α-MeHA. Therefore we also calculated hydration enthalpies for methylamine and ethylamine. Results presented in Table II indicate that the inherent error is implemented in the calculation, because the difference between the values of experimental hydration enthalpies of methylamine and ethylamine is *ca.* 2.2 kcal/mol (last row of Table II) (Aue *et al.*, 1976). The results suggest that for α-MeHA a higher value of the total hydration enthalpy should be expected if the method could yield correct values for methylated and unmethylated ethylamine side chain. Thus calculated total hydration enthalpies allow only for qualitative estimation of hydration effects. The ranking of stabilization energies calculated based on quantum chemical approach should therefore be preserved in an aqueous medium. However, a hydrophilic solvent tends to increase vacuum stabilization energies for intramolecular hydrogen bonding. With other words, the nature of the solvent (or receptor environment) seems to be a crucial factor that governs the existing equilibrium between extended and hydrogen-bonded forms. Furthermore, this property could be a decisive factor in the recognition stage at histamine H_2 and H_3 receptors.

At physiological pH-protonated forms are present in considerable concentrations and by this fact they mainly contribute to the H_3 histaminic activity. Calculations performed at the 6-31 G level yield fairly strong stabilization of protonated forms with side chain closed by intramolecular hydrogen bonding (Table III). In each case the most stable form has a proton at the imidazole ring (structure **1d**) rather than on amine nitrogen at the side chain (structure **1e**). Structure **1d,** however, represents situation in which the imidazole ring is prepared to release a proton to the receptor site. Obviously, with that form interaction through an amine group of the side chain with the slightly negatively charged receptor would be significantly

Table II. Hydration energies (in kcal/mol) calculated for N(1)–H tautomeric forms of histamine analogs and for methylamine and ethylamine.

Structure		E_{HYDR}	ΔE_{HYDR}
HA:	extended	−13.64	–
	H-bonded	−14.17	0.53
α-MeHA:	extended	−13.98	–
	H-bonded	−14.49	0.51
2-MeHA:	extended	−13.62	–
	H-bonded	−14.98	1.36
4-MeHA:	extended	−13.94	–
	H-bonded	−14.95	1.01
Methylamine:		−5.88	–
Ethylamine:		−6.49	0.61
	experimental	2.23	

Table III. Total molecular energies (in hartrees) and hydrogen bond stabilization energies (in kcal/mol) calculated at the 6-31 G//6-31 G level for protonated N(3)–H tautomers of histamine analogs.

Structure		Total energy	ΔE_{hb}
HA:	extended	−358.134458[a]	–
	H-bonded	−358.169025[b]	21.69
		−358.159808[c]	15.91
α-MeHA:	extended	−397.157494	–
	H-bonded	−397.191576	21.39
		−397.184671	17.05
2-MeHA:	extended	−397.164527	–
	H-bonded	−397.204488	25.08
		−397.190815	16.50
4-MeHA:	extended	−397.159900	–
	H-bonded	−397.197342	23.50
		−397.186450	16.66

[a] Structure **1c;** [b] structure **1d;** [c] structure **1e.**

weakened. Therefore species with a protonated amino group should be considered to be recognized by the receptor during the first stage of interaction. One can see that α-MeHA in such a protonated form (structure **1e**) is again slightly more stable than others methylated analogs of histamine (by *ca.* 1.2 kcal/mol than histamine). Hydration effects stabilize better a protonated extended form in which a positive charge is well localized within the $-NH_3^+$ vicinity (Table IV). The α-MeHA, however, remains more stable than histamine by *ca.* 1.3 kcal/mol. Those effects discriminate histamine and α-MeHA. Results in Table V show that α-MeHA can be directly involved in the receptor activation mechanism of H_2 receptor proposed for histamine (Ganellin and Parsons, 1982; Topiol *et al.*, 1984; Weinstein *et al.*, 1976, 1985; Mazurek *et al.*, 1987; Mazurek and Kukawska-Tarnawska, 1991; Haaksma *et al.*, 1991; Pardo *et al.*, 1991; Giraldo *et al.*, 1992). Like in histamine neutralization of the cationic side chain of α-MeHA causes a shift in tautomeric preference from N(3)–H to N(1)–H by 10.59 kcal/mol (Table V). In H_2 receptor the anionic site is probably negative enough to open even a strong intramolecular hydrogen bond through electrostatic interaction with a positively charged $-NH_3^+$ group at the side chain. Also from hydration results it appears that the environment should be more hydrophilic in the H_2 receptor than in H_3 because these compound are not considerably different in their H_2 agonistic activity (Ganellin and Parsons, 1982; Haaksma *et al.*, 1991). The hydrophobic environment in the H_3 receptor may render all incoming species to exist mostly in the dehydrated form. The population of an opened form would be the lowest for α-MeHA. In a hydrophobic region no anionic sites are present that could eventually open a relatively strong intramolecular hydrogen bond like in α-MeHA. Breaking of hydrogen bonding can therefore interfere with recognition of imidazole portion of molecule at H_3 receptor. The fact that α-MeHA can adopt *R* and *S* conformation has its reflection in activity of particular isomers (Arrang *et al.*, 1987). This phenomenon, however, seems to be due to steric effects, rather than to hydrogen-bonding properties.

Table IV. Hydration energies (in kcal/mol) calculated for protonated N(3)–H tautomers of histamine analogs and for protonated methylamine and ethylamine.

Structure		E_{HYDR}	ΔE_{HYDR}	ΔH_{TOT}
HA:	extended	−65.94[a]	–	–
	H-bonded	−55.37[b]	−10.57	11.12
α-MeHA:	extended	−63.50	–	–
	H-bonded	−54.56	−8.94	12.45
2-MeHA:	extended	−65.62	–	–
	H-bonded	−53.83	−11.79	13.29
4-MeHA:	extended	−65.64	–	–
	H-bonded	−53.55	−12.09	11.41
$CH_3NH_3^+$		−68.96	–	–
$C_2H_5NH_3^+$		−65.63	3.33	–

[a] Structure **1c**; [b] structure **1d**.

Table V. Total molecular energies (in hartrees) and tautomer stabilization (in kcal/mol) calculated for α-MeHA with STO-3G basis set at equilibrium geometry.

	Total energy	$E_{N(3)-H} - E_{N(1)-H}$
Cation		
N(3)–H	−392.495451	–
N(1)–H	−392.480368	−9.47
Neutral		
N(3)–H	−392.045035	–
N(1)–H	−392.046827	1.12

The accessibility of the active conformation is also a very important factor. When the equilibrium between interconverting forms is considered, two factors determine the process. First the thermodynamic one, expressed as the difference between total energies of substrate (starting conformation) and the product (final conformation), and second, the barrier for the process. The second factor is therefore describing the kinetics of the process. At the quantum chemical level the thermodynamic term is easy to calculate in its enthalpy part. Here it is simply the difference between total energies for hydrogen-bonded and the opened form of histamine derivatives. To describe properly and precisely the equilibrium also the entropy term should be estimated to yield the ΔG value rather than ΔH. If we assume that process occurs in an infinite time the thermodynamic description, based on ΔG value, is satisfactory. However, for biological processes not always a full equilibrium of the system is possible. At this point also kinetics of the process becomes an important factor. The rate of the process and accessibility of particular conformation depends on the energetic barrier for interconversion. For histamine analogs studied here

such a barrier is coupled to rotation of the side chain. A fully extended form can be achieved through rotation of the side chain of the conformer in which amino nitrogen of the side chain is hydrogen-bonded to N(1)–H of imidazole ring. This barrier is expected to be higher for α-methylhistamine due to steric hindrance of $-CH_3$ group slightly interacting with the N(1) of imidazole ring.

Conclusion

The analysis of the intramolecular hydrogen bonding in HA, 2-MeHA, 4-MeHA, and α-MeHA both in vacuum and in aqueous medium, provides a rationale for their different recognition at the H_3 receptor that is consistent with mechanism proposed for the H_2 receptor. The influence of the solvent on the equilibrium between extended and hydrogen-bonded structures, along with data form pharmacological tests, allow to identify for these compounds specific receptor properties that prevent their recognition at H_3 receptor. The H_3 receptor seems to be less hydrophilic than H_2 receptor and characterized by moderately negatively charged regions.

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