Studies on Activation of H_3 Histamine Receptor: A Mechanistic Model

Grażyna Karpińska and Aleksander P. Mazurek

Drug Institute, 30/34 Chelmska Str., 00–725 Warsaw, Poland

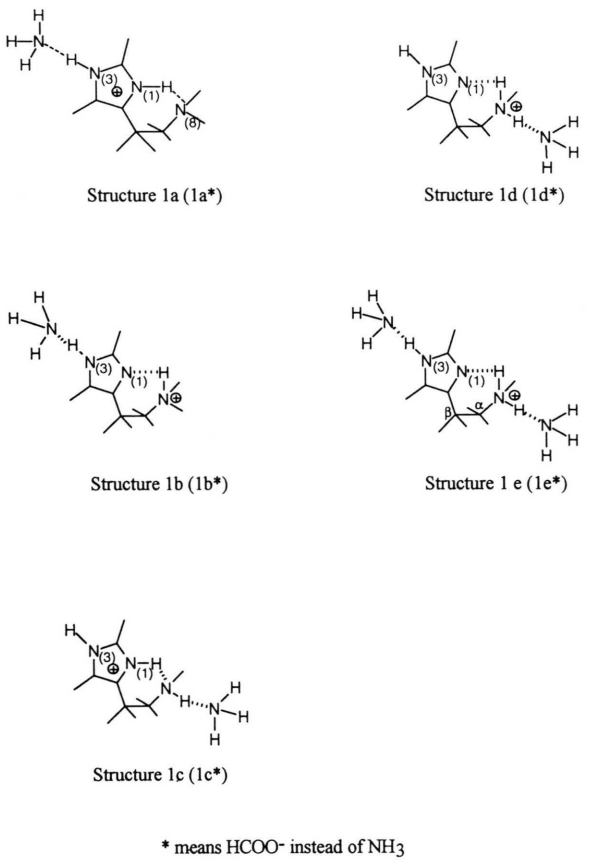
Z. Naturforsch. **50 c,** 143–147 (1995); received June 9/September 19, 1994

Histamine, α-Methylhistamine, H_3 Receptor Activation, Molecular Orbital Calculations

The recognition and the activation mechanism of the H_3 histamine receptor was studied based on quantum-chemical calculations. A mechanistic model proposed both for recognition and activation stage clarifies different properties of histamine and α-methylhistamine at the H_3 receptor. Interaction with a hypothetical receptor sites leads to the opening of the intramolecular hydrogen bonding in histamine, whereas the α-methylhistamine remains in closed conformation.

Introduction

From the early experimental (Black *et al.*, 1972) and theoretical (Weinestein *et al.*, 1976) studies on the H_2 histamine receptor it has been made a significant progress in getting more insight into recognition and activation stage (Ganellin *et al.*, 1982; Topiol *et al.*, 1984; Weinstein *et al.*, 1985; Mazurek *et al.*, 1987; Mazurek and Kukawska-Tarnawska, 1991; Haaksma *et al.*, 1991; Pardo *et al.*, 1990; Pardo *et al.* 1991; Giraldo *et al.*, 1992). Recently we have focused on the molecular determinants responsible for different recognition of histamine analogs at the H_2 and H_3 receptors (Mazurek and Karpińska, 1994). The main molecular feature discriminating histamine (HA) and its methylated analogs appeared to be their ability to form an intramolecular hydrogen bonding (Scheme 1). For the recognition stage also steric properties are important, but they rather discriminate particular isomers of the α-methylhistamine (α-MeHA) than the histamine and α-methylhistamine itself. Obviously the optical isomerism is expected to be fairly less important for energetics of activation process. Our previous findings rationalize hypothesis that similar mechanistic model for the H_3 receptor activation stage can be proposed as for H_2 receptors (Mazurek and Karpińska, 1994). In both cases the proton-relay process is triggered smoothly if incoming species approaches receptor in the most spatially fitted conformation. Any variations of structure at the recognition stage would lead to



Scheme 1.

situation in which the proton transfer is disturbed due to distorted spatial arrangement within the proton-relay portion of the receptor (Mazurek *et al.*, 1983). This would of course directly relate

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0939–5075/95/0100–0143 $ 06.00

to the ability of intramolecular hydrogen bond opening what creates different spatial situation. Therefore here we probe both various recognition and activation properties of the HA and α-MeHA.

Findings from previous studies (Karpińska and Mazurek, 1994) on the intramolecular hydrogen bonding of histamine suggested to select as potential receptor sites both the moderately proton accepting NH_3 molecules as well as the strongly negative formate anions (HCOO-).

Methods

The *ab-initio* molecular orbital calculations at the Hartree-Fock level were performed with GAUSSIAN 92 system of programs (Frish *et. al.*, 1992), using the split valence 6–31G basis set (Hehre *et al.*, 1972). The Hartree -Fock (Hehre *et al.*, 1986) allows to find the best orbitals describing electron distribution that yields minimum energy for particular wave function. This method is based on the one-electron approximation. It means that each electron is considered to be moving in the potential field of nuclei and averaged field of other electrons. Geometry optimizations were performed with the optimization procedures in the GAUSSIAN 92 package, based on the analytical calculation of the first derivatives of energy. All the programs were run on 4D/35 Personal Iris and Challenge M Silicon Graphics computers.

The HF/6–31G//HF/6–31G calculation is according to notation by Curtiss (Curtiss *et al.*, 1984) and means single-point calculation using 6–31G basis set in the 6–31G optimized geometry at the Hartree-Fock (HF) level.

Results and Discussion

Recognition stage

The results from simulation of intramolecularly hydrogen bonded HA and α-MeHA interacting with one NH_3 molecule are collected in Table I. At first the proton-donor and acceptor sites were modelled by the ammonia molecule with the lone electron pair facing towards the one of N(8) and/or N(3) attached hydrogens. There is a significant preference for recognition from the N(3) side.

The analysis of results shows that for the α-MeHA interaction is stronger than for histamine by ca. 0.9 kcal/mol if proton resides at the N(8) (rather than at N(1)). Interaction through N(3)H....NH_3 does not discriminate those two molecules yielding 9.52 and 9.60 kcal/mol of stabilization energy for HA and α-MeHA, respectively.

Table I. Total molecular energies (in hartrees[a]) and stabilization energies (in kcal/mol) calculated at HF/6–31G//HF/6–31G level for monocationic forms of histamine and α-methylhistamine interacting with one or two NH_3 molecules.

Total energy	Histamine	α-Methylhistamine
Structure **1a**	−414.364965	−453.387317
Structure **1b**	−414.349102[b]	−453.373752
Structure **1c**	−414.349797	−453.372024
Structure **1d**	−414.355790	−453.379428
Structure **1e**	−470.543497	−509.567011
Stabilization energy		
$E_{1b}-E_{1a}$	9.95	8.51
$E_{1c}-E_{1a}$	9.52	9.60
$E_{1d}-E_{1a}$	5.76	4.95
$E_{1b}-E_{1c}$	3.76	4.65
$E_{1d}-E_{1b}$	4.20	3.56

[a] 1 hartree = 627.5095 kcal/mol.
[b] $E(NH_3)$ = −56.165521; E(HA)= −358.159808 hartrees.

The transfer of the proton to the N(8) causes reversal of stabilization energy for the interaction from N(8) side over N(3) side by 4.20 kcal/mol (HA) and 3.56 kcal/mol (α-MeHA). Making the HA or α-MeHA molecule interacting with more negative receptor site, modelled by the HCOO- anion, yields the strongest interaction from the N(8) side (Table II). This is consistent with H_2 receptor recognition proposed previously based on the less frame background (Weinstein *et al.*, 1976).

Table II. Total molecular energies (in hartrees) and stabilization energies (in kcal/mol) calculated at HF/6–31G//HF/6–31G for monocationic forms of histamine and α-methylhistamine interacting with $HCOO^-$ anion.

Total energy	Histamine
Structure **1a***	−546.420773
Structure **1b***	−546.383992
Structure **1c***	–
Structure **1d***	−546.430178
Stabilization energy	
$E_{1b^*}-E_{1a^*}$	3.08
$E_{1d^*}-E_{1a^*}$	−5.90

The most stable is complex in which positive charge is localized at the imidazole ring (structure **1a**) and the NH_3 is interacting with N(3)-H. If all the sites of the receptor would be moderately negative (here mimicked by the lone pair of NH_3 molecule) the strongest interaction would occur through the N(3)-H without opening of the side chain. Therefore the HA and α-MeHA should be expected to be recognized in the same manner because energetically interaction is similar both for the HA and α-MeHA, irrespectively on the interaction site. Only structure **1c** *vs.* **1a** is less stable by 0.08 kcal/mol for the HA than for α-MeHA. This state is relevant however to the early steps of interaction with the receptor site. Performed calculations confirm that relatively weak interaction with the receptor site can not make up for eventual opening of an intramolecular hydrogen bond. Full optimization of N(3)-H tautomer protonated at the side chain and interacting with two NH_3 molecules (structure **1e**) also did not lead to the break of the intramolecular hydrogen bond. The total stabilization energy for such a NH_3...HA...NH_3 complex is 33.04 kcal/mol, whereas stabilization energies for the N(3)-H histamine cation interacting with one NH_3 molecule from the N(3)-H side or N(1)-H side is 14.92 and 19.11 kcal/mol, respectively. Thus formation of this complex is energetically additive. Similar stabilization like for HA we obtained for α-MeHA interacting with two ammonia molecules: 32.19 kcal/mol. Therefore stabilization energy is not a discriminative factor for the interaction of the HA or α-MeHA with the receptor sites modelled by the ammonia molecules. The interaction with the formate anion yields fairly larger stabilization energies and unlike with NH_3 molecule the strongest interaction is from the side of the ethylamine chain through -NH_3^+ group (structure **1d***) rather than N(3)-H hydrogen (structure **1a***). During optimization course of structure **1c*** the N(1)-H proton was transferred to the amine chain yielding structure **1d***. The same behavior we observed for the complex with α-MeHA. It means that the negative potential reduces the barrier for proton transfer from the N(1) to N(8) both in HA and α-MeHA to similar extent.

At this point we performed simulation of the HA side chain opening as a result of expected interaction with the rigid receptor site. We separated the oxygens of two formate anions by 10.487Å (distance for the fully extended side chain of histamine), 10.887Å, 11.387Å and 12.387Å. The full side chain opening occurred at the O....O distance of 12.387Å yielding conformation with τ_1=60.21° and τ_2=178.05°. For histamine the side chain opens gradually with simultaneous rotation over the Cβ-C5 (τ_1) and Cα-Cβ (τ_2) bond. At the O....O distance of 10.887Å the opening of the side chain is noticeable, with τ_1=4.97° and τ_2=85.24°. The N(1)...N(8) and N(1)...H-N(8) distances are 3.530Å and 3.073Å, respectively.Therefore we performed at that distance simulation with α-MeHA inside the receptor model. Under the same constrains like in HA the α-MeHA remains closed by intramolecular hydrogen bonding with proton residing on the N(1) imidazole nitrogen. At the equilibrium geometry the τ_1 and τ_2 angles for α-MeHA are −31.84° and 77.72°, respectively. The N(1)...N(8) distance is 3.124Å and N(1)...H-N(8) is 2.500Å. In the isolated hydrogen bonded α-MeHA molecule the respective distances and bond angles are the following: N(1)...N(8) 2.639Å, N(1)...H-N(8) 1.773Å, τ_1=-37.90°, τ_2=59.90°.

Activation stage

To activate the H_2 histamine receptor the proton relay must be triggered. For any proton transfer there are three characteristics of the process: the geometry of the system, the driving force (defined as difference between energies of starting and endpoint of proton transfer) an the energy barrier for transition state. In the intramolecularly hydrogen bonded N(3)-H tautomer of histamine the proton can move from the N(8) to N(1) and then be released from N(3) to the receptor site. From our previous findings it appeared that histamine at the physiological pH exists as the N(3)-H monocation with strong intramolecular hydrogen bonding in which proton is at the distance of 1.719Å from the N(1) imidazole nitrogen. Assuming that this conformation is an active one after dehydration at the receptor site (as also the most stable form in vacuum) the barrier for back proton transfer to the N(8) nitrogen becomes an important issue. In vacuum the barrier for proton transfer back to the N(8) is higher for HA by 0.9 kcal/mol but driving force larger by 1.45 kcal/mol than for α-MeHA (Table III). Here no receptor surrounding is considered.

Table III. Total molecular energies (in hartrees) and stabilization energies (in kcal/mol) calculated for monocationic forms of histamine and α-methylhistamine at the HF/6–31G//HF/6–31G level.

Total energy	I. [N(3)–H, N(1)–H···N(8)H_2][a]	II. [N(3)–H, N(1)···H···N(8)H_2]	III. [N(3)–H, N(1)···H–N(8)H_2][b]
		Transition state	
Histamine	–358.169025	–358.155063	–358.159808
α-Methylhistamine	–397.191576	–397.179052	–397.184671
Stabilization energy	$E_{II}-E_I$	$E_{III}-E_I$	$E_{II}-E_{III}$
Histamine	8.76	5.78	2.98
α-Methylhistamine	7.86	3.93	3.53

[a] Histamine conformation like in structure **1c.**
[b] Histamine conformation like in structure **1d.**

The analysis of data collected in Table III shows that both the driving force and the barrier for proton transfer from the N(8) to N(1) is preferential for HA rather than for α-MeHA. The final stage of the activation would be represented in this case by situation in which proton is released from the N(3) nitrogen, e.g. by the intramolecularly hydrogen bonded N(1)-H tautomer. The presence of the receptor sites lowers the barrier for such a proton transfer. It seems therefore that discriminative factor comes from distortion of the proton relay due to different recognition affecting the intramolecular hydrogen bonding.

Conclusion

The conformational and energetical analysis of the HA and α-MeHA indicates that both compounds can be recognized in conformation closed through intramolecular hydrogen bonding. To yield the best interaction from the N(8) side the one of the receptor sites must be fairly negative, as mimicked here by the $HCOO^-$ anion. If spatial arrangement in the receptor site is not restricted by the molecular structure of the receptor, both the HA and α-MeHA can interact in almost the same manner with respect to stabilization energy. Although in the isolated α-MeHA monocation the proton shift from the N(1) to N(8) is more difficult than for HA, this factor itself cannot probably account for experimentally observed differences in activity. However, since α-MeHA is known to be more active than HA, a flexible H_3 receptor model becomes unlikely. Within the rigid receptor the intramolecularly hydrogen bonded HA can be opened due to the strong electrostatic interaction with negatively charged receptor sites. This would significantly lower the possibility of proton release from the N(3) imidazole atom, rendering HA less active at the H_3 receptor than α-MeHA.

Acknowledgment

Support of this work by Grant No. 401059101 from Scientific Research Committee (KBN) in Poland is gratefully acknowledged.

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