A Proton Relay Process as the Mechanism of Activation of the Histamine H_3-Receptor Determined by ^1H NMR and ab Initio Quantum Mechanical Calculations

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Abstract: This study proposes a new mechanism of activation of the histamine H_3-receptor based on stabilization of the active state of the receptor protein by a proton relay process. A series of histamine H_3-receptor agonists and one antagonist, all containing an imidazole and a side chain amino group, were studied using ^1H NMR and ab initio quantum mechanical calculations. A significant correlation ($r = -0.87$) between the formation energy of the active reaction intermediate and the agonistic activity is found. The calculated proton-transfer energies and pK_a values of the reaction intermediates (at the MP2/6-31+G*//HF/6-31+G* level in the gas and aqueous phases), as well as, ^1H NMR conformational analysis, enable a qualitative and quantitative determination of intramolecular hydrogen bonding and its effect on proton release from the imidazole N(τ)-atom. The results indicate that the histamine H_3-receptor is not activated by a proton release from imidazole N(τ)-atom but through adoption of a folded conformation of the ligand which stabilizes the active state of the receptor by an intramolecular hydrogen bond.

Introduction

The histamine H_3-receptor[1] has been described as a presynaptically located auto- and heteroreceptor in histaminergic and nonhistaminergic neurones in the central and autonomic nervous system. Furthermore, the histamine H_3-receptor is involved in the regulation of the release of several other neurotransmitters (i.e., acetylcholine, dopamine, γ-aminobutyric acid (GABA), glutamate, noradrenaline, and 5-hydroxytryptamine (5-HT)). The histamine H_3-receptor is involved in control of sleep and wakefulness, food intake, and inhibition of seizures. Thus, many possible therapeutic targets for H_3-receptor ligands have been suggested, i.e., asthma, migraine, septic shock, heart failure, acute myocardial infarction, learning and memory degenerative disorders (like Alzheimer's disease), attention deficit hyperactive disorder (ADHD), epilepsy, and obesity.[2]

The histamine H_3-receptor belongs to the superfamily of G-protein coupled receptors. Recently the human histamine H_3-receptor was cloned; however, the localization and topography of the binding sites were not resolved.[3] Therefore, approaches to study the molecular determinants for receptor binding and activation have to be focused on methods that utilize the information from molecular structures and structure activity relationships of known ligands.

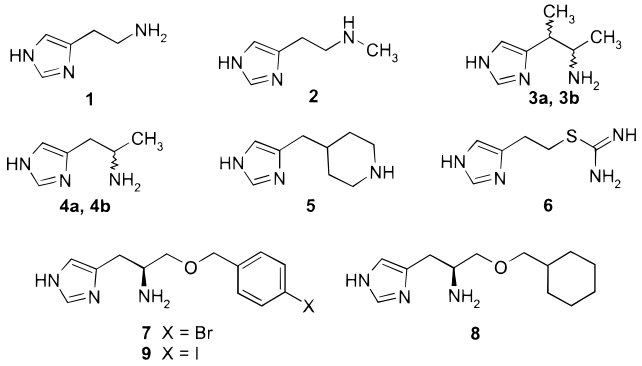

Figure 1. Histamine H_3-receptor activating compounds: histamine **1**, N^{α}-methyl histamine **2**, *R*(α),*S*(*β*)-dimethyl histamine **3a**, *S*(α),*R*(*β*)-dimethyl histamine **3b**, *R*(α)-methyl histamine **4a**, *S*(α)-methyl histamine **4b**, 4-(1*H*-imidazol-4(5)-ylmethyl)piperidine (immepip) **5**, *S*-(2-(1*H*-imidazol-4(5)-yl)ethyl)isothiourea (imetit) **6**, 2(*S*)-amino-3-(1*H*-imidazol-4(5)-yl)propyl 4-bromobenzyl ether dihydrochloride **7** (exhibits also H_3-antagonistic activity[36]), 2(*S*)-amino-3-(1*H*-imidazol-4(5)-yl)-propyl cyclohexylmethyl ether **8**, 2(*S*)-amino-3-(1*H*-imidazol-4(5)-yl)-propyl 4-iodobenzyl ether **9** (exhibits only H_3-antagonistic activity[36]).

All known potent histamine H_3-receptor agonists are small molecules. Common properties for these agonists are a 4(5)-substituted imidazole and an ionizable group in the side chain (Figure 1). The imidazole group plays a role in the activation mechanism of the histamine H_2-receptor and 5-HT-receptor.[4−7] Because of the unique role of the imidazole group as a proton-transfer device,[8−10] its chemical and physical properties have widely been studied with many theoretical and experimental approaches.[11−15]

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Two distinct hypotheses for receptor binding and one for receptor activation have been proposed for histamine H_3-receptor agonists. The assumption that the environment of the binding site is complementary to the structure of the ligand allowed the determination of steric properties of the pharmacophore by elucidation of the structural characteristics of potent and selective agonists. Sippl et al.[16] generated a pharmacophoric model in which the imidazole ring and the protonated side chain nitrogen could be superimposed by calculating local energy minima of a group of potent histamine H_3-receptor agonists. This model was then used for the development of a pseudoreceptor model with hypothetical binding sites[17] suggesting that histamine H_3-receptor agonists are recognized by a receptor in the extended trans conformation.[16] Although this approach describes receptor binding of several histamine H_3-receptor ligands, it does not give any information about the activation mechanism of the H_3-receptor.

The approach of Mazurek and Karpinska[18] focuses on the major physicochemical properties of molecules that can discriminate between histamine H_2- and H_3-receptors. Calculations of energies at different conformations, hydrogen bond stabilization energies, and solvation energies of some histamine H_2- and H_3-receptor ligands at the STO-3G and 6-31G levels indicated that the form most likely to be recognized by the H_3-receptor is a folded gauche conformation with an intramolecular hydrogen bond.[18] This is in accordance with combined ab initio calculations and Monte Carlo simulations of the conformational equilibrium for physiological existing forms of histamine (**1**) and *R*(α),*S*(*β*)-dimethylhistamine (**3a**) by Nagy et al.[13] These calculations revealed that in aqueous solution 64% of histamine (**1**) is in the folded gauche conformation and that for both compounds the gauche N(*τ*)H conformation is more stable than the extended trans N(*τ*)H conformer by 8.4 kJ/mol.[13] Recently it was shown by ^{1}H NMR studies that 52% to 61% of the H_3-receptor agonists **4a** and **8** occur in a folded conformation at physiological pH and temperature.[19] Ab initio calculations of histamine (**1**) and *R*(α)-methylhistamine (**4a**) at the HF/6-31G level indicated an energy of −24.2 and −18.1 kJ/mol, respectively, for proton transfer from the side chain amino group to the imidazole group.[20] The process became more favorable (by 17.5 kJ/mol) by placing a proton acceptor near the imidazole group (a NH_3-group as a proton acceptor). Although these results are only based on two H_3-receptor ligands, a mechanistic model for H_3-receptor activation was proposed.[20] After H_3-receptor binding a ligand activates the receptor by transferring a proton from the cationic side chain amino group to the receptor protein via a tautomeric shift over the imidazole group (Figure 2). However, a more thorough analysis of the molecular determinants responsible for histamine H_3-receptor activation is required to accomplish drug design. The aim of this study was to examine qualitatively and quantitatively a proton relay process as a putative mechanism of activation of the histamine H_3-receptor. The conformational behavior of known H_3-receptor agonists and one antagonist was studied by ^{1}H NMR. The molecular energy of the reaction intermediates was calculated with ab initio quantum mechanical methods. The effect of aqueous solvation on the reaction energies was included by using a continuum solvation model. This computational approach, based on the quantum mechanically calculated gas-phase proton affinities (PA(gas)) and solvation energies (ΔG_{solv}), allows the calculation of pK_a values for each reaction intermediate after calibration with a set of compounds with known pK_a values.[21,22] The proton-transfer energies, the pK_a values, and ^{1}H NMR conformational data allow a qualitative and quantitative determination of intramolecular hydrogen bonding and its effect on proton release from the imidazole N(*τ*)-atom. This study introduces a new hypothesis for the mechanism of activation of the histamine H_3-receptor based on stabilization of the active state of the receptor by agonists.

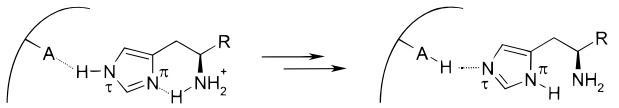

Figure 2. Putative mechanism of activation of the histamine H_3-receptor (A = hydrogen acceptor).

Results and Discussion

^{1}H NMR Analysis. The environmental effect on the conformational behavior of the histamine H_3-receptor agonists **4a** and **8** and antagonist **9** was studied by analysis of proton−proton couplings in a few characteristic solvents: C_6D_6, $CDCl_3$, d_6-acetone, d_6-DMSO, H_2O, D_2O, and CD_3OD. The chemical properties of each of these solvents are different, and can therefore be roughly classified on the basis of dielectric constants into polar protic (H_2O, D_2O, and CD_3OD), polar aprotic (d_6-acetone, d_6-DMSO), and nonpolar aprotic (C_6D_6, $CDCl_3$) environments. Polar protic solvents mimic the extracellular fluid. Nonpolar aprotic solvents mimic a hydrophobic receptor environment with aromatic−aromatic interactions and weak hydrogen bonding potential. Polar aprotic solvents mimic a hydrophobic receptor environment with strong hydrogen bonding potential. However, the selected solvents provide only a rough estimate of the cell's and receptor's parameters. The solvent effects are not able to mimic any specific receptor−ligand interaction as also other parameters such as steric effects of the binding site should be considered.

The assumption was made that the compounds may exist in three conformers (Figure 3), of which two are in a conformation where weak intramolecular hydrogen bonding may be possible

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Table 1. Populations (%) and Free Energies (kJ/mol) of Compounds **4a**, **8**, and **9** in Different Solvent Environments Obtained from ^{1}H NMR at 302K

		nonpolar aprotic				polar aprotic				polar protic						
		C_6D_6		$CDCl_3$		d_6-acetone		d_6-DMSO		H_2O		D_2O		CD_3OD		EQUILA
compd	rotamer	%	ΔG	%	ΔG	%	ΔG	%	ΔG	%	ΔG	%	ΔG	%	ΔG	analysis [%]
4a	I	–[a]	–[a]	11	0	2	0	3	0	–[a]	–[a]	–[a]	–[a]	43	0	
	II			59	−4.2	98	−9.8	97	−8.7					42	0.1	
	III			30	−2.5	0	–[b]	0	–[b]					15	2.6	
	I + III			41		2		3						58		
8	I	10	0	8	0	4	0	4	0	39	0	42	0	44	0	
	II	47	−3.9	47	−4.4	93	−7.9	93	−7.9	47	−0.5	49	−0.4	48	−0.2	
	III	43	−3.7	45	−4.3	3	0.7	3	0.7	13	2.8	9	3.9	8	4.3	
	I + III	53		53		7		7		52		51		52		
9	I	17	0	12	0	2	0	0	–[b]	44	0	46	0	46	0	47[c]
	II	49	−2.7	55	−3.8	95	−9.7	96	−8.0	47	0.2	49	0.2	47	0.1	47[c]
	III	34	−1.7	33	−2.5	3	−1.0	4	0	9	4.0	5	5.6	6	5.1	6[c]
	I + III	51		45		5		4		53		51		52		

[a] Could not be analyzed due to degenerated spectra. [b] The energy cannot be estimated from the data. [c] Average in H_2O, D_2O, and CD_3OD.

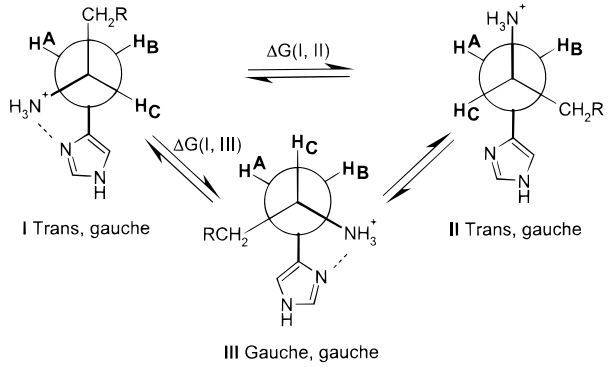

Figure 3. The three main rotamers of compound **9**. R = O-(4-I-benzyl).

(I and III).[23] The compounds exist as monocations in water and methanol, and as neutral molecules in the other solvents.

The observed couplings J_{AC} and J_{BC} allow the determination of the two thermodynamic parameters between the conformers (ΔG(I,II) and ΔG(I,III)) if the theoretical couplings are known for the conformers I, II, and III. These couplings were predicted using the Haasnoot equation.[24] The relative populations of the conformers given in Table 1 were solved with the following equations:

$$X_I + X_{II} + X_{III} = 1.0$$

$$J_{AC}(\text{Obs}) = X_I J_{AC}(\text{I}) + X_{II} J_{AC}(\text{II}) + X_{III} J_{AC}(\text{III})$$

$$J_{BC}(\text{Obs}) = X_I J_{BC}(\text{I}) + X_{II} J_{BC}(\text{II}) + X_{III} J_{BC}(\text{III})$$

Because the theoretical couplings given by the Haasnoot equation are not accurate, the reliability of the coupling constant approach was verified by additional analysis of the temperature dependence of the couplings J_{AC} and J_{BC} of compound **9** (in H_2O, D_2O, and CD_3OD). The iterative algorithm of the program EQUILA[25] was used for this analysis as discussed before.[19] The coupling constant analysis gives fair estimates of the relative energies of the conformers, although the absolute values of the energies may vary significantly depending on the applied model.[19] Therefore the simplest described model was used to obtain the results shown in Table 2. The confidence limits given by the standard statistics of the program are also given in Table 2. It should be noted that the confidence limits do not contain the uncertainty arising from the assumption made for the applied model.

Table 2. Thermodynamic Parameters between the Rotamers of **9** Calculated with the Program EQUILA[25]

solvent	ΔG(I,II) [kJ/mol]	ΔG(I,III) [kJ/mol]
H_2O	-0.2 ± 0.01[a]	5.2 ± 0.09
D_2O	-0.2 ± 0.01	5.5 ± 0.11
CD_3OD	-0.1 ± 0.01	4.6 ± 0.07

[a] Standard deviations predicted by the program. The standard deviations are far too small because they do not account for the uncertainties of the model.

The analysis indicates that the ΔG(I,II) values are very small (−0.2 to −0.1 kJ/mol). The ΔG(I,III) is ca. 5 kJ/mol (Table 2). The ca. 1 kJ/mol difference in the calculated ΔG(I,III) from the populations obtained by the "single temperature model" and the ΔG(I,III) from the EQUILA analysis in H_2O is typical in the thermodynamic parameter analysis of NMR data. The difference has its origin in the theoretical couplings: the values for the "single temperature model" were calculated by the Haasnoot equation[24] based on the optimized molecular structures, whereas in the EQUILA model the couplings were optimized by the program. It is notable that the accuracy of the energy estimation (and populations) increases for lower free energies. For example, the experimental ratio for I and II is 44:47 in H_2O (Table 1), leading to ΔG(I,II) = −0.17 kJ/mol at 302 K. The value differs only by 0.03 kJ/mol from the result of the EQUILA analysis (−0.2 kJ/mol, Table 2). Thus, a good qualitative picture of the conformational behavior of the compounds in the solvent systems can be obtained by analysis of the couplings at one single temperature.

The small ΔG(I,II) values (Table 2) indicate that the intramolecular hydrogen bond between the side chain amino group and the N(π)-atom of the imidazole group (possible for rotamers I and III) is weak and does not stabilize rotamer I in polar protic solution at physiological pH. Therefore, the populations of rotamers I and II are nearly equal (Table 1). The population of rotamers I + III with a folded *gauche* conformation was 53% (at 310 K) for compound **9**. These results are in accordance with the data from ^{1}H NMR analysis of the histamine H_3-receptor agonists **4a** and **8**.[19] Thus, there is no qualitative or quantitative correlation between the biological activity and

(23) Conformer I: N(π)- - -HN(terminal) = 1.877 Å, N(π)- - -H- - N(terminal) = 138.93° (at the HF/6-31G* level). Conformer III: N(π)- - -HN(terminal) = 1.875 Å, N(π)- - -H- -N(terminal) = 138.94° (at HF/6-31G* level).

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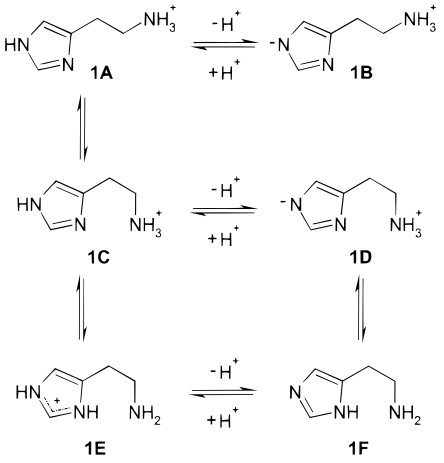
Scheme 1. Reaction Intermediates Involved in the Proposed Mechanism of Activation of the Histamine H_3-Receptor



the populations of the folded gauche and extended trans conformations in solution at physiological pH.

The Effect of Solvent Environment. The results in Table 1 show that in both nonpolar aprotic (C_6D_6, $CDCl_3$) and polar protic (H_2O, D_2O, and CD_3OD) environments the population of the hydrogen bonded conformers (I and III) of all compounds is about 50%. In the polar aprotic (d_6-acetone, d_6-DMSO) environment the population of I and III is 0−10%, meaning that a carbonyl group of the solvent competes with intramolecular hydrogen bonding effectively. Analysis of the populations in two mixtures of $CDCl_3$ and d_6-acetone for compound **9** (see Supporting Information, Table S8) shows that 33% of d_6-acetone in $CDCl_3$ extinguishes the folded conformation (I + III) and breaks the intramolecular hydrogen bond due to an efficient solvation of the imidazole and the side chain amino groups.

The populations of the conformers I and III are inversed in polar protic and nonpolar aprotic environments. In energy terms this means that the ΔG(I,III) is ca. 4 kJ/mol in water and methanol, while the ΔG(I,III) is ca. −4 kJ/mol in benzene and chloroform.

Conformation−activity relationships in a polar protic environment suggest the importance of the folded conformation (structure C, Scheme 1) in the receptor activation process. For compound **4a** (the most potent agonist in this series) the population of rotamer III and the total population of the folded conformation (rotamers I + III) were the highest. For the less potent agonist **8** the population of rotamer III and the total population of the folded conformation were reduced. For antagonist **9** the population of III was the lowest while the total population of the folded conformation was the same as for compound **8** (Table 1). This conformation−activity relationship is absent in nonpolar aprotic environment supporting the finding of Mazurek et al.[18,20] that the biologically active form of the agonist is the monocation (which is also the most abundant form in solution at physiological pH (97%)[26]) rather than the neutral form. However, a change from a polar protic environment (extracellular fluid mimic) to a more hydrophobic nonpolar aprotic (receptor mimic) environment favors conformer III. The change involves also dehydrogenation of structure C resulting in structure F (Scheme 1), which is proposed to occur during receptor deactivation (see the Mechanism of Activation section). The prevalence of this gauche, gauche conformation of structure F (Scheme 1) in a nonpolar aprotic environment might indicate the importance of conformer III in the decomposition process of the proposed active state of the receptor.

^{1}H NMR analysis produces valuable information of the conformational behavior of the ligand in different environments. A hydrophobic nonpolar environment (C_6D_6, $CDCl_3$) with aromatic−aromatic interactions and weak hydrogen bonding potential favors conformer III. A hydrophobic polar environment (d_6-acetone, d_6-DMSO) with strong hydrogen bonding potential breaks intramolecular hydrogen bonds and favors strongly conformer II. A polar protic environment with strong hydrogen bonding potential (CD_3OD) favors conformer I when the ligand exists as a monocation.

Relative Energies. The reaction intermediates involved in the proposed mechanism of activation of the histamine H_3-receptor are presented in Scheme 1. Antagonist **9** could not be included in the ab initio calculations as the applied program does not include parameters for an iodine atom. The relative energies of the reaction intermediates calculated at HF6-31+G* (gas phase) and MP2/6-31+G*//HF/6-31+G* (gas and aqueous phase) levels are listed in Table 3. Calculations revealed that transformation A−C is favored in the gas phase (−43.7 to −55.4 kJ/mol at HF6-31+G* and −48.6 to −61.0 kJ/mol at MP2/6-31+G*//HF/6-31+G* levels). This result indicates the conformational stabilizing effect of intramolecular hydrogen bonding in the gas phase. In the aqueous phase the calculated transformation energies A−C (MP2/6-31+G*//HF/6-31+G* + $\Delta\Delta G_{solv}$) were higher for all compounds (−42.6 to −5.4 kJ/mol for **1**−**4**, and 10.1 and 19.3 kJ/mol for **7** and **8**, respectively). These results indicate a more equal distribution of the folded and extended conformation in solution while a more hydrophobic environment favors the folded conformation as a result of intramolecular hydrogen bonding. The transformation energies C−E range from 4.0 to 64.8 kJ/mol in the gas phase at the MP2/6-31+G*//HF/6-31+G* level, and from 18.4 to 47.9 kJ/mol in the aqueous phase (except for **7** where it was −14.2 kJ/mol). These results show a positive energy difference for proton transfer from the side chain amino group to the imidazole N(π)-atom. However, reaction intermediate E has an equal or lower gas-phase energy than intermediate A for all compounds (1.2 to −54.8 kJ/mol), except **4b** (11.4 kJ/mol). In the aqueous phase the transformation energies A−E range from −4.2 to 67.2 kJ/mol. Thus, in the gas phase proton transfer from the side chain amino group to the imidazole ring is possible due to the negative energy difference. In the aqueous phase this proton transfer is less likely because the energy difference is positive. Transformation D−F is highly favored in both the gas and aqueous phase.

Proton Affinity (PA) and pK_a. The proton affinities were 45.6 to 53.6 kJ/mol (PA(gas)) and 12.0 to 93.0 kJ/mol (PA(aq)) lower for reaction intermediate C than for A except for **3a** and **4a** which showed 0.8 and 22.1 kJ/mol higher aqueous proton affinities (Table 4). Furthermore, the proton affinities were 94.3 to 158.4 kJ/mol (PA(gas)) and 43.6 to 79.6 kJ/mol (PA(aq)) lower for reaction intermediate E than for C except for **7**, which showed only a 1.2 kJ/mol lower aqueous proton affinity (Table 4). The proton affinities were 139.9 to 207.5 kJ/mol (PA(gas) and 42.8 to 172.6 kJ/mol (PA(aq)) lower for reaction intermediate E than for A. Based on earlier structure activity relationship studies,[27] it can be assumed that one of the first interactions between the histamine H_3-receptor and a ligand is the formation of a hydrogen bond between a hydrogen acceptor and the imidazole N(τ)H-atom. Since, within a series

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Table 3. The Relative Transformation Energies (kJ/mol) of the Reaction Intermediates (Scheme 1) Calculated for the Histamine H_3-Receptor Ligands

	4b	**3b**	**1**	**2**	**4a**	**3a**	**7**	**8**
				HF/6-31+G* (gas)				
pD_2[a]	6.3	6.5	7.4	7.8	8.4	8.5	*b*, *c*	*b*
Δ(A,C)	−44.0	−43.7	−48.9	−46.4	−54.3	−55.4	−45.5	−47.0
Δ(C,E)	44.3	26.1	−11.0	12.2	−1.6	−3.3	30.4	32.0
Δ(A,E)	0.3	−17.6	−60.0	−34.2	−55.9	−58.7	−15.2	−15.0
Δ(D,F)	−129.7	−117.6	−149.7	−131.5	−138.1	−120.5	−120.7	−110.8
				MP2/6-31+G*//HF/6-31+G* (gas)				
Δ(A,C)	−53.4	−50.2	−58.8	−57.3	−61.0	−61.0	−48.6	−51.4
Δ(C,E)	64.8	41.7	4.0	25.2	9.5	10.5	49.8	51.5
Δ(A,E)	11.4	−8.5	−54.8	−32.0	−51.5	−50.6	1.2	0.1
Δ(D,F)	−93.6	−82.1	−116.0	−96.7	−105.5	−83.9	−82.4	−73.0
				MP2/6-31+G*//HF/6-31+G* + $\Delta\Delta G_{solv}$ (aq)				
Δ(A,C)	−7.3	−8.9	−5.4	−24.1	−42.6 −6.7[d]	−10.8 −17.5[d]	10.1	19.3
Δ(C,E)	44.0	44.1	18.4	28.0	20.4 23.4[d]	23.7 21.0[d]	−14.2	47.9
Δ(A,E)	36.7	35.2	13.0	3.9	−22.2 16.7[d]	12.9 3.5[d]	−4.2	67.2
Δ(D,F)	−30.0	−24.8	−38.4	−31.9	−51.5 −31.2[d]	−19.9 −15.1[d]	−15.4	−31.7

[a] Histamine H_3-agonistic activity published by Leurs et al.[27] The pD_2 refers to the agonistic activity determined by evaluation of the influence of the compound on K + stimulated [^{3}H]histamine release on rat cortex.[1] [b] Compounds **7** And **8** activate the H_3-receptor in a semiquantitative assay.[36] [c] Exhibits also antagonistic activity.[36] [d] PCM-model used for aqueous solvation energy.

Table 4. Calculated Proton Affinities in the Gas (PA(gas), kJ/mol) and Aqueous Phases (PA(aq), kJ/mol) and pK_a Values of the Reaction Intermediates (Scheme 1)

	4b	**3b**	**1**	**2**	**4a**	**3a**	**7**	**8**
PA(gas, AB)	1177.0	1176.2	1178.4	1184.7	1182.1	1177.4	1158.9	1180.3
PA(gas, CD)	1127.9	1129.0	1127.6	1131.1	1131.9	1131.8	1141.1	1132.9
PA(gas, EF)	969.5	1005.1	1007.6	1009.2	1016.9	1037.5	1008.9	1008.4
PA(aq, AB)	1284.3	1254.2	1260.5	1263.3	1229.2	1240.5	1301.8	1311.1
PA(aq, CD)	1243.1	1242.2	1243.8	1246.3	1251.4	1241.3	1249.1	1218.1
PA(aq, EF)	1169.2	1173.2	1187.0	1186.5	1179.6	1197.7	1247.9	1138.5
pK_a(AB)	17.5	14.6	15.2	15.5	12.1	13.2	19.3	20.2
pK_a(CD)	13.5	13.4	13.5	13.8	14.3	13.3	14.0	11.0
pK_a(EF)	6.2	6.6	7.9	7.9	7.2	9.0	13.9	3.1

of similar compounds, lower PA's usually mean stronger hydrogen bonding interactions,[28] the calculated PA's indicate that this intermolecular hydrogen bond is stronger for reaction intermediate C than for A. The hydrogen bond is the strongest for reaction intermediate E.

The calculated aqueous PA's were related to their corresponding pK_a values by using an earlier reported correlation equation.[21] The predicted pK_a values for the reaction intermediates are listed in Table 4. Calculations revealed that pK_a(AB) is the highest varying from 12.1 to 20.2, pK_a(CD) from 11.0 to 14.3, and pK_a(EF) from 3.1 to 13.9 in aqueous solution (Table 4). Thus, changing from an extended to a folded conformation or transferring a proton from the side chain amino group to the imidazole N(π)-atom both increase the proton-donating capacity of the imidazole N(τ)H-atom.

The calculated Δ(A,C) and PA(EF) values of **4a** in aqueous solution deviate from the general trend of the other molecules (Table 3, Figures S5 and S6 in the Supporting Information). These deviations, which do not affect the conclusions of this work, are due to the low solvation energy calculated for conformation I of **4a**. It is probable that the IPCM solvation model does not produce the solvation energy for this conformer correctly. This suggestion is supported by the polarized continuum model (PCM)[29,52] calculations for **3a** and **4a** listed in Table 3. Similar kinds of difficulties were encountered in earlier IPCM calculations[30,31]

Mechanism of Activation. The partial opposite biological activities of the dicationic compounds **7** and **8** cannot be explained by the X-ray structures as both compounds exist in

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an almost identical extended conformation.[32] This indicates that the dicationic extended form of the compounds is not responsible for the receptor activation.

The populations of the folded and the extended conformations are almost equal in solution at physiological pH and temperature based on ^{1}H NMR experiments (Table 1). Ab initio quantum mechanical calculations in the aqueous phase predict slightly more stable folded conformations compared to the NMR experiments. Larger basis set, inclusion of electron correlation correction at the higher level, and inclusion of thermodynamic corrections are needed to achieve better agreement with the experiments.[33] Since we are here interested in relative energies of a series of similar compounds, the computational level used is thought to be adequate (Table 3). Thus, both the folded and extended forms of the ligand are equally available for receptor binding. According to earlier structure activity relationship studies the 4(5)-substituted imidazole is essential for histamine H_3-receptor activity.[27] Isosteric replacement or substitution of the imidazole group diminishes the histamine H_3-receptor activity. This indicates a strict binding site for the imidazole ring. Intermolecular hydrogen bonding between the imidazole N(τ)H-atom and a hydrogen bond acceptor at the receptor protein may be one of the main interactions during the receptor binding process. Site-directed mutagenesis studies of several G-protein coupled receptors revealed that a binding site is located at the transmembrane domain (for review see Bikker et al.[34]). In case this finding is also valid for the H_3-receptor, then it can be assumed that the hydrophobicity of the environment surrounding the ligand increases during the receptor binding process. It must be noted that the cellular receptor environment is not homogeneous as the cavities in transmembrane domains of the receptor might have hydrophobic or hydrophilic areas ("pockets"), as well as very polar or nonpolar areas depending on the side chains of the amino acids nearby.

^{1}H NMR experiments showed that a change of environment from a polar protic to a more hydrophobic, nonpolar aprotic one increased the population of the folded gauche, gauche conformation (rotamer III, Table 2). Furthermore, ab initio calculations indicated that transformation from an extended to a folded conformation is energetically favored (Table 3, Δ(A,C)), suggesting that after initial formation of an intermolecular hydrogen bond (receptor binding) the ligand adopts a folded conformation. This is supported by a considerable reduction of the desolvatation energy as a result of a delocalized positive charge. The existence of the folded conformation during receptor activation is supported by a significant correlation ($r = -0.92$) between the gas-phase transformation energy A−C (MP2/6-31+G*//HF/6-31+G*) and the agonistic activity. No correlation ($r = -0.59$) between the aqueous phase transformation energy A−C and the agonistic activity was observed. This might be due to an interfering effect of the solvent environment which presumably is not present in the receptor environment. A conformational change from the extended to the folded conformation strengthens the intermolecular hydrogen bond between the receptor protein and the imidazole group as a result of a reduced PA of the imidazole N(τ)-atom (Table 4). The folded conformation allows intramolecular hydrogen bonding between the imidazole N(π)-atom and the side chain amino group. The calculated PA values of the reaction intermediates C and E (Scheme 1) indicate that transferring a proton from the side chain amino group to a position closer to the imidazole N(π)-atom will also strengthen the intermolecular hydrogen bond between the N(τ)H-atom and the receptor protein due to a reduced PA of the imidazole N(τ)-atom (Table 4). Although ab initio calculations indicated that proton transfer from the side chain amino group to the imidazole ring increases the energy of the molecule (Table 3, Δ(C,E)), the intermolecular hydrogen bond between the imidazole N(τ)-atom and the receptor protein reduces the reaction energy[20] and may make this process more facile. A significant negative correlation (gas phase $r = -0.87$ and aqueous phase $r = -0.84$) between the transformation energy A−E and the agonistic activity indicates that the reaction intermediate E with an imidazolium moiety is an important molecular species in histamine H_3-receptor activation. Significant positive correlations (gas phase: $r = 0.84$, aqueous phase: $r = 0.80$[35]) between the calculated PA(EF) values and the agonistic activity and between the calculated pK_a(EF) and the agonistic activity ($r = 0.80$[35]) indicate that the proton is not released from the imidazole N(τ)-atom of reaction intermediate E during receptor activation. However, as the calculated pK_a values indicate, such a proton release would be possible after receptor activation. Release of the proton from the imidazole N(τ)-atom and simultaneous breaking of the intermolecular hydrogen bonds could lead to dissociation of the ligand. Both the gas and aqueous phase transformation energies C−E are reduced for the most potent compounds (Table 3). This indicates an easier proton transfer between the side chain amino group and the imidazole N(π)-atom, enabling strengthening of two putative intermolecular hydrogen bonds. These hydrogen bonds, which stabilize the active state of the receptor, are between the imidazole N(τ)H-atom and the receptor protein, and between the side chain amino group and the receptor protein (Figure 4). This theory is supported by a significant negative correlation between the transformation energies C−E and the agonistic activity (gas phase $r = -0.83$ and aqueous phase $r = -0.84$).

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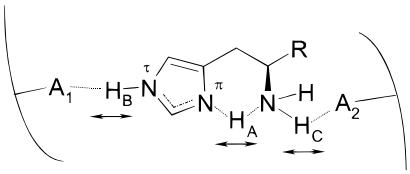

Figure 4. Stabilization of the active state of the histamine H_3-receptor by optimization of the strength of two intermolecular hydrogen bonds gained by proton transfer between the side chain amino group and the imidazole N(π)-atom. A is a putative hydrogen bond acceptor of the receptor. Transfer of proton H_A from the side chain amino group to the imidazole N(π)-atom will increase the H_B-A_1 interaction and transfer of proton H_A from the imidazole N(π)-atom to the side chain amino group will increase the H_C-A_2 interaction.

Compounds **7**, **8**, and **9** are recently described as histamine H_3-receptor ligands having (partial) agonistic and/or antagonistic effects.[36] However, these compounds were screened on a semiquantitative assay[37] while all other compounds mentioned in this study are tested in the same pharmacological assay according to Arrang et al.[1] Therefore, only a qualitative comparison is possible. Interestingly, compound **7** exhibits both (partial) agonistic and antagonistic activity.[36] The dual activity of **7** has its origin in the high pK_a(EF) of the imidazole N(τ)-atom and the lipophilic 4-Br-benzyl group. The 4-Br-benzyl group is a common moiety among histamine H_3-receptor antagonists.[27] Increased basicity of the imidazole N(τ)-atom does not allow long-term stabilization of the active state of the receptor due to weak hydrogen bonding between the imidazole N(τ)H-atom and receptor protein. However, the lipophilic 4-Br-benzyl group of **7** prevents the dissociation of the ligand from the receptor leading to antagonistic activity.

The high transformation energy A−E (Table 3) and the low pK_a(EF) (Table 4) of **8** indicate a low population of the active reaction intermediate E, and a facile proton release from the imidazole N(τ)-atom suggesting weak agonistic activity.

Some compounds are reported to have H_3-agonistic activity, despite being unable to form an intramolecular hydrogen bond.[38−41] It might be that these ligands operate by a different mechanism and/or activate a different subtype of histamine H_3-receptor. The existence of histamine H_3-receptor subtypes has not yet been confirmed; however, they have been indicated by several pharmacological studies.[42−48] On the other hand, it might be that these ligands share the proposed activation mechanism. In this case formation of a hydrogen bonded water chain between the side chain amino group and the imidazole N(π)-atom might allow strengthening of intermolecular hydrogen bonds. Formation of a hydrogen-bonded water chain has recently been described in the mechanism of proton transfer in a carbonic anhydrase enzyme containing an imidazole ring.[10,49]

Determination of the ligand properties in the gas phase or solution assists in understanding the activation mechanism of the histamine H_3-receptor. However, deductions on the basis of indirect methods are hypothetical, and do not give an absolute picture of the complicated biological process.

Conclusions

Ab initio quantum mechanical calculations in the gas phase indicate a favorable transformation from an extended to a folded conformation for histamine H_3-receptor agonists. Although less pronounced, this transformation still occurs in the aqueous phase. ^{1}H NMR studies revealed only a small preference for the folded conformation as a result of weak intramolecular hydrogen bonding in solution at physiological pH. These results indicate that, in extracellular fluid, both the extended and folded conformation are available for receptor binding at nearly equal ratio. ^{1}H NMR studies show that a change from a polar protic environment (H_2O, D_2O, or CD_3OD) to a nonpolar aprotic one (C_6D_6 or $CDCl_3$) increases the population of the gauche, gauche rotamer III considerably. Ab initio calculations show that the molecule adopts a folded conformation more readily when going from an aqueous to hydrophobic aprotic environment. Adoption of a folded conformation and an increase in inter- and intramolecular hydrogen bond formation during receptor binding is also indicated by a considerable reduction of the desolvation energy of the positive charge of the ligand. Both a folded conformation and an increase in intramolecular hydrogen bond formation decrease the calculated PA of the imidazole N(τ)-atom. This indicates strengthening of the putative intermolecular hydrogen bond between the N(τ)H-atom and the receptor protein. As a consequence, the energetic driving force for proton transfer from the side chain amino group to the imidazole N(π)-atom increases considerably. The importance of this folded imidazolium conformation (E) in the receptor activation process is supported by a significant negative correlation between the gas-phase transformation energy A−E and the agonistic activity ($r = -0.87$). According to the ab initio quantum mechanical calculations, transferring a proton from the side chain amino group to the imidazole N(π)-atom reduces the pK_a value of the imidazole N(τ)H-atom to a range where the proton can be released. However, a significant positive correlation between the pK_a value of the imidazole N(τ)H-atom and the agonistic activity indicates that release of a proton from the imidazole is not responsible for receptor activation. These results suggest that proton transfer between the side chain amino group and the imidazole N(π)-atom leads to stronger binding of the ligand and increased stability of the active state of the receptor. Proton release from the imidazole N(τ)-atom deactivates the receptor by dissociating the ligand from the receptor as a result of broken intermolecular hydrogen bonds.

This study proposes a proton-transfer process between the side chain amino group and the imidazole N(π)-atom in the mechanism of activation of the histamine H_3-receptor. The results could indicate that the histamine H_3-receptor is not activated by a proton release from the imidazole N(τ)-atom but through adoption of a stable folded conformation which stabilizes the active state of the receptor. Experimental information on the nature of the ligand receptor interaction could show if the proposed hypothesis for the receptor binding and activation is justified.

Experimental Section

Compounds. The 2-(*S*)-amino-3-(1*H*-imidazol-4(5)-yl)propyl cyclohexylmethyl ether dihydrochloride (**8**), 2-(*S*)-amino-3-(1*H*-imidazol-4(5)-yl)propyl 4-bromobenzyl ether dihydrochloride (**7**), and 2-(*S*)-amino-3-(1*H*-imidazol-4(5)-yl)propyl 4-iodobenzyl ether dihydrochloride (**9**) were synthesized by Kovalainen et al.[36] *R*(α)-Methylhistamine dihydrobromide (**4a**) was purchased by Tocris Cookson.

^{1}H NMR. The pH and pD of 0.03−0.07 M H_2O and D_2O solutions of **4a**, **8**, and **9** were adjusted to 7.4 using 0.1 M HCl/DCl and NaOH/NaOD affording the monocations.[26] The CD_3OD samples were converted to the monocationic form by adding 1 equiv of NaOH. The $CDCl_3$, C_6D_6, $(CD_3)_2CO$, and d_6-DMSO samples were converted to the free bases by adding 2.3 equiv of NaOH. After evaporation of the solvent, the residues were redissolved in deuterated solvents and filtrated. ^{1}H NMR spectra in H_2O, D_2O, and CD_3OD at 284, 302, 313, and 333 K and in $CDCl_3$, C_6D_6, d_6-acetone, and d_6-DMSO at 302 K were recorded with a Bruker AM 400 WB spectrometer operating at 400.1 MHz. The spectra were analyzed with the program PERCHit.[50]

Theoretical coupling constants of different rotamers (Figure 3, dihedral angle: rotamer I, −60°, rotamer II, 180°; and rotamer III,

60°) were calculated for the optimized structures (molecular dynamics, HYPERCHEM) with the Haasnoot equation.[24] Signals were assigned on the basis of theoretical couplings (see Supporting Information).

Computational Details. Ab initio quantum mechanical calculations were performed with the Gaussian 98 program[51] on a Sun Enterprise 450 workstation. The geometries of the molecules studied were optimized at the HF/6-31+G* level. The energies were further calculated at the MP2/6-31+G* level for the species optimized at the HF/6-31+G* (MP2/6-31+G*//HF/6-31+G*) level. The isodensity surface-polarized continuum model (SCRF=PCM option of Gaussian 98[52] was employed to estimate the effect of aqueous solvation on the relative energies. In the solvation calculations (IPCM-HF/6-31+G*//HF/6-31+G*) a dielectric constant of 78.5 (water) and a value of 0.0004 e/au^3 for the charge density were applied to determine the solute boundary. The computational approach applied for the calculation of the pK_a values is based on the quantum mechanically calculated gas-phase proton affinities (PA(gas)) and solvation energies (ΔG_{solv}). The calculated aqueous proton affinities (PA(aq) = PA(gas) + $\Delta\Delta G_{solv}$) were calibrated by using a set of compounds with known pK_a values.[21,22] All folded conformations were based on the minimum energy conformation I of the potent histamine H_3-receptor agonist *R*(α)-methylhistamine (**4a**). The conformation of *S*(α)-methylhistamine **(4b)** was obtained from the R-enantiomer by keeping the side chain amino group fixed and inverting the α-carbon substituents (hydrogen and methyl).

Statistical Analysis. Statistical analysis of the quantum chemical data and biological activity was carried out by using the standard linear regression method. Since the size of the data set is small, there was no reason to carry out more complicated analyses, such as PLS or cross-validation.

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Supporting Information Available: ^{1}H NMR signal assignation and chemical shifts, theoretical, observed, and calculated (EQUILA[25]) couplings, ΔG_{solv} energies, and statistical details of the correlations found (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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