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Tautomerism and the Receptor Action of Histamine: a Mechanistic Model

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SUMMARY

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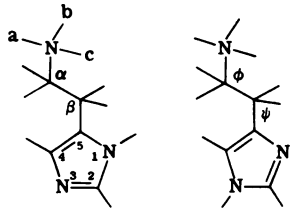
The effect of deprotonation of histamine monocation on the dominant tautomeric forms of the molecule was analyzed theoretically from molecular orbital calculations *ab initio*. The electron charge redistribution caused by the interaction of the monocation with a negatively charged group. In both cases the result is a shift in the dominant tautomeric form caused by a change in the reactivity of the ring nitrogens, as demonstrated by the molecular electrostatic potentials and total molecular energies. A model for the role of this induced tautomerism in the activation of the histamine H_2 receptor is proposed on the basis of these findings.

INTRODUCTION

Experimental findings in a variety of media (1-5), as well as theoretical considerations of the molecular structure of histamine (2, 6), indicate that in its neutral form the molecule is found mainly as the N(1)H tautomer (I), whereas in the monocationic form (in which the side chain nitrogen is protonated) the N(3)H tautomer (II) is dominant. Results from theoretical calculations of the tautomeric preference have been shown to be quite independent of side chain conformation and of the geometry chosen for the imidazole ring (6).

An analysis of the chemical structure of histamine analogues prompted the suggestion that tautomerism is important in the action of histamine agonists, especially on the H_2 receptor (1, 2). In order to test a possible mechanism by which tautomerism could be involved in receptor activation, we studied the changes that occur in the electronic structure of the histamine molecule upon deprotonation of the side chain nitrogen. Since these are the circumstances under which the tautomeric preference has been shown to change, we compared these changes in electronic structure with those following a simulated interaction of the cationic head with a neg-



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atively charged group (OH^-) serving as a model for a negatively charged biological receptor site. The analysis of the redistribution of electronic charge in the molecule and the changes in the reactivity of the ring nitrogens caused by this redistribution suggests a model for the molecular mechanism of the interaction of histamine with a receptor containing such an anionic site. This model also provides a basis for studying other systems in which the imidazole ring could function by ionic and hydrogen bonds, such as in imidazole catalysis or in histidine at the active site of enzymes.

We present here the striking changes that occur in the reactivity characteristics of the ring nitrogens and the electronic properties of the entire imidazole ring when the side chain of histamine cation is deprotonated and when the cation interacts with a negatively charged species. An immediate result of these changes is a calculated shift in tautomer stability which corresponds to experimental observations. A mechanism is proposed by which the transition between the two tautomeric forms could trigger receptor activation.

METHODS

We investigated the changes induced by protonation and deprotonation of the side chain on the comparative reactivity of the 2 ring nitrogens using two related criteria: (a) the redistribution of electronic charge density and (b) the electrostatic potential generated in the vicinity of the reactive sites. The changes in the electrostatic potential (b) are induced by the rearrangement in (a) and by the presence of the new nuclear center (H^+). The observations of (b) are expected to yield the reactivity information, i.e., to identify the site that will be preferentially nucleophilic. The observation of (a) should provide an explanation of the mechanism by which this change in reactivity is obtained. The conclusions on the comparative tautomer stability and ring nitrogen reactivity are also checked by *ab initio* calculations of the total energy of the tautomers. The electronic density is represented as a continuous function in real space (coordinate points $\bar{r}$), and is obtained as

$$\rho(\bar{r}) = N\int\Psi^*(r_1 \ldots r_N)\Psi(r_1 \ldots r_N)\, dr_2 \ldots dr_N$$

from the molecular wave function Ψ (function of the coordinates of all N electrons). In all calculations reported here, Ψ is the result of *ab initio* computations using the POLYATOM program.[2] The atomic basis function is that of Whitman and Hornback (7), with 5*s* 3*p* Gaussian orbitals on heavy atoms and two *s*-type gaussians on hydrogens. In this LCAO representation, the electron density generated by a molecule A at a certain point $\bar{r}$ is given as

$$\rho_A(r) = \sum_i n_i\phi_i(r)\phi_i(r)$$

where ϕ_i is the set of real occupied molecular orbitals and n_i is their occupation. The density maps represent contours connecting points at which the electron density is identical (isodensity maps). Density difference maps $[\rho_{A-B}(\bar{r})]$ represent isodensity contours obtained from the difference between the density at a given point as generated by molecule A and the density that molecule B would generate at the same point. For example, in our calculations A could be the neutral species while B is the protonated species, or A and B could represent two different tautomers.

The molecular electrostatic potentials $[\phi(\bar{r})]$ are calculated from the same *ab initio* wave function Ψ, using a method described previously (8, 9). The potential maps represent contours connecting points at which the energy of interaction of the unperturbed molecule with an attacking proton is identical (isopotential) (10). These maps have been shown to provide reliable information on the interaction sites of molecules with point charges and on the comparative reactivities of these sites (11–13; for an extensive review, see ref. 10). The geometry used for these calculations was obtained from crystallographic data (4, 5), with the side chain in the ($\psi = 90°$, $\phi = 180°$) conformation. This conformation is well within the region of the non-hydrogen-bonded minima of both the neutral species and the

[2] Obtained from the Quantum Chemistry Program Exchange, Indiana University.

monocation in both tautomers (see ref. 14 and references therein). The entire set of calculations was performed both with the imidazole ring geometry of histamine monocation (4) and with that of the free base (5). The maps were drawn with the aid of the PROPHET system, which is a specialized computer resource developed by the Chemical/Biological Information-Handling Program of the National Institutes of Health (15).

RESULTS AND DISCUSSION

The results show the electronic structure and reactivity in transitions between three different situations: the neutral species, the cation, and the cation interacting with the negatively charged group (OH^-).[3]

Figure 1 shows the density difference map of the N(3)H cation compared with the N(3)H tautomer of the neutral species. It can be considered to represent the redistribution of the electronic charge density upon deprotonation, with the molecular geometry remaining unperturbed.

The main characteristics of the redistribution are that deprotonation increases the charge density on the ring hydrogens and causes a decrease in the charge density in the C–H and N–H bond regions. Notably, the 2 ring nitrogens are not affected in an identical manner by the transition from cation to neutral species.

These main characteristics of the charge redistribution are repeated in Fig. 2, which shows the difference between the density in the neutral and protonated species of the N(1)H tautomer. An important difference between Figs. 1 and 2 appears, however, in the way the ring nitrogens are affected. While the redistribution on N(1) in the N(1)H tautomer parallels that on its counterpart, which is N(3) in the N(3)H tautomer, the redistribution on N(3) in the N(1)H tautomer is quite different from that on its equivalent, N(1) in the N(3)H tautomer. Thus N(3) appears to gain much more charge density upon neutralization of the N(1)H tautomer than does N(1) upon neutralization of the N(3)H tautomer.

[3] Unless stated otherwise, the density maps and numerical results refer to geometry (5).

In comparison with these findings on changes following protonation and deprotonation, Figs. 3 and 4 show the charge density redistribution following the interaction of the N(1)H cation (Fig. 3) and the N(3)H cation (Fig. 4) with the negatively charged group OH^-. The group is placed

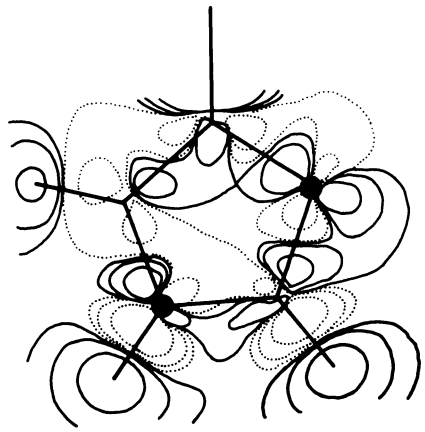

FIG. 1. *Density difference (ρ_{A-B}) map between neutral (molecule A) and monocation (molecule B) forms of N(3)H tautomer of histamine*

●, ring nitrogens. Contours are in the imidazole plane. Solid contours have positive values (higher density in *A*). Dotted contours have negative values (higher density in *B*). The values are 0.01 for the innermost contour and 0.003 and 0.001 for the second and third. In the regions of the contours, these values represent a change of close to 10% in the total density. See the text for details of calculation procedures and numbering scheme of atoms in the imidazole ring.

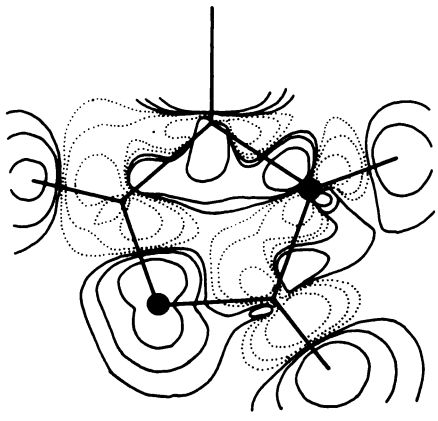

FIG. 2. *Density difference map (ρ_{A-B}) between neutral (molecule A) and cation (molecule B) forms of N(1)H tautomer of histamine*

See the legend to Fig. 1 for details.

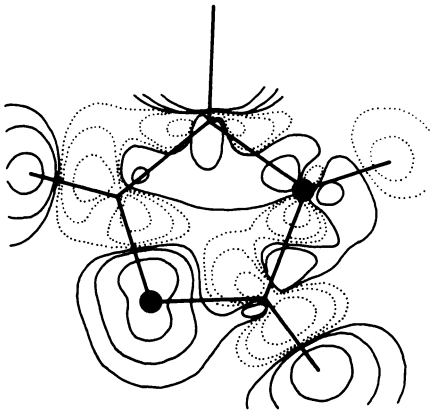

FIG. 3. *Density difference map (ρ_{A-B}) between monocation of N(1)H tautomer of histamine interacting with OH^- group (molecule A) and isolated N(1)H monocation (molecule B)*

See the text for geometry and details of calculation, and Fig. 1 for details on contour values.

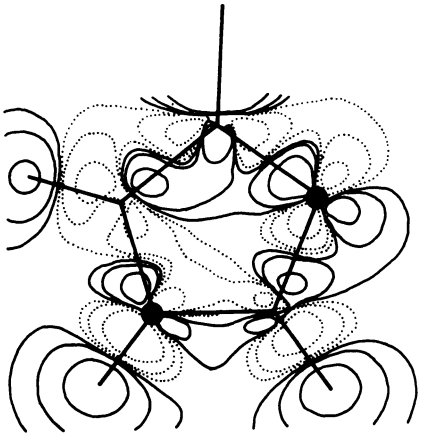

FIG. 4. *Density difference map (ρ_{A-B}) between monocation of N(3)H tautomer of histamine interacting with OH^- (molecule A) and isolated monocation (molecule B)*

See Fig. 3 for details.

at an N—O distance of 2.7 A in a hydrogen bond-like geometry. The wave function for the system thus created was recalculated and the density obtained as before. It is immediately evident that the charge redistribution pattern following deprotonation (Figs. 1 and 2) is similar in all its features to the redistribution upon interaction with OH^- (Figs. 3 and 4). As a direct result of this striking similarity, an identical effect on the reactivity of the imidazole ring is expected in the two cases: (a) when the positive side chain is deprotonated and (b) when the histamine cation interacts with a negatively charged site. Consequently an identical tautomeric shift is to be expected in both cases.

The calculations performed with the imidazole ring in the geometry of histamine monocation (4) yielded maps which were indistinguishable from Figs. 1 and 2, respectively. The resulting conclusions concerning changes in ring reactivity are therefore identical and further support the proposition that the electron density redistribution following neutralization represents an intrinsic characteristic of the histamine molecule.

The electrostatic potential maps and the total energy differences calculated for the various species further support the mechanistic conclusions. The general pattern of the electrostatic potential maps in the ring plane of each tautomer is shown in Fig. 5. The main features of these maps vary insignificantly upon deprotonation or interaction with OH^-, but the values of the contours and of the minima near the nitrogens are strongly affected. This reflects the fact that while the sites of electrophilic attack remain unchanged, the energies of interaction of the molecule with species interacting at the different sites will depend on the ionization state of the molecule. The variation in the numerical values of the potential minima near the nitrogen (Table 1) is representative of the intrinsic changes in the proton affinity of these nitrogens in the various states of the molecule. As expected, protonation of the side chain amine reduces the proton affinity of the corresponding imine nitrogen in each tautomer. This decrease is reflected in the less negative (less proton-attractive) values of the potentials. Clearly, the N(3) position in the N(1)H tautomer of the cation is more proton-attractive than the N(1) position in the N(3)H tautomer. This corresponds to the experimental findings showing that the monocation is in the N(3)H tautomeric form. Table 1 also shows that in the neutral form these reactivity characteristics are reversed and the N(1) position in the

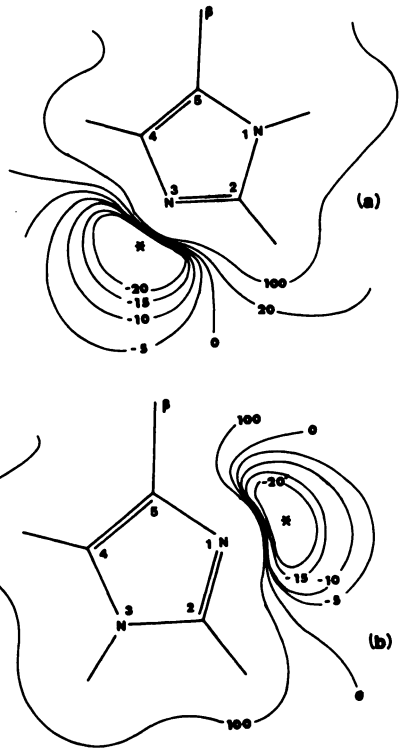

FIG. 5. *General pattern of electrostatic potential maps of N(1)H tautomer (a) and N(3)H tautomer (b) of histamine monocation in plane of imidazole ring*

The locations of corresponding minima in the potentials are indicated by asterisks.

TABLE 1

Electrostatic potential minima

Species	N(1)H tautomer minimum near N(3)	N(3)H tautomer minimum near N(1)
	kcal/mole	*kcal/mole*
Cation	−37.9	−28.2
Neutral (free base)	−96.5	−98.7
Cation + negative group (OH^-) at 2.7 A	−89.9	−95.0

N(3)H tautomer is more nucleophilic than the N(3) position in the N(1)H tautomer. Here again we find the theoretical result to be consistent with the experimentally observed change in tautomeric preference, which makes N(1) the prevalent tautomer in the neutral molecule.

More noteworthy is the finding that the cation interacting with the negative group shows the same reactivity characteristics as the neutral species. Thus, upon interaction with OH^-, the more nucleophilic site becomes N(1) and not N(3) as in the cation. Clearly, the electron charge redistribution in the ring following the interaction has had the same effects on reactivity as the redistribution caused by transition from the cation to a deprotonated species. It follows that in a reactive situation in which the 2 nitrogens compete as nucleophilic sites, N(3) would form a bond and N(1) would interact less effectively through its lone pair regions if the molecule is protonated on the side chain amine. However, if deprotonation or neutralization occurs, the N(3) bond to the electrophile will weaken and N(1) will form a stronger bond. The entire process is reversed when the interaction with the negative group is weakened by increasing the distance.

These results are also reflected in the total energy calculations for the tautomers. Using three different imidazole ring geometries available at the time, the calculations by Kang and Chou (6) have shown that the theoretical conclusions on the difference in tautomeric preference between the free base and the monocation of histamine is independent of the particular geometry assumed for the imidazole ring. With the recently published geometry of the monocation (4), we have calculated the energy difference between the N(3)H and N(1)H tautomers of the monocation to be −13 kcal/mole. The energy difference between the same two tautomers of the free base, calculated with the corresponding geometry (5), is opposite in sign: +7 kcal/mole. It appears, therefore (Table 2), that the conclusions on a switch in the theoretically calculated tautomeric preference associated with the different ionic states remain valid even when the different crystal structures of the corresponding species are used for the calculation.

It may be important to stress that these conclusions would not have been apparent from a simple Mulliken population analysis. Thus Table 3 shows that N(1) in the N(3)H tautomer remains more negative than N(3) in the N(1)H tautomer in

both the monocation and the free base. Net atomic charges having found such a wide and frequent use as reactivity criteria for biological molecules (16–18), the findings in Table 3 might have been misinterpreted to predict that N(1) would remain more attractive, and N(1)H might have been the prevalent tautomer predicted for all cases. The discrepancy in these predictions merely illustrates that the change in reactivity related to the tautomeric shift is caused by the combined electrostatic contributions from electron density changes in the whole molecule and from all nuclei. Incorporation, to a first order, of all the electrostatic contributions to the interaction energy, such as the electrostatic potential, can be expected to provide a more accurate criterion for such reactivity considerations (10, 13, 19, 20).

TABLE 2

Total molecular energies and tautomer stabilization

Histamine species	Total energy[a]	E[N(3)H] − E[N(1)H]
	a.u.	*kcal/mole*
Cation[b]		
N(3)H	−356.672212	
N(1)H	−356.651385	−13.1
Neutral[c]		
N(3)H	−356.331551	
N(1)H	−356.343254	7.3
Cation and OH^-[c]		
N(3)H	−431.859924	
N(1)H	−431.874440	9.1

[a] One atomic unit = 627.7 kcal/mole.
[b] Geometry from ref. 4 and extended side chain.
[c] Geometry from ref. 5 and extended side chain.

CONCLUSIONS

Our results show that the interaction of the "cationic head" of histamine with a simulated negative receptor subsite or deprotonation of the side chain amine has a nearly identical effect on the electronic charge distribution in the structurally remote region of the imidazole ring. In the transition from cation to neutralized species, the comparative nucleophilic character of the N(1) and N(3) ring nitrogens is altered. These changes in reactivity parallel the shift in predominant tautomeric forms found both experimentally and theoretically.

For tautomerism to be directly involved in receptor activation in the manner described above, one has to assume that histamine interacts through its cationic head with anionic site I, while the interaction of the N(3) group with a matching site, II, and that of the imine nitrogen N(1) at site III could provide the additional points of attachment. Figure 6a shows such an initial interaction scheme. It then follows from our findings that as the protonated amine on the side chain approaches anionic site I, the interaction with this site will cause a switch in the reactivities of the ring nitrogens that can induce the *modified* binding scheme shown in Fig. 6b.

The mechanism proposed here depends on the assumption that the receptor con-

TABLE 3

Distribution of charges on ring atoms (gross atomic population)

Atom	N(1)H tautomer			N(3)H tautomer		
	Cation	Neutral	Cation + OH^-	Cation	Neutral	Cation + OH^-
N(1)	7.4252	7.4293	7.4264	7.2691	7.2759	7.2695
C(2)	5.9879	6.0097	6.0030	5.9915	6.0073	6.0032
N(3)	7.2507	7.2693	7.2672	7.4290	7.4367	7.4352
C(4)	6.1614	6.1890	6.1821	6.1289	6.1503	6.1448
C(5)	5.9424	5.9208	5.9267	5.9801	5.9640	5.9676
H(1)	0.6482	0.6575	0.6445			
H(2)	0.7379	0.7690	0.7606	0.7404	0.7690	0.7608
H(3)				0.6156	0.6414	0.6362
H(4)	0.7784	0.7906	0.7905	0.7754	0.7853	0.7853

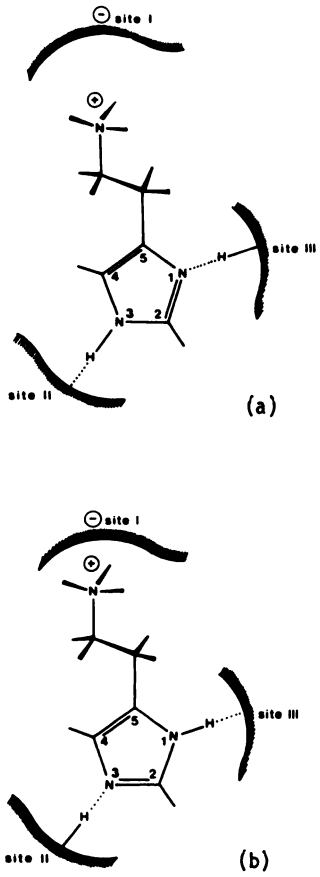

FIG. 6. *Proposed interaction scheme for histamine at the receptor site*
a. Initial state. b. Modified binding scheme.

tains an anionic or strongly polar site. That biological receptors contain anionic sites at which the cationic fragments of drugs interact represents one of the most basic and common hypotheses in receptor models (21, 22). It is a result of analyses of structure-activity relationships on a large variety of drug families that contain either permanently charged molecules (e.g., when quaternary amines are present) or bases that are considered almost fully protonated at physiological pH. Nevertheless, the possibility that a charged amine, such as acetylcholine, could interact with a hydrophobic rather than an anionic site in acetylcholinesterase has been proposed on the basis of experimental data (23) and theoretical calculations (24), although the evidence is by no means conclusive (23). The bulk of evidence for most drugs seems to support an anionic or highly polar site (21, 22). Even when models supporting a nonionic side chain interaction were considered theoretically for acetylcholine (24), the experimental structure-activity data for histamine were singled out as a counter-example, in which successive methylation at the side chain nitrogen decreases the measured potency of the drug (25)[4] and supports the assumption of an ionic interaction. The ionic interaction is defined as one in which the main contribution is coulombic, such as with separate charges or certain types of hydrogen bonds. The energy calculable from our model for the ionic interaction between the cationic head and the negative group cannot be considered a measurement of drug-receptor affinity, nor can it provide an indication for the reversibility of the reaction or the rate at which the molecule dissociates from the receptor. The main reason is the neglect of many thermodynamic parameters, such as those representing nonspecific interactions and entropy effects, solvation of both drug and receptor, and competition of the drug with endogenous counterions that are in the vicinity of the receptor and interact with the anionic site in the absence of drug. However, the mechanistic conclusions from our model, regarding the intramolecular changes following the proposed drug-receptor interaction and the binding scheme in Fig. 6, should remain independent of these total energy considerations in the complex system, and will hold for strong ionic interactions.

The chain of interactions proposed in Fig. 6 is not unique. It has been suggested for the active site of α-chymotrypsin. Crystallographic studies show that the imidazole ring of His-57 engages in hydrogen bonding (26–28) similar to that suggested here. Our findings identify the mechanism by which the interaction of histamine with a receptor could induce a "charge relay system," similar to the one suggested for the enzyme (26, 28), to trigger a biological response.

[4] C. R. Ganellin, personal communication.

In a very recent discussion of the possible role of tautomerism in H_2 receptor activation, Ganellin and co-workers (29) reported that all H_2 receptor agonists which had a reasonable level of activity in their assays were capable of undergoing the characteristic 1,3-tautomerism. Accordingly, they suggested a scheme which resembles the one emerging here from actual calculations. The mechanism by which the changes in reactivity of the 2 ring nitrogens can occur and the relationship of these changes to interaction of the side chain with the receptor become evident, however, only from analysis of the charge redistribution and subsequent modifications of the electrostatic potential. Moreover, the other reported H_2 agonists, for which the molecular structure does not directly suggest a possibility of tautomerism, could still by involved in the binding schemes proposed here, on the basis of an appropriate electron charge redistribution.

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