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Theoretical Studies on the Histamine H_2 Receptor: Molecular Mechanism of Action of Antagonists

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SUMMARY

The previously defined sites in the histamine H_2 receptor model [*Mol. Pharmacol.* **40**:980–987 (1991)] were used to elucidate the pharmacological mechanism of action of compounds that act as antagonists at the receptor. In this model, a formate anion is used both as the negative site at which the histamine cation is anchored to the receptor and as a proton acceptor site. An ammonium cation is used as a proton donor site. The proposed model of recognition of cimetidine, tiotidine, and ranitidine suggests that the monocationic form of the antagonists is the most favorable species to bind the receptor. Moreover, the mode of recognition follows the same trends obtained for compounds that act as agonists; the protonated site of the molecule, i.e., imidazolium in cimetidine, guanidinium in tiotidine, or substituted ammonium in ranitidine, anchors at the negative site of the receptor, whereas the nonbasic part, i.e., cyanoguanidine in cimetidine and tiotidine and nitrodiaminoethene in ranitidine, is located between the proton donor and acceptor sites. An energetic analysis of the interaction between the antagonists and the receptor model, including the energies of ligand desolvation, shows that histamine cannot compete effectively with cimetidine, tiotidine, or ranitidine for binding to the H_2 receptor. The predicted order of antagonist potencies, based on differences of formation enthalpies ($\Delta\Delta H_f$), reproduces qualitatively the experimental rank order.

Recently, the gene that encodes the HA H_2 receptor was cloned (1). Computer analysis of the amino acid sequence revealed extensive homology to other known guanine nucleotide-binding regulatory protein-coupled receptors and also considerable homology with the transmembrane helices in bacteriorhodopsin, if the sequential order of the helices is ignored (2). The proposed transmembrane topologies of these proteins consist of a helical bundle containing seven helices, arranged in such a way that they maintain the same cross-sectional area, as determined by the low-resolution electron-density map of bacteriorhodopsin (3). Nevertheless, current understanding of receptor function is still hampered by the lack of detailed structural information at the atomic level about the tertiary structure of the receptor protein. This difficulty explains the interest in the insight into receptor processes that can be obtained from models of receptor systems. A computational simulation (4) of the recognition complex between HA and the HA H_2 receptor suggested a mechanism by which the receptor performs its biological function; the interaction of the monocationic side chain of HA with a negatively charged region of the receptor (site I) induces the N(1) atom of the IM ring to attract a proton from a proton donor site on the receptor (site III), and the N(3)-H of HA acts as a proton donor to a proton acceptor site (site II). The receptor model (5, 6) used to investigate the activation stage of the HA H_2 receptor is composed of three sites. Sites I and II are represented by FRM^- anions, which are the smallest groups that can mimic the desired chemical properties of glutamate or aspartate. These residues may serve as the anchoring residue for the HA cation (site I) and a proton acceptor site (site II). Additionally, an AM^+ ion is used to model a lysine, arginine, or histidine residue, which may serve as the proton donor site (site III).

Sequence alignments of the third transmembrane domain of the guanine nucleotide-binding protein-coupled receptors that bind protonated amine ligands show the existence of a conserved aspartate residue located in the middle of the helix (1, 2). It was shown by mutational analysis that Asp-113 of the β-adrenergic receptor is involved in both agonist and antagonist binding (7, 8). Therefore, it can be suggested that Asp-98 of the HA H_2 receptor is the proposed negatively charged region of the receptor (site I) at which HA anchors (4–6). Gantz *et al.* (1) noted the absence of the two serine residues present in the fifth transmembrane region of the adrenergic and dopaminergic receptors. Mutagenesis studies of the β-adrenergic receptor showed that substitutions at Ser-204 and Ser-207 affect agonist but not antagonist binding to the receptor (9). These serine residues are suggested to be sites of hydrogen bonding to the hydroxyl groups present in the catechol ring of adrenergic

ABBREVIATIONS: HA, histamine; NAGHA, N^{α}-guanylhistamine; CMTDN, cimetidine; TTDN, tiotidine; RNTDN, ranitidine; IM, imidazole; IM^+, imidazolium; GNDN, guanidine; $GNDN^+$, guanidinium; AM, neutral amine; AM^+, protonated amine; CNGNDN, cyanoguanidine; $CNGNDN^+$, cyanoguanidinium; NTRDMNETH, nitrodiaminoethene; FRM^-, formate; Min I, minimum I; Min II, minimum II; TS, transition state.

agonists. A nonconserved Asp-186 is found in helix 5 of the HA H_2 receptor at the position of the serine residue (1). This residue could be the proposed proton acceptor site (site II) at which the N(3)-H of the IM ring of HA is hydrogen bonded (5, 6). However, mutational analysis of the sites and further receptor modeling are required for the validation of the model.

The definition of the sites that model the essential functional parts of the receptor can be used to explore the connection between the structure and the pharmacological activity of molecules that act as agonists, partial agonists, or antagonists. A wide range of compounds (10, 11) that act as agonists were investigated with quantum chemical methods, which provided an explanation for the pharmacological activity. However, the molecular determinants of the action of molecules that act as antagonists at the HA H_2 receptor remain unknown. As a working hypothesis, we assume that antagonists of the HA H_2 receptor occupy the same receptor sites that agonists do, thus preventing agonists from entering the active site and triggering the proton-relay mechanism that leads to the biological response. This hypothesis has already been examined for the congener N^{α}-guanylhistamine and was shown to explain the molecular determinants of the partial agonism and antagonism of this compound (6).

CMTDN (12), TTDN (13), and RNTDN (14) are known as potent and selective H_2 receptor antagonists. CMTDN inhibits HA-stimulated gastric acid secretion with a 50% inhibitory dose of 1.4 μmol/kg (12). Various ionic and tautomeric species of CMTDN can exist in aqueous solution. The neutral form, predominant at physiological pH, has two tautomeric forms of the IM ring; they are refered to as the N(1)-H and N(3)-H tautomers. In contrast to HA, the only site of CMTDN that can be protonated is the IM ring, which leads to the formation of an IM^+ ring. TTDN differs from CMTDN only by the replacement of IM by a GNDN-substituted thiazole ring, while the CNGNDN side chain remains the same; this change increases the potency of TTDN, compared with CMTDN, by at least 10 times (13). RNTDN replaces the CNGNDN moiety of CMTDN and TTDN by a NTRDMNETH group and includes a dimethylamino (AM) methyl-substituted furan ring at the other side of the molecule. The substituents of the aromatic heterocyclic rings of TTDN and RNTDN are more basic than the IM ring of CMTDN. Therefore, significant amounts of both of them will be, at physiological pH, in their $GNDN^+$ and AM^+ forms. The molecular conformations of different antagonists have been studied by X-ray crystallography and IR spectroscopy (15). The flexibility of the side chain of CMTDN and RNTDN allows for the formation of an intramolecular hydrogen bond between the two terminal moieties of the molecules. Due to the intramolecular hydrogen bond formed between the GNDN and the thiazole ring, the conformational preference of the side chain of TTDN is the extended conformation (16).

The relationship between the structure and the activity of such analogs is of special importance. The similarities among these compounds must be formulated in terms of chemical, rather than structural, properties. Following this approach, structure-activity relationships revealed that the HA H_2 receptor antagonists are mainly formed by two terminal groups of different chemical properties, connected by a common methylthioethyl chain. The protonated site of these molecules corresponds to IM^+ in CMTDN, $GNDN^+$ in TTDN, and AM^+ in RNTDN. Similarly, the other sides of the molecules are formed by neutral, planar, π-electron systems, i.e., the common CNGNDN moiety of CMTDN and TTDN and the NTRDMNETH side chain of RNTDN. The guiding assumption is that these elements play an important role in the recognition of these compounds at the receptor, because a change in structure results in a major change in activity.

Analysis of the interaction between these compounds and the receptor model offers an opportunity to probe the validity of the proposed receptor model, as well as to elucidate the molecular mechanism of action of the antagonists at the HA H_2 receptor.

Methods

Quantum mechanical calculations were performed with the GAUSSIAN (17, 18) and MOPAC (19) systems of programs. Structural optimization of the drug-receptor complexes were carried with the AM1 hamiltonian function (20).

The dimethylcyanoguanidine molecule was used as a model system of CMTDN and TTDN, to study the proton-transfer process proposed for the activation stage of the HA H_2 receptor. The potential energy curve for the proton transfer from AM^+ (site III) to the CNGNDN moiety of CMTDN and TTDN was calculated with the 4–31G basis set (21), according to a scheme developed for the construction of a proton-transfer curve from the separately optimized components of a hydrogen bond system (22). The curve was constructed from the two half-curves that correspond to the $AM^+/CNGDN/FRM^-$ complex and the $AM/CNGNDN^+/FRM^-$ complex. The crossover point from one half-curve to the other was obtained by performing calculations with both complexes and then selecting the one with the lower energy. The singular points of the potential energy curve for proton transfer (Min I, Min II, and TS) were obtained from a fourth degree polynomial that was fitted to the calculated points. Min I is the minimum with the proton near site III, and Min II is the minimum with the proton near the CNGNDN moiety. Bond and dihedral angles of the moving proton were optimized at each point of the movement with the STO-3G(NHN:4–31G) basis set. This 'mixed basis set' consists of the 4–31G basis set on the atoms involved in the proton transfer system and the minimal basis set on the rest of the system. This choice was based on the ability of the mixed basis set to reproduce the main properties of the hydrogen bond, i.e., interatomic hydrogen bond distance, energy of interaction between the members of the hydrogen bond, and energies of activation and reaction of the proton-transfer process (23). The parameters chosen to characterize the proton-transfer process are E_{act}, which represents the barrier to proton transfer and is defined as the difference in energy between the TS and Min I, and E_{react}, which represents the energy of reaction of the proton transfer and is defined as the difference in energy between Min II and Min I.

Hydration enthalpies (E_{solv}) of isolated ligands were calculated as the sum of two terms, the energy required for cavity formation and the electrostatic interaction energy between the charge distribution of the molecule and the reaction field induced in the surrounding medium, represented as continuous, with a dielectric constant of water (24).

Results and Discussion

Molecular Determinants for Recognition

Conformational analysis of CNGNDN and NTRDMNETH. The interaction of CNGNDN and NTRDMNETH groups with a negatively charged site on the receptor is thought to be part of the molecular recognition of CMTDN, TTDN, and RNTDN by the HA H_2 receptor. These moieties can assume different conformations according to the relative orientation of the methyl and methylthioethyl groups, *Z,Z*, *Z,E*, *E,Z*, and *E,E* (see Ref. 15 for nomenclature). It has been

determined (15) that the most stable forms are the *Z,E* and *E,Z* conformers, in which the two alkyl groups and either the cyano or the nitro group point in opposite directions. However, energetically, the best alignment for the binding with a negatively charged site in the receptor is the *Z,Z* conformation, in which the two amino groups are hydrogen bonded with the FRM^- of the receptor site. To evaluate which conformation of the CNGNDN and NTRDMNETH groups binds the receptor, the planar structures of the different conformers (*Z,E* and *Z,Z*) and their interaction with the FRM^- group of the receptor were optimized with the semiempirical AM1 method. The dimethylcyanoguanidine and dimethyldiaminonitroethene molecules were used to model the CNGNDN and NTRDMNETH moieties of CMTDN and TTDN and of RNTDN, respectively.

The energies of the isolated conformers, as well as their energy differences, are summarized in Table 1. The most stable form of the isolated CNGNDN and NTRDMNETH groups is the *Z,E* conformer, as expected. The *Z,Z* form is energetically less stable by 2.38 and 10.31 kcal/mol for the CNGNDN and NTRDMNETH groups, respectively. Table 1 also lists the total energy of the model conformer-FRM^- system, together with the energy differences between the *Z,E* and *Z,Z* conformers. The *Z,Z* form of CNGNDN is found to be energetically more stable than the *Z,E* form, by 4.69 kcal/mol. This is attributed to the larger energy of interaction with the FRM^- group of the *Z,Z* conformer, compared with the *Z,E* conformer, due to the extra amino-FRM^- hydrogen bond. This finding suggests that the H_2 receptor environment can affect the conformation of the CNGNDN moiety of CMTDN and TTDN, upon their interaction with the recognition site. These considerations are opposite if we consider the NTRDMNETH group. The *Z,E* conformer remains the most stable when it is interacting with the FRM^- group. The disfavored steric interaction between the methyl and nitro groups is not compensated by the stronger interaction with the FRM^- group.

These considerations point to the conclusion that the CNGNDN moiety of CMTDN and TTDN interacts with the receptor in a different conformation than the NTRDMNETH group of RNTDN.

Recognition of CMTDN. The structural characteristics of the CNGNDN moiety of CMTDN led us to propose (see above) its interaction with the FRM^- group of the HA H_2 receptor model. Therefore, the interaction of the CNGNDN side chain can occur with both negatively charged regions in the receptor, site I and site II. Because of the various ionic and tautomeric species of CMTDN in aqueous solution and the two modes of binding within the receptor, eight different possibilities were identified for the recognition of CMTDN by the receptor, which were designated as **1** to **8** in Table 2 (see Figs. 1, 2, and 3). Structures **1** to **4** correspond to the monocationic species (Fig. 1), whereas **5/6** and **7/8** correspond to the tautomers N(1)-H (Fig. 2) and N(3)-H (Fig. 3) of the neutral form, respectively. The recognition elements for CMTDN at the receptor are shown in Table 2. In the **1** (Fig. 1a), **2** (Fig. 1b), **5** (Fig. 2a), and **7** (Fig. 3a) structures, the CNGNDN part of CMTDN is recognized by site I, and either the N(1)-H or N(3)-H moiety of neutral or protonated IM functions as a hydrogen donor in the hydrogen bond to site II. The situation is reversed in the **3** (Fig. 1c), **4** (Fig. 1d), **6** (Fig. 2b), and **8** (Fig. 3b) structures. Site I forms the hydrogen bond with either N(1)-H or N(3)-H of IM or IM^+, and site II with the CNGNDN part of CMTDN. The total energy of structures **1** to **8** and the relative energy ΔE, calculated with respect to the most stable mode of binding of CMTDN with the receptor model for a given ionic and tautomeric species, are also shown in Table 2. The AM1 semiempirical method was used for the complete optimization of the system. Only the structure of the receptor model and linear hydrogen bonds were constrained. A monocationic form of CMTDN that is recognized by the receptor through the interaction of the CNGNDN moiety with site II and the hydrogen bond of N(1)-H with site I (see structure **3** in Table 2 and Fig. 1c) has the lowest total energy and is more stable than binding modes **1**, **2**, and **4**, by 24.6, 20.0, and 4.0 kcal/mol, respectively (Table 2). Between the two possible tautomers of neutral CMTDN, we found that the N(1)-H tautomer of IM is recognized by the receptor through the IM··site I and CNGNDN·· site II interactions (see structure **6** in Table 2 and Fig. 2b), whereas the mode of binding of the N(3)-H tautomer is reversed, i.e., the CNGNDN part interacts with site I and IM is hydrogen bonded to site II (see structure **7** in Table 2 and Fig. 3a).

To discriminate between the active ionic and tautomeric forms of CMTDN at the HA H_2 receptor represented in Table 2, we considered the contribution of solvation energies to the stabilization of the various complexes. The following equilibrium represents the process that takes place in the formation of the drug-receptor complex

$$\text{Drug}_{solv} + R \leftrightarrows \text{Drug-R}\ \Delta H_f^{drug}$$

where R is the HA H_2 receptor. The enthalpy of formation of the drug-receptor complex can be easily calculated with the proposed HA H_2 receptor model as

$$\Delta H_f^{drug} = E_{int}^{drug} - E_{solv}^{drug}$$

where E_{solv}^{drug} is the energy obtained by solvating the ligand and E_{int}^{drug} is the interaction energy of the complex formed between the ligand molecule and the receptor model. Results in Table 2 show E_{int}, E_{solv}, ΔH_f, and the total energy of the isolated ligand for the different species of CMTDN. The molecular conformation of the tautomeric and ionic forms of isolated CMTDN corresponds to that with the intramolecular hydrogen bond between the two terminal moieties of the molecule, IM or IM^+ and CNGNDN. In the monocationic and in the neutral N(1)-H tautomer the N(1)-H part functions as a hydrogen donor in the hydrogen bond to the lone pair of the sp^2 nitrogen of the CNGNDN moiety (see Figs. 1e and 2c), whereas in the neutral N(3)-H tautomer the N(1) nitrogen serves as a hydrogen acceptor in the hydrogen bond formed

TABLE 1

Total energies and energy differences between *Z,E* and *Z,Z* conformers for isolated dimethylcyanoguanidine (CNGNDN) and dimethylnitrodiaminoethene NTRDMNETH and their interaction with a FM^- group

The AM-1 hamiltonian function was used in the calculations.

Species	Site II	Total energy		ΔE
		Z,Z	*Z,E*	
		eV		kcal/mol
CNGNDN		−1448.647	−1448.750	2.38
	FM^-	−2233.910	−2233.706	−4.69
NTRDMNETH		−1894.170	−1894.617	10.31
	FM^-	−2679.559	−2679.703	3.33

TABLE 2

E_{int} (energy of interaction), E_{solv} (energy of solvation), and ΔH_f (enthalpy of formation of the drug-receptor complex) for the different ionic and tautomeric forms of CMTDN

Site I[a]	Site II[a]	Total energy		ΔE[d]	E_{int}	E_{solv}	ΔH_f
		Complex[b]	Ligand[c]				
		eV		kcal/mol	kcal/mol	kcal/mol	kcal/mol
Monocation							
1 CNGNDN	N(1)-H	−4816.771		24.6			
2 CNGNDN	N(3)-H	−4816.974		20.0			
3 N(1)-H	CNGNDN	−4817.839	−2986.048	0.0	−133.9	−57.7	−76.2
4 N(3)-H	CNGNDN	−4817.666		4.0			
Neutral, N(1)-H							
5 CNGNDN	N(1)-H	−4807.109		5.2			
6 N(1)-H	CNGNDN	−4807.335	−2978.385	0.0	−68.4	−20.8	−47.6
Neutral, N(3)-H							
7 CNGNDN	N(3)-H	−4807.484	−2978.423	0.0	−71.0	−22.0	−49.0
8 N(3)-H	CNGNDN	−4807.155		7.6			

[a] Molecular determinants of recognition of CMTDN by site I and site II of the receptor.
[b] Total energies of the ligand-receptor complex (see Figs. 1, 2, and 3), calculated with the semiempirical AM1 method. The energy of the histamine H_2 receptor model is −1825.982 eV (6).
[c] Total energies of the optimized isolated CMTDN, calculated with the semiempirical AM1 method. The molecular conformation of the ligand includes the intramolecular hydrogen bond between the two terminal moieties of the molecule (see Figs. 1e, 2c, and 3c).
[d] Relative energies are calculated with respect to the most stable mode of binding of CMTDN with the receptor model, for a given ionic and tautomeric species.

with the most external amino group of the CNGNDN moiety (see Fig. 3c). It is important to clarify that E_{int} includes the energy needed for opening the intramolecular hydrogen bond, because it has been calculated with respect to the hydrogen-bonded conformation. Consequently, E_{solv} was also calculated in this closed conformation.

Analysis of the entalphy of formation of the drug-receptor complex, ΔH_f, for the ionic and tautomeric forms of CMTDN shows that the monocationic form is the most favorable species to bind the HA H_2 receptor. As expected, the energy of solvation of the protonated form of CMTDN is greater than for the neutral forms, −57.7 versus −20.8 or −22.0 kcal/mol (Table 2). However, this energy is compensated by the stronger interaction with the high polar sites on the receptor, −133.9 versus −68.4 or −71.0 kcal/mol (Table 2). It is worth noting that, given these large energy differences among ΔH_f (−76.2 versus −47.6 or −48.9 kcal/mol), the presence of the electrostatic field generated by the various components of the protein receptor structure, which have not been taken into account in the present work, and a more accurate representation of the wavefunction with *ab initio* methods and extended basis sets are not expected to modify the obtained preference of protonated CMTDN over any neutral tautomer.

The proposed model of recognition of CMTDN, in which the FRM^- group of site I at the HA H_2 receptor recognizes the IM^+ moiety of CMTDN and the CNGNDN side chain is recognized by site II and site III, makes the IM^+ part of CMTDN equivalent to the AM^+ terminal side chain of HA and the CNGNDN side chain of CMTDN equivalent to IM of HA. Thus, the determinants for recognition of HA and CMTDN are the same; the protonated site of the molecule (AM^+ or IM^+) interacts with the negatively charged site of the receptor (site I), whereas the neutral part (IM or CNGNDN) is located between site II and site III.

Recognition of TTDN. Structure-activity relationships among TTDN analogs revealed the importance of the terminal GNDN thiazole ring part of TTDN for the recognition of the drug by the receptor (16). For instance, 4-methyl-2-guanidinethiazole, in which the CNGNDN side chain of TTDN has been replaced by a methyl, has properties of antagonism by itself (16). The GNDN moiety of TTDN is a common feature in the structures of most H_2 receptor antagonists discovered so far (25, 26).

At physiological pH, a significant amount of TTDN exists in its $GNDN^+$ form. The positively charged group can interact with a matching anionic site for molecular recognition. The interaction of $GNDN^+$ with site II would not be feasible, because of the size of the group and the repulsion between the positive charge and the charge of the same sign on site III. Therefore, the FRM^- group at site I of the HA H_2 receptor model is responsible for the interaction. Results in Table 3 show the total energy of the isolated ligand and the ligand-receptor complex for the protonated form of TTDN, with the AM1 hamiltonian function. Linear hydrogen bonds were constrained, as was done for CMTDN. The complete optimization of the drug-receptor complex is depicted in Fig. 4a. The flexibility of the side chain allows the CNGNDN group of TTDN to form a hydrogen bond with the FRM^- group of site II. Due to the structural properties of the $GNDN^+$ group, there are two possible modes of binding with site I, analogous to those of NAGHA (6). However, the other mode of binding is not relevant, because the CNGNDN side chain cannot be accommodated inside the active center of the HA H_2 receptor model (results not shown). As was found for CMTDN, isolated TTDN can also form an intramolecular hydrogen bond between the two terminal parts of the molecule. However, due to the intramolecular hydrogen bond present between the $GNDN^+$ NH and the thiazole ring nitrogen, the sp^2 nitrogen of CNGNDN must form the hydrogen bond with the $GNDN^+$ part of TTDN. Thus, the $GNDN^+$ NH_2 would act as a hydrogen donor to both the thiazole and the CNGDN nitrogens. The length of the TTDN side chain cannot reach the position of the $GNDN^+$ NH to form a linear hydrogen bond and, consequently, the extended conformation depicted in Fig. 4b is more stable than the close conformation, by 13.0 kcal/mol, when the effect of the solvent is included in the calculation. This finding is in perfect agreement with the X-ray structure obtained for TTDN (16).

Despite the different molecular sizes of HA and TTDN, both molecules can fit in the same HA H_2 receptor cavity and can be recognized by the sames sites. The $GNDN^+$··site I and CNGNDN··site II–III interactions of TTDN correspond to the

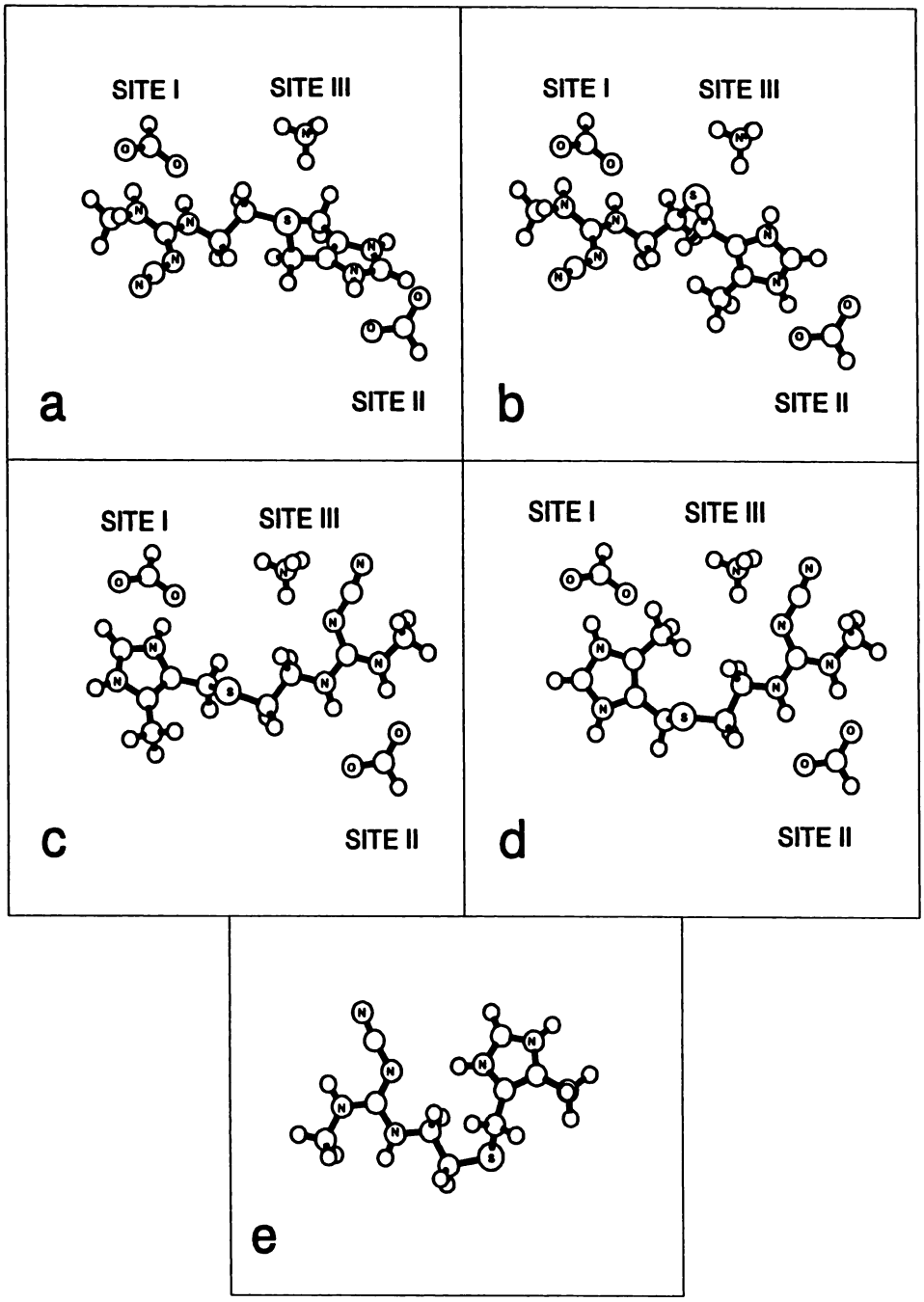

Fig. 1. Models of recognition of CMTDN in the monocationic form. a, Site I recognizes the CNGNDN side chain, whereas the N(1)-H moiety of the IM^+ ring is hydrogen bonded to site II (structure **1** in Table 2). b, Site I recognizes the CNGNDN side chain, whereas the N(3)-H moiety of the IM^+ ring is hydrogen bonded to site II (structure **2** in Table 2). c, Site I recognizes the N(1)-H moiety of the IM^+ ring, whereas site II and site III recognize the CNGNDN side chain (structure **3** in Table 2). d, Site I recognizes the N(3)-H moiety of the IM^+ ring, whereas site II and site III recognize the CNGNDN side chain (structure **4** in Table 2). e, Isolated CMTDN. The molecular conformation corresponds to that with the intramolecular hydrogen bond between the N(1)-H part of IM^+ and the sp^2 nitrogen of CNGNDN.

AM^+··site I and IM··site II–III interactions of HA, respectively.

Recognition of RNTDN. Similarly to TTDN, structure-activity relationships among RNTDN analogs show that replacement of the tertiary amine always leads to reduction in H_2 antagonist activity (27). This consideration, and the correspondance to the recognition model proposed before for CMTDN and TTDN, served to establish the hypothesis that molecular recognition involves the interaction of the positively charged AM^+ group with the matching anionic site I and of the NTRDMNETH moiety with the FRM^- group at site II. It was shown above that the *Z,E* conformer of NTRDMNETH is more stable than the *Z,Z* in its interaction with the FRM^- group. However, both conformations were taken into account in the complete optimization of RNTDN inside the receptor model. The AM1 hamiltonian function was used for the optimization in which the receptor model and linear hydrogen bonds were constrained. The interaction of the *Z,E* conformer of NTRDMNETH is more favorable than that of the *Z,Z* form, by 10.8 kcal/mol. Therefore, these calculations reinforce the previous conclusion of different conformations in the molecular recognition of the CNGNDN and NTRDMNETH moieties of CMTDN and TTDN and RNTDN. The total energies of the isolated RNTDN, in its intramolecularly hydrogen-bonded conformation (Fig. 5b), and the drug-receptor complex (Fig. 5a) are also shown in Table 3.

Simulation of the Activation Mechanism at the HA H_2 Receptor

The molecular determinant for the process of activation of the HA H_2 receptor by HA is assumed to be proton movement from the proton donor site (site III) to the proton acceptor site

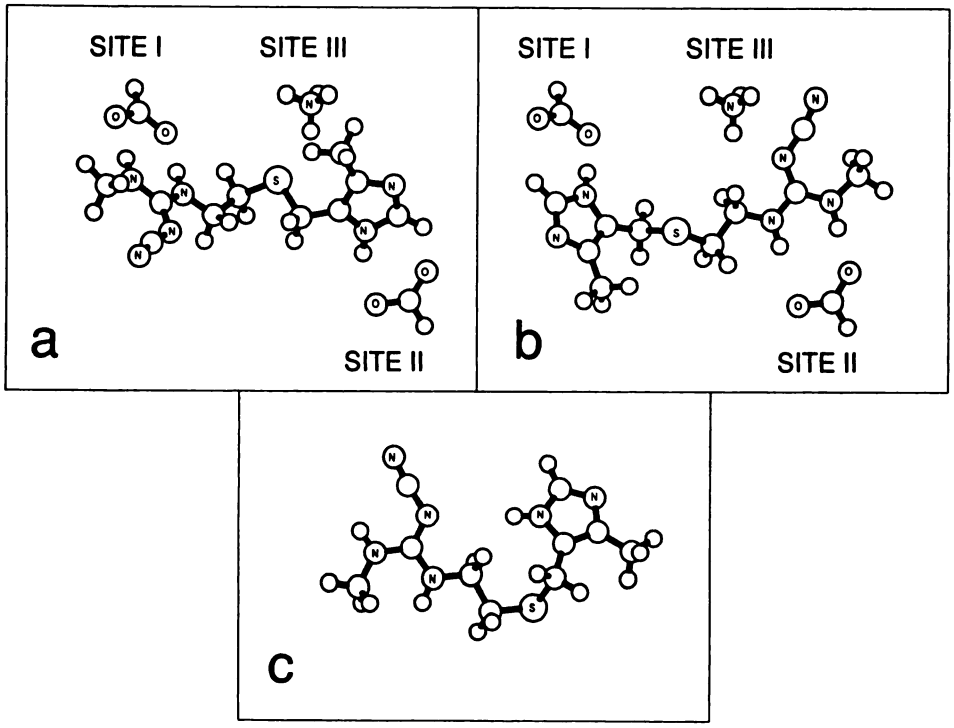

Fig. 2. Models of recognition of the N(1)-H tautomer of CMTDN in the neutral form. a, Site I recognizes the CNGNDN side chain, whereas the N(1)-H moiety of the IM ring is hydrogen bonded to site II (structure 5 in Table 2). b, Site I recognizes the N(1)-H moiety of the IM ring, whereas site II and site III recognize the CNGNDN side chain (structure 6 in Table 2). c, Structure of isolated CMTDN. The molecular conformation corresponds to that with the intramolecular hydrogen bond between the N(1)-H part of IM and the sp^2 nitrogen of CNGNDN.

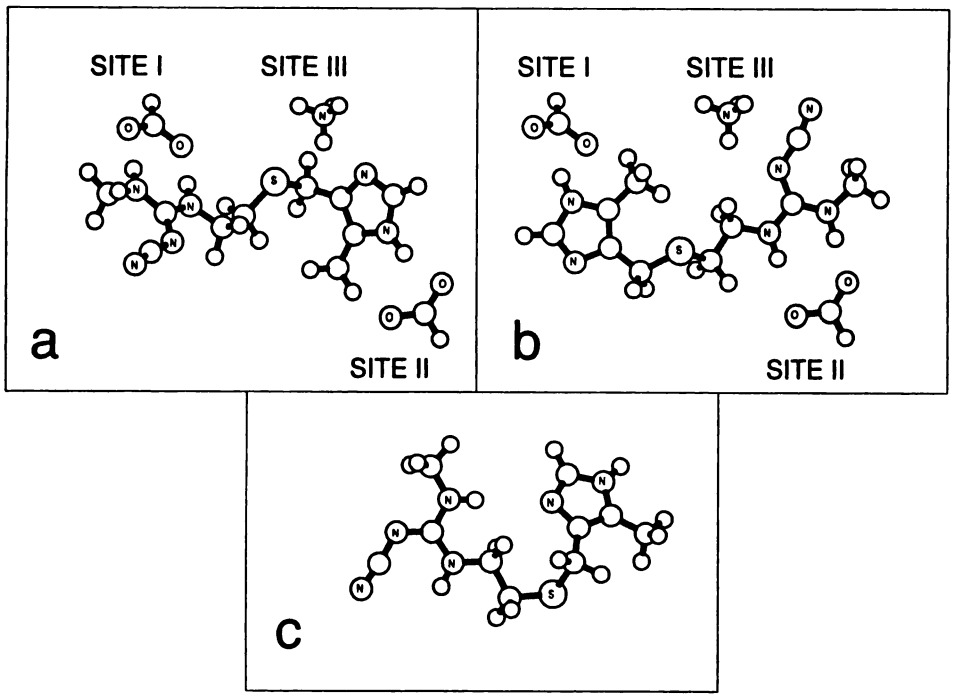

Fig. 3. Models of recognition of the N(3)-H tautomer of CMTDN in the neutral form. a, Site I recognizes the CNGNDN side chain, whereas the N(3)-H moiety of the IM ring is hydrogen bonded to site II (structure 7 in Table 2). b, Site I recognizes the N(3)-H moiety of the IM ring, whereas site II and site III recognize the CNGNDN side chain (structure 8 in Table 2). c, Structure of isolated CMTDN. The molecular conformation corresponds to that with the intramolecular hydrogen bond between the N(1) part of IM and the most external amino group of CNGNDN.

(site II), assisted by the tautomeric shift in the IM ring of HA (4, 5). Thus, it is necessary to study the viability of such a proton transfer as a result of the interaction of CMTDN, TTDN, or RNTDN with the receptor. The neutral CNGNDN or NTRDMNETH moieties, located between site II and site III, could act as the transducer of the proton from the proton donor site to the proton acceptor site. It seems clear that the structural characteristics of NTRDMNETH do not allow such a proton transfer, whereas the sp^2 nitrogen of CNGNDN can act as a proton acceptor from the proton donor site (site III) and any of the amino groups of CNGNDN can act as a proton donor to the proton acceptor site (site II). Despite the structural differences in the binding of CMTDN and TTDN to the receptor, we assume that the relative position of CNGNDN between site II and site III is the same for both molecules. The dimethylcyanoguanidine molecule was used as a model system of CMTDN and TTDN, to study the proposed proton transfer process (see Methods for computational details).

The transfer of the proton from site III (AM^+/CNGNDN/FRM^-) to CNGNDN (AM/$CNGNDN^+$/FRM^-) is characterized by a double-well potential. The results modeling the proton transfer are shown in Fig. 6. The barrier to the movement, E_{act}, is 45.4 kcal/mol, calculated with the 4–31G basis set. The process is found to be exothermic, with an energy of reaction,

TABLE III

E_{int} (energy of interaction), E_{solv} (energy of solvation), ΔH_f (enthalpy of formation of the drug-receptor complex), $\Delta\Delta H_f$ (the difference in formation entalphy between the drug and HA receptor complexes), and K_f^{TTDN}/K_f^{DRUG} (the ratio of formation constants between TTDN and the drug-receptor complexes) for the monocationic form of HA, CMTDN, RNTDN, and TTDN

Drug	Total energy		E_{int}	E_{solv}	ΔH_f	$\Delta\Delta H_f$	K_f^{TTDN}/K_f^{DRUG}
	Complex[a]	Ligand[b]					
	eV		kcal/mol	kcal/mol	kcal/mol	kcal/mol	
HA[c]	−3222.906	−1391.868	−116.6	−62.0	−54.6		
CMTDN	−4817.839	−2986.048	−133.9	−57.7	−76.2	21.6	21.9
RNTDN	−5829.314	−3997.638	−131.3	−54.1	−77.2	22.6	4.3
TTDN	−5469.501	−3637.052	−149.1	−71.0	−78.1	23.5	

[a] Total energies of the ligand-receptor complex (see Fig. 2 in Ref. 6 and Figs. 1c, 4a, and 5a), calculated with the semiempirical AM1 method.
[b] Total energies of the optimized isolated ligands, calculated with the semiempirical AM1 method. The molecular conformation of the ligands corresponds to that with the intramolecular hydrogen bond between the two terminal moieties of the molecule for HA, CMTDN, and RNTDN and to the extended conformation for TTDN (see Figs. 1e, 4b, and 5b).
[c] From Ref. 6.

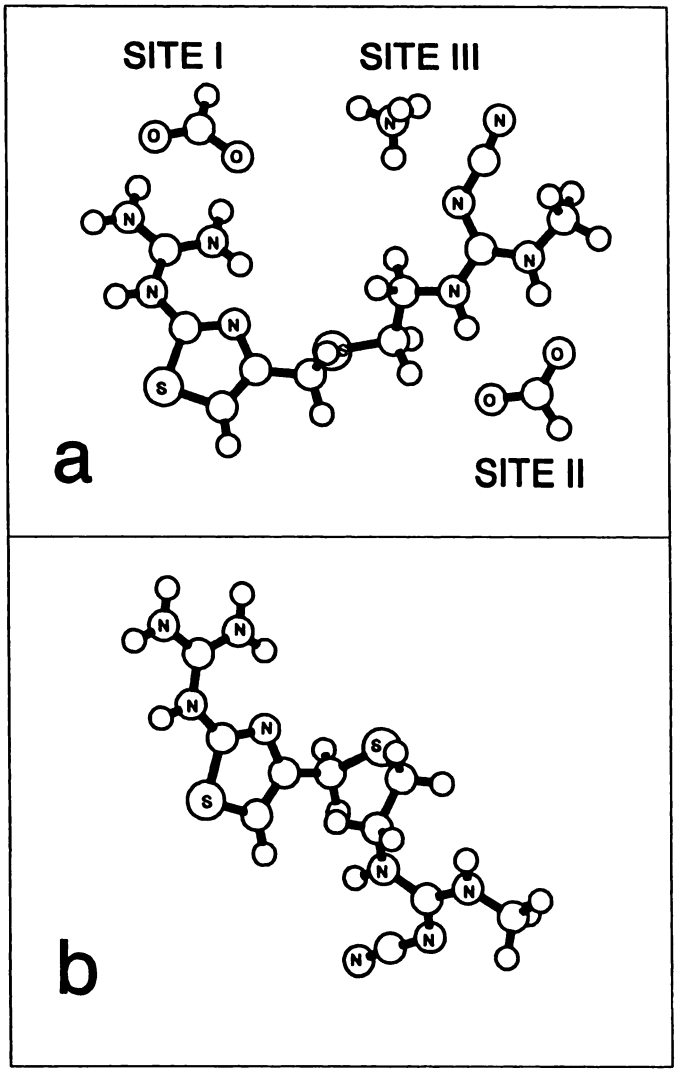

Fig. 4. Model of recognition of TTDN in the monocationic form. a, Site I recognizes the $GNDN^+$-substituted thiazole ring, whereas site II and site III recognize the CNGNDN side chain (Table 3). b, Structure of isolated TTDN. The molecular conformation corresponds to the extended conformation of the side chain.

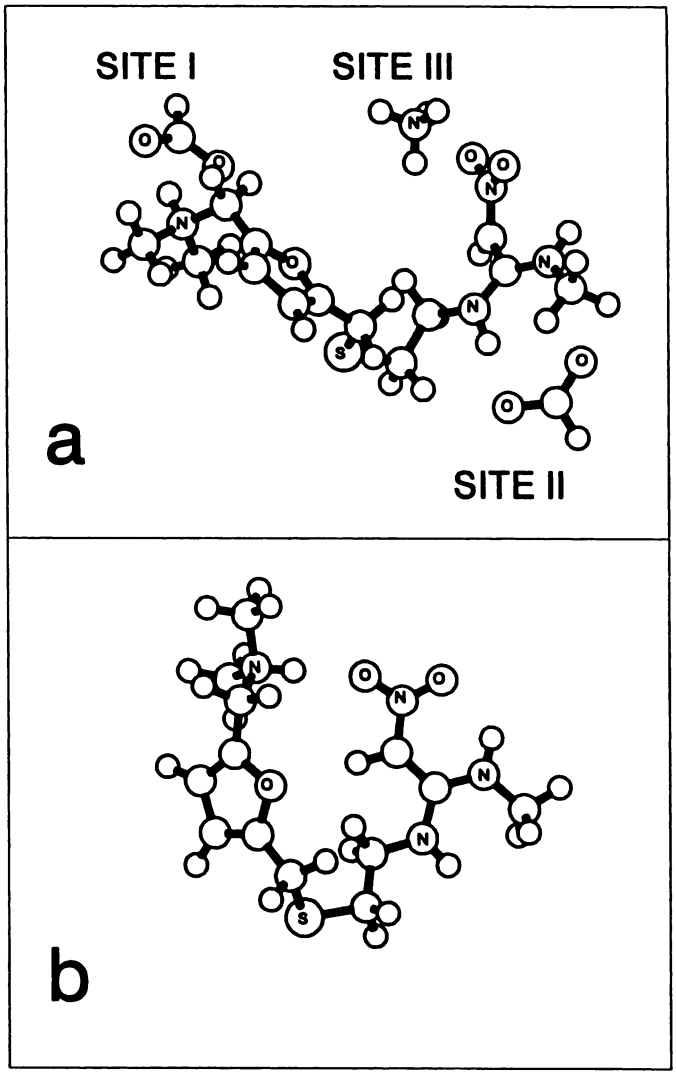

Fig. 5. Model of recognition of RNTDN in the monocatinic form. a, Site I recognizes the dimethylammonium-methyl-substituted furan ring, whereas site II and site III recognize the NTRDMNETH side chain (Table 3). b, Structure of isolated RNTDN. The molecular conformation corresponds to that with the intramolecular hydrogen bond between the dimethylammonium and the oxygen of the nitro group.

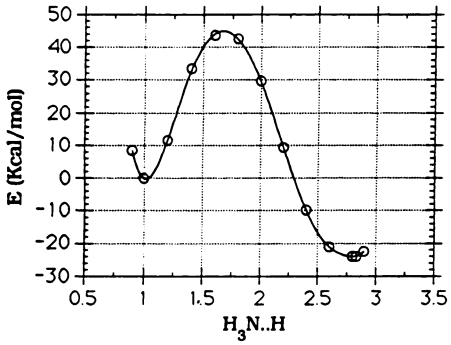

Fig. 6. Potential energy curve for proton transfer from site III (AM^+/CNGNDN/FRM^-) to the CNGNDN moiety of either CMTDN or TTDN (AM/$CNGNDN^+$/FRM^-), calculated with the 4–31G basis set.

E_{react}, of −24.5 kcal/mol. Comparison of E_{act} with the value obtained for HA with the same basis set (7.4 versus 45.4 kcal/mol; see Table 1 in Ref. 6) indicates that the proton transfer process, assumed to be the trigger of the biological response, is not likely to occur when CMTDN or TTDN is interacting with the HA H_2 receptor. The electron-withdrawing ability of the cyano group is responsible for the destabilization of the positive charge developed in the CNGNDN group. These findings suggest that a minor change in the structure of the CNGNDN moiety can fulfill the energetic requirements for the proton transfer process from site III to site II and can, therefore, produce a major change in the activity of the compounds, because they will be converted from antagonists to agonists at the HA H_2 receptor.

Molecular Mechanism of Action of Antagonists

Antagonist activities were determined against HA stimulation, and the concentrations of HA needed to produce half-maximal responses in the absence and presence of different concentrations of antagonist were measured. Under the assumption of competitive antagonism, the following equilibria take place

$$\begin{array}{lll} \text{Drug}_{solv} + R \rightarrow & \text{Drug} - R & \Delta H_f^{drug} \\ & \downarrow \quad \Delta\Delta H_f & \\ HA_{solv} + R \rightarrow & HA - R & \Delta H_f^{HA} \end{array}$$

From a molecular point of view, once HA is added to the tissue it has to compete with the drug for the receptor, to give the pharmacological response. The potency of the drug is related to the difference of formation free energies, $\Delta\Delta G_f$, between the complex of the receptor with HA and with the drug. It is difficult to evaluate this quantity by free energy perturbation analysis (28) without knowing the tertiary structure of the receptor. However, $\Delta\Delta G_f$ can be estimated from the difference of formation enthalpies, $\Delta\Delta H_f$, if cancellation of entropic effects is assumed. This assumption is reasonable, because there are no major differences in the process of drug binding. On the other hand, the difference of formation enthalpies between the complex with HA and with the drug, $\Delta\Delta H_f$, can be determined theoretically as

$$\Delta\Delta H_f = \Delta H_f^{HA} - \Delta H_f^{drug}$$
$$= (E_{int}^{HA} - E_{solv}^{HA}) - (E_{int}^{drug} - E_{solv}^{drug})$$

A negative sign in $\Delta\Delta H_f$ indicates that, for equal concentrations of HA and antagonist, there will be a higher fraction of receptors occupied by HA. A positive sign indicates that the drug-receptor complex is stronger than the HA-receptor complex. Therefore, higher concentrations of HA will be needed to trigger the activation process responsible for the biological response. Table 3 shows the obtained value of $\Delta\Delta H_f$ for the active species of CMTDN, TTDN, and RNTDN, relative to HA, together with the values needed for its calculation, E_{int}, E_{solv}, and ΔH_f. The total energy of isolated compounds corresponds to the intramolecular hydrogen-bonded conformation for CMTDN and RNTDN and to the extended conformation for TTDN (see above). Consequently, the total energy (6) of isolated HA has been calculated for the closed conformation of the tautomer N(3)-H, in which the protonated side chain is hydrogen bonded to the N(1) nitrogen of IM.

A comparison of the enthalpies of formation of the drug-receptor complexes, ΔH_f, reveals that TTDN is the most favorable species to bind the receptor, followed by RNTDN and then by CMTDN (see ΔH_f in Table 3). Therefore, the predicted order of antagonist potencies, based on differences in formation enthalpies [from highest to lowest, $\Delta\Delta H_f$ = 23.5, 22.6, 21.6, and −1.4 kcal/mol for TTDN, RNTDN, CMTDN, and NAGHA (6), respectively] reproduces qualitatively the experimental rank order (12–15, 26, 29). It is worth mentioning that the relative differences among the values of $\Delta\Delta H_f$ obtained are rather small. Therefore, more accurate calculations of these processes, by including the difference of formation entropy, could either reinforce the obtained order of potencies or change it. However, given the large positive values of $\Delta\Delta H_f$ obtained for CMTDN, TTDN, and RNTDN, calculation of $\Delta\Delta G_f$ is not expected to modify the obtained preference of the drug-receptor complexes over the HA-receptor complex.

In addition, relative affinities of two drugs can be evaluated from the ratio of formation constants, by

$$K_f^{TTDN}/K_f^{drug} = \exp(-(\Delta H_f^{TTDN} - \Delta H_f^{drug})/RT)$$

where R = 1.987 cal/deg·mol and T = 310 K. Thus, relative affinities will depend only on the difference of formation enthalpies if, once again, the cancellation of entropic effects is assumed. Table 3 shows the ratio of formation constants relative to TTDN, the most potent antagonist considered. These theoretical results predict TTDN to be 4.3 times more potent than RNTDN and 21.9 times more potent than CMTDN. Experimental relative affinities obtained from the literature (30) are 11.5, 10.0, and 3.2 for K_f^{TTDN}/K_f^{RNTDN} and 13.8, 39.5, and 13.0 for K_f^{TTDN}/K_f^{CMTDN}, if inhibition of [^{3}H]TTDN binding to guinea pig cerebral cortex membranes, activation of adenylate cyclase from guinea pig gastric mucosa, or chronotopic activity in guinea pig right atrium, respectively, is considered. As can be seen, experimental results on relative affinities vary by as much as a factor of 3.6, depending on the method employed. It is remarkable that calculated theoretical values of relative affinities fall within the ranges determined experimentally (4.3, within 3.2–11.5, for K_f^{TTDN}/K_f^{RNTDN} and 21.9, within 13.0–39.5, for K_f^{TTDN}/K_f^{CMTDN}). Another fact that must be taken into account in order to explain the lower affinity of CMTDN is that IM is less basic than GNDN or AM. Thus, a smaller fraction of the molecules of CMTDN, at physiological pH, would be in the monocationic form necessary for competing with HA for binding to the H_2 receptor.

These molecular determinants of action provide a basis for a rational approach to the design of new, structurally active,

compounds. Accordingly, a potent HA H_2 antagonist is obtained by maximizing the value of $\Delta\Delta H_f$. This is achieved by decreasing the values of E_{int} (more negative) and increasing the value of E_{solv} (less negative).

Conclusion

The present study of the recognition complexes between the different cationic and tautomeric forms of CMTDN, TTDN, and RNTDN and the receptor indicates that the monocationic form is, in all the cases, the most favorable species to bind the receptor. Moreover, the mode of recognition of antagonists follows the same trends obtained for HA; the protonated site of the molecule (IM^+ of CMTDN, $GNDN^+$ of TTDN, or AM^+ of RNTDN and HA) anchors at the negatively charged site of the receptor (site I), whereas the less basic or nonbasic part (CNGNDN of CMTDN and TTDN, NTRDMNETH of RNTDN, or IM of HA) is located between site II and site III. The different protonation state of the common IM ring of HA and CMTDN implies that these similar structures serve different functions. The protonated IM ring of CMTDN acts as a matching cationic moiety with site I, whereas the neutral IM ring of HA acts as a transducer of the proton from site III to site II, to trigger the pharmacological response. This finding emphasizes the importance of formulating the molecular determinants of action in terms of chemical reactivity, rather than in terms of structural properties.

We can conclude that antagonists of the HA H_2 receptor bind to the same receptor sites that the agonists do and act by blocking the access of agonists to receptor sites. Therefore, higher concentrations of HA will be needed to trigger the cascade of events that finally lead to the biological response. Moreover, the differences of formation enthalpies, $\Delta\Delta H_f$, between the drug-receptor and HA-receptor complexes reveal the mechanism of action, at the molecular level, of these compounds. According to this mechanism, an increase in the value of $\Delta\Delta H_f$ will yield an increase in the antagonist potency of the compound. These findings provide the tools for predicting the biological activity of untested compounds and designing new, structurally active, HA H_2 receptor antagonists.

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References

1. Gantz, I., M. Schaffer, J. Del Valle, C. Logsdon, V. Campbell, M. Uhler, and T. Yamada. Molecular cloning of a gene encoding the histamine H_2 receptor. *Proc. Natl. Acad. Sci. USA* **88**:429–433 (1991).
2. Pardo, L., J. A. Ballesteros, R. Osman, and H. Weinstein. On the use of the transmembranal domain of bacteriorhodposin as a template for modeling the 3-D structure of G-protein coupled receptors. *Proc. Natl. Acad. Sci. USA*, **89**:4009–4012 (1992).
3. Henderson, R., J. M. Baldwin, T. A. Ceska, F. Zemlin, E. Beckmann, and K. H. Downing. Model for the structure of bacteriorhodopsin based on high-resolution electron cryomicroscopy. *J. Mol. Biol.* **213**:899–929 (1990).
4. Weinstein, H., D. Chou, C. L. Johnson, S. Kang, and J. P. Green. Tautomerism and the receptor action of histamine: a mechanistic model. *Mol. Pharmacol.* **12**:738–745 (1976).
5. Weinstein, H., A. P. Mazurek, R. Osman, and S. Topiol. Theoretical studies on the activation mechanism of the histamine H_2-receptors: the proton transfer between histamine and a receptor model. *Mol. Pharmacol.* **29**:28–33 (1986).
6. Pardo, L., J. Giraldo, M. Martin, and M. Campillo. Theoretical studies on the histamine H_2-receptor: the construction of a receptor model based on the structural properties of dimaprit and N^{α}-guanylhistamine. *Mol. Pharmacol.* **40**:980–987 (1991).
7. Strader, C. D., I. S. Sigal, M. R. Candelore, M. R. Rands, W. S. Hill, and R. A. F. Dixon. Conserved aspartic acid residues 79 and 113 of the β-adrenergic receptor have different roles in receptor function. *J. Biol. Chem.* **263**:10267–10271 (1988).
8. Strader, C. D., I. S. Sigal, and R. A. F. Dixon. Structural basis of β-adrenergic receptor function. *FASEB J.* **3**:1825–1832 (1989).
9. Strader, C. D., M. R. Candelore, W. S. Hill, I. S. Sigal, and R. A. F. Dixon. Identification of two serine residues involved in agonist activation of the β-adrenergic receptor. *J. Biol. Chem.* **264**:13572–13578 (1989).
10. Reggio, P., S. Topiol, and H. Weinstein. Molecular determinants for the agonist activity of 2-methylhistamine and 4-methylhistamine at H_2-receptors. *J. Med. Chem.* **29**:2412–2415 (1986).
11. Mazurek, A. P., R. Osman, and H. Weinstein. Molecular determinants for recognition of triazole and tetrazole analogs of histamine at H_2-receptors. *Mol. Pharmacol.* **31**:345–350 (1987).
12. Brimblecombe, R. W., W. A. M. Duncan, G. J. Durant, J. C. Emmet, C. R. Ganellin, and M. E. Parsons. Cimetidine: a non thiourea H_2 receptor antagonist. *J. Intern. Med. Res.* **3**:86–92 (1975).
13. Yellin, T. O., S. H. Buck, D. J. Gilman, D. F. Jones, and J. M. Wardleworth. ICI 125211: a new gastric antisecretory agent acting on histamine H_2-receptors. *Life Sci.* **25**:2001–2009 (1979).
14. Bradshaw, J., R. T. Brittain, J. W. Clitherow, M. J. Daly, D. Jack, B. J. Price, and R. Stables. AH 19065: a new potent, selective histamine H_2-receptor antagonist. *Br. J. Pharmacol.* **66**:P464 (1979).
15. Ganellin, C. R. Chemistry and structure-activity relationships of drugs acting at histamine receptors, in *Pharmacology of Histamine Receptors* (C. R. Ganellin and M. E. Parsons, eds.). Wright PSG, Bristol, U.K., 10–102 (1982).
16. Gilman, D. J., D. F. Jones, K. Oldham, J. M. Wardleworth, and T. O. Yellin. 2-Guanidinothiazoles as H_2-receptor antagonists, in *The Chemical Regulation of Biological Mechanisms* (A. M. Creighton and S. Turner, eds.). The Royal Society of Chemistry, London, U.K., 58–76 (1982).
17. Frisch, M. J., J. S. Binkley, H. B. Schlegel, K. Raghavachari, C. F. Melius, R. L. Martin, J. J. P. Stewart, F. W. Bobrowicz, C. M. Rohlfing, L. R. Kahn, D. J. Defrees, R. Seeger, R. A. Whiteside, D. J. Fox, E. M. Fleuder, and J. A. Pople. *Gaussian 86*. Carnegie-Mellon Quantum Chemistry Publishing Unit, Pittsburgh PA (1984).
18. Frisch, M. J., M. Head-Gordon, G. W. Trucks, J. B. Foresman, H. B. Schlegel, K. Raghavachari, M. A. Robb, J. S. Binkley, C. Gonzalez, D. J. Defrees, D. J. Fox, R. A. Whiteside, R. Seeger, C. F. Melius, J. Baker, R. L. Martin, L. R. Kahn, J. J. P. Stewart, S. Topiol, and J. A. Pople. *Gaussian 90*. Gaussian, Inc., Pittsburgh PA (1990).
19. Stewart, J. J. P. MOPAC 4.0. *Quantum Chem. Program Exc. (QCPE) Bull.* 455.
20. Dewar, M. J. S., E. G. Zabish, E. F. Healy, and J. J. P. Stewart. AM1: a new general purpose quantum mechanical molecular model. *J. Am. Chem. Soc.* **107**:3902–3909 (1985).
21. Ditchfield, R., W. J. Hehre, and J. A. Pople. Self-consistent molecular orbital methods IX. An extended gaussian-type basis for molecular orbital studies of organic molecules. *J. Chem. Phys.* **54**:724–728 (1971).
22. Topiol, S., G. Mercier, R. Osman, and H. Weinstein. Computational schemes for modeling proton transfer in biological systems. *J. Comput. Chem.* **6**:581–586 (1985).
23. Pardo, L., A. P. Mazurek, and R. Osman. Computational models for proton transfer in biological systems. *Int. J. Quantum Chem.* **37**:701–711 (1990).
24. Rashin, A., and K. Namboodiri. A simple method for the calculation of hydration enthalpies of polar molecules with arbitrary shapes. *J. Phys. Chem.* **91**:6003–6012 (1987).
25. Donetti, A., E. Cereda, E. Bellora, A. Gallazzi, C. Bazzano, P. Vanoni, P. D. Soldato, R. Micheletti, F. Pagani, and A. Giachetti. (Imidazolylphenyl)formamidines: a structurally novel class of potent histamine H_2-receptor antagonists. *J. Med. Chem.* **27**:380–386 (1984).
26. Goot, H. v. d., and H. Timmerman. Structural requirements for histamine H_2 agonists and H_2 antagonists. *Handb. Exp. Pharmacol.* **97**:573–748 (1991).
27. Bradshaw, J., M. E. Butcher, J. W. Clitherow, M. D. Dowle, R. Hayes, D. B. Judd, J. M. McKinnon, and B. J. Price. Aminoalkylfurans as H_2-receptor antagonists, in *The Chemical Regulation of Biological Mechanisms* (A. M. Creighton and S. Turner, eds.). The Royal Society of Chemistry, London, 45–57 (1982).
28. McCammon, J. A., and S. C. Harvey. *Dynamics of Proteins and Nucleic Acids*. Cambridge University Press, New York (1987).
29. Durant, G. J., M. E. Parsons, and J. W. Black. Potential histamine H_2-receptor antagonists: 2,N^{α}-guanylhistamine. *J. Med. Chem.* **18**:830–833 (1975).
30. Gajtkowski, G. A., D. B. Norris, T. J. Rising, and T. P. Wood. Specific binding of ^{3}H-tiotidine to histamine H_2 receptors in guinea pig cerebral cortex. *Nature (Lond.)* **304**:65–67 (1983).

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