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Theoretical Studies on the Histamine H_2 Receptor: Construction of a Receptor Model Based on the Structural Properties of Dimaprit and N^{α}-Guanylhistamine

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SUMMARY

A histamine H_2 receptor model was constructed based on the receptor sites previously proposed by Weinstein *et al.* [*Mol. Pharmacol.* **29**:28–33 (1986)]. In this model, a glutamate or aspartate residue, simulated by a formate anion, is proposed both as the negative site at which the histamine cation is anchored to the receptor and as a proton-acceptor site. A proton-donor site, simulated by an ammonium cation, is proposed to model either a lysine, arginine, or histidine residue. The simulation of the activation mechanism of the histamine H_2 receptor, inside the proposed receptor model, includes structure optimizations of stationary points and transition states with a split valence basis set. The proton movement from the proton donor site to the proton acceptor site, mediated by the imidazole ring of histamine, was found to be sequential in the potential energy surface. Results of the calculations reveal that both proton transfers are feasible from an energetical point of view. However, the proton movement from N(3) to the proton acceptor site has a higher energy of activation and, therefore, will be the rate-limiting step in the starting process that triggers the cascade of events that finally leads to a biological response. This model also provides a basis for explaining the molecular determinants of the pharmacological activity of N^{α}-guanylhistamine. The structural properties of the guanidinium group allow N^{α}-guanylhistamine to interact with the proposed receptor in two different modes. The proton-relay process, proposed as the trigger of the activation of the histamine H_2 receptor, is likely to occur in only one of these binding modes. In the other case, N^{α}-guanylhistamine acts as an antagonist because the barrier to proton transfer in this mode is too high. The partial agonism of N^{α}-guanylhistamine is related to the ability of the drug to bind with the receptor in two different modes with similar affinity. An energetic analysis of the interaction between a ligand and the receptor model, including the energies of ligand desolvation, shows that histamine can compete with N^{α}-guanylhistamine for the binding to the H_2 receptor.

HA [2-(4-imidazolyl)ethylamine] is a neurotransmitter known to act on three different receptors, namely H_1, H_2, and H_3 receptors (1, 2). Experimental evidence (3), as well as structure-activity considerations, led to the formulation of a mechanistic model for the activation process at the HA H_2 receptor (4, 5). The model assumes that HA approaches the receptor as the N(3)-H tautomer of the monocationic form. The cationic side chain interacts with a negative region of the receptor (site I) and, as the side chain is anchored, the neutralization causes a shift in the tautomeric preference of the imidazole ring to N(1)-H. The attachment of HA at the receptor site thus causes N(1) to attract a proton from a proton-donor site on the receptor (site III), while the proton on N(3) is transferred to a proton-acceptor site (site II). This mechanistic hypothesis was explored with theoretical calculations and was shown to provide an explanation for the pharmacological activity of compounds that act as agonists at the HA H_2 receptor (6, 7).

Because there is a lack of structural information about the receptor, structure-activity relationships can be useful in order to assess the structural properties of the receptor required for receptor recognition and activation. Thus, extended series of structurally related compounds, known to act at the same receptor, can be used to construct a model of the receptor, based on their physicochemical properties. Subsequent simulations of the interaction of these compounds with the receptor models can be used to characterize the nature of drug-receptor interactions and to identify the molecular requirements needed at the receptor sites to explain the pharmacological properties such as agonism, partial agonism, and antagonism.

A model system to study the proton-transfer process as a trigger for receptor activation was constructed (8) by placing the HA molecule among hydroxyl (site I), ammonia (site II), and ammonium (site III) groups. Alternatively, hydroxyl instead of ammonia was used to model site II. These choices represent the minimal model with the desired molecular properties. In order to improve the characterization of the sites of the HA H_2 receptor, two compounds are being studied, dimaprit

ABBREVIATIONS: HA, histamine; NAGHA, N^{α}-guanylhistamine; TS, transition state(s).

[*S*-[3-(*N*,*N*-dimethylamino)-propyl]isothiourea] and NAGHA [2-(4-imidazolyl)ethylguanidine].

Dimaprit is known as a highly selective HA H_2 receptor agonist. Pharmacological studies *in vitro* have found it to have approximately 17.5% of the potency of HA on the rat uterus and 71% on the guinea pig right atrium, with similar maximal responses (1, 9). Dimaprit stimulates gastric acid secretion in the rat, dog, and cat, with potencies of 19, 58, and 400–500%, respectively, compared with HA (1, 9). The activation of the HA H_2 receptor by dimaprit (1, 10–12) was considered to follow the same mechanism proposed for HA (4, 5, 8), in spite of the ostensible differences in their molecular structures. In this mechanism, the monocationic form of dimaprit, protonated at the side chain, interacts with the negative region of the receptor. Because the isothiourea moiety of the dimaprit molecule embodies the same electronic characteristics that govern the reactivity properties of the imidazole ring, it can act as the transducer of the proton transfer from site III to site II.

NAGHA differs from HA only in that it replaces the amine group in the side chain by a guanyl group. This change, however, gives NAGHA very different properties, compared with HA. It is a partial agonist and, therefore, in the presence of HA it will appear as an antagonist (13). In the studies that led to the design of the first H_2 antagonist, NAGHA served as the lead compound for the transition from agonist structures to antagonists (1, 13). We, therefore, studied NAGHA in order to elucidate the molecular determinants for partial agonism and antagonism. In a previous study (14), the tautomeric equilibrium of neutral and protonated NAGHA was studied. It was shown that, as in HA, the ionization state of the side chain of NAGHA affects the tautomeric preference of the imidazole portion of the molecule. The strong tautomeric preference for the N(3)-H form of the monocation is reversed to N(1)-H in the neutral (free base) form of NAGHA. It appears, therefore, that the imidazole portion of NAGHA can be recognized at the H_2 receptor in the same way as HA. Following the model proposed for the interaction of HA with the receptor, the positively charged side chain must also interact with a matching anionic site to complete the process of molecular recognition.

Therefore, the chemical structures of dimaprit and NAGHA are useful tools for the construction of a more realistic model for the HA H_2 receptor. We explore the interaction of NAGHA with the obtained receptor model to characterize the structural and molecular properties of its pharmacological activity.

Methods

Ab initio quantum mechanical calculations were performed with the GAUSSIAN system of programs (15, 16). Structural optimizations were carried out using analytical first derivatives of the energy at the Hartree Fock level, with the standard STO-3G (17), 4-31G (18), and 3-21G* basis sets. The 3-21G* basis set consists of the 3-21G (19) augmented by a set of 6d functions with exponents 0.65 (20) on the sulfur atom. The 'mixed basis set' STO-3G(NHONHO:3-21G) consists of the 3-21G basis set on the atoms involved in the proton transfer system and the minimal basis set (STO-3G) on the rest of the system. In this way, the preceding notation represents the basis set used for the atoms surrounding the proton-transfer system and, within parenthesis, the basis set on the atoms engaged in the hydrogen bond. 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 (21).

Semiempirical quantum mechanical calculations were performed with the MOPAC (22, 23) and GAUSSIAN programs, using the AM-1 hamiltonian function (24).

The methylisothiourea molecule was used as a model for dimaprit in the characterization of site II with the 3-21G* basis set. The protonated propylguanidine molecule was used as a model for NAGHA in the characterization of site I with the STO-3G(NHONHO:3-21G) basis set. The 5-methylimidazole molecule, in which the ethylamine side chain of HA has been replaced by a methyl, was used as a model system to study the proton-transfer process proposed for the activation stage of the HA H_2 receptor, by HA, with the 4-31G basis set. It has been shown (8) that replacement of ethylamine side chain in HA by a methyl group does not change the energies of activation and reaction for the proton-transfer process. Similarly, the simulation of the activation process of the H_2 receptor, by NAGHA, was carried out with 5-methylimidazole, in which the ethylguanyl side chain of NAGHA has been replaced by a methyl, as a model.

The simulation of the activation mechanism of the HA H_2 receptor, by HA, includes structure optimizations of stationary points and TS with the 4-31G basis set. The TS is defined as the structure that has a Hessian matrix of force constants with one negative eigenvalue. The approximate positions of the TS of the proton movement from site III toward N(1) and from N(3) to site II were located by calculating a limited portion of the potential energy surface using the H_3N-H and N(3)-H distances as reaction coordinates (see Fig. 3). During the course of the optimizations, the relative orientation of the ammonium (site III) and formate (site II) groups of the receptor model and the distance between the nitrogen of site III and N(1) were kept fixed at the positions found on the optimization of HA inside the receptor model. A split valence basis set plus a set of diffuse functions on the heavy atoms of the formate group (4-31 + G) was used in energy calculation of the proton movement from site III toward N(1) and from N(3) to site II.

The potential energy curves for the proton transfer from ammonium (site III) to the imidazole moiety of NAGHA, proposed for the activation stage of the HA H_2 receptor by NAGHA, were calculated with the 4-31G basis set, according to a scheme developed for the construction of a proton-transfer curve from the separately optimized components of a hydrogen bond system (25). The curves were constructed from the two half-curves that correspond to the ammonium-imidazole-formate ($AM^+/IM/FRM^-$) complex and the ammonia-imidazolium-formate ($AM/IM^+/FRM^-$) 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 (the two minima and the TS) were obtained from a quartic polynomial that was fitted to the calculated points. For the nonlinear proton transfer, the bond and dihedral angles of the moving proton were optimized at each point of the movement with the 4-31G basis set.

The parameters chosen to characterize the activation mechanism of the HA H_2 receptor are as follows: E_{act}^{PT1}, which represents the barrier to proton transfer from site III to N(1) and is defined as the difference in energy between the TS ammonia-proton-imidazole-formate ($AM/H^+/IM/FRM^-$) and the initial minima complex $AM^+/IM/FRM^-$; E_{react}^{PT1}, which represents the energy of reaction of the proton transfer from site III to N(1) and is defined as the difference in energy between the minima $AM/IM^+/FRM^-$ and $AM^+/IM/FRM^-$; E_{act}^{PT2}, which represents the barrier to proton transfer from N(3) to site II and is defined as the difference in energy between the TS ammonia-imidazole-proton-formate ($AM/IM/H^+/FRM^-$) and the minima $AM/IM^+/FRM^-$; and E_{react}^{PT2}, which represents the energy of reaction of the proton transfer from N(3) to site II and is defined as the difference in energy between the minima ammonia-imidazole-formic acid (AM/IM/FRM) and $AM/IM^+/FRM^-$.

E_{int} represents the stabilization of the complex and is defined as the difference in energy between the complex and the sum of the energies of the optimized isolated molecule and the receptor model. Semiempir-

ical optimizations of the drug-receptor complex and the isolated drug were carried out with the AM-1 hamiltonian function.

In addition, 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 (26).

Results and Discussion

Construction of the HA H_2 Receptor Model

Characterization of site I. Previous calculations on NAGHA (14) showed that the same considerations that led to the identification of the N(3)-H tautomer of the HA monocation as the species recognized at the HA H_2 receptor apply to the NAGHA monocation in the N(3)-H tautomeric form. It is of interest to compare the structural properties of HA and NAGHA and seek the similarities that define the action at the HA H_2 receptor. As a working hypothesis, we can assume that NAGHA acts as an agonist, with the imidazole ring and the side chain at the same position as HA when it is interacting with the HA H_2 receptor. The interaction of the guanidinium side chain of the N(3)-H cationic form with the negative site in the receptor, analogously to HA, is thought to be the first element in the molecular recognition of NAGHA by the HA H_2 receptor. Assuming that the negatively charged residues that form the polypeptide structure of the receptor are aspartate and glutamate, we suggest a formate group to model site I. The formate anion represents a more realistic negative model than hydroxyl as the anchoring site at the HA H_2 receptor.

The size of the system in which NAGHA interacts with formate would be prohibitively large for good quality calculations. We, therefore, represent NAGHA by propylguanidinium, in which the imidazole ring has been replaced by a methyl group. In this system, we carried out geometry optimization of the propylguanidinium-formate complex with the mixed basis set STO-3G(NHONHO:3-21G), in which both oxygens of the formate group, the two hydrogen-bonded nitrogens in the guanidinium group, and both protons were represented with the 3-21G basis set and the rest of the system with the minimal basis set. During the course of the optimization, the propyl group of propylguanidinium was kept fixed in the conformation obtained from previous calculations on the HA molecule (4, 5), and the imidazole ring, site II, and site III were omitted. The oxygen of the formate group, which corresponds to the oxygen of the hydroxyl group in the previous model (8), was also kept fixed to the values obtained in the calculations on the HA H_2 receptor activation. The completely optimized system is shown in Fig. 1a.

Characterization of site II. The structural properties of the isothiourea moiety of dimaprit led us to propose a carboxyl group as site II at the HA H_2 receptor. This group was modeled by a formate anion, which can act as a proton acceptor. The methylisothiourea molecule, used as a model for the isothiourea moiety of dimaprit (12), was placed between the ammonium (site III) and the formate (site II) ions. The distance between the nitrogen of ammonium and the oxygen of formate was taken as 7.9 Å. This value was obtained from the distance between site II and site III, in previous calculations on the HA H_2 receptor activation model (8). We assumed a linear hydrogen bond between site III and the sulfur atom of the isothiourea moiety, with an internuclear N··S distance of 3.5 Å (27). The hydrogen bond was kept fixed because the interaction between the protonated side chain and site I is omitted. Fig. 1b shows the optimized system with the 3-21G* basis set. The interaction between isothiourea and the formate group is another important element in the recognition of dimaprit by the HA H_2 receptor. The absence of such a formate moiety in the binding sites of other HA receptors may explain why dimaprit is selective for the HA H_2 receptor.

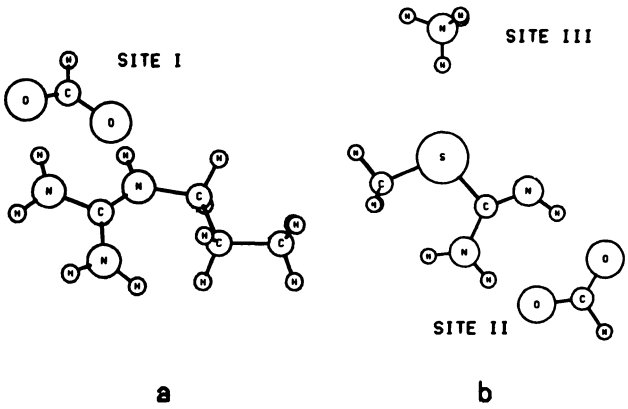

Fig. 1. a, Characterization of site I. The propylguanidinium molecule used as a model of NAGHA was used for the characterization of the formate group acting as anchoring site at the HA H_2 receptor. b, Characterization of site II. The methylisothiourea molecule used as a model of dimaprit was placed between ammonium (site III) and formate (site II) ions for the characterization of the formate group that acts as a proton-acceptor site at the HA H_2 receptor.

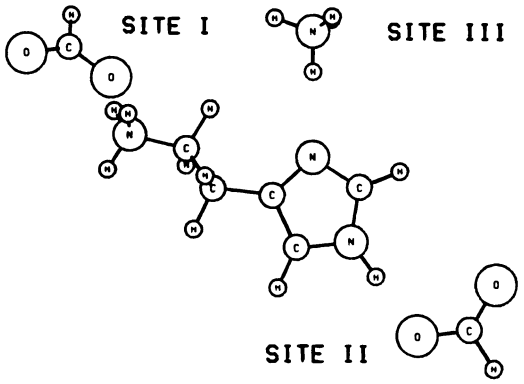

Fig. 2. Complete model system proposed for the activation stage of the HA H_2 receptor, by HA. The HA molecule was placed between the molecules of formate (anchoring, site I), formate (proton acceptor, site II), and ammonium (proton donor, site III).

The complete model system of the HA H_2 receptor, constructed in the way described above, is shown in Fig. 2. The semiempirical method AM-1 was used for the optimization of HA inside the model. During the course of the optimization, we kept the conformation of the ethylamine side chain fixed to the values obtained in the optimization of the isolated N(3)-H cationic form of HA (5). The structure contains linear hydrogen bonds between site III and N(1), between N(3)-H and site II, and between site I and the nitrogen side chain of HA, at the optimized distances of 3.09, 2.89, and 2.54 Å, respectively. These results are very close to the values obtained in the previous optimization (8) with hydroxyl, ammonia, and ammonium as receptor sites. Owing to the fact that during the construction process the constraints imposed were taken from the properties of the previous receptor optimization (8), the

HA molecule could be placed inside the receptor anchoring the cationic side chain to site I, positioning the N(3)-H next to site II and N(1) next to site III. Such an arrangement upholds the recognition and activation processes of the HA H_2 receptor.

The orientation of the formate group representing site I in the HA H_2 receptor has been derived from the structural properties of the side chain of NAGHA. The orientation of the formate representing site II has been derived from the isothiourea properties of dimaprit. Thus, the definition of the sites that model the essential functional parts of the receptor can be used in further studies to explore the connection between the structure and the pharmacological activity of other molecules that act as agonists, partial agonists, or antagonists.

Simulation of the Activation Mechanism at the HA H_2 Receptor

The molecular determinant for the activation process at the HA H_2 receptor is assumed to be the proton movement from the proton-donor site (site III) to the proton-acceptor site (site II) (3, 4, 8). We used the 5-methylimidazole molecule, as a model of HA, to study this activation mechanism in the receptor model.

The proton movement from site III to site II was found to be sequential in the potential energy surface (see Methods for computational details). The structures of the local minima and of the TS found on the potential energy surface are depicted in Fig. 3. Structural parameters and total energies are shown in Table 1. The transfer of the proton from ammonium (AM^+/IM/FRM^-) to imidazole (AM/IM^+/FRM^-) is characterized by a double-well potential. Both minima are connected by a TS structure (AM/H^+/IM/FRM^-), which corresponds to the proton being between site III and N(1) at a H_3N-H distance of 1.413 Å (Fig. 3b). The structure found has the characteristics of a TS, with a single negative eigenvalue in the matrix of force

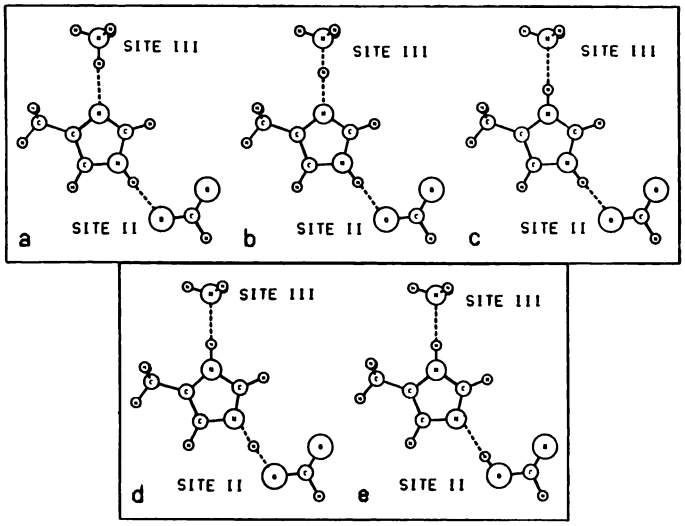

Fig. 3. Simulation of the activation mechanism of the HA H_2 receptor by HA. The 5-methylimidazole molecule was used as a model of HA. a, b, and c, Local minima (a and c) and TS (b) of proton-transfer movement from the proton-donor site (site III) to the N(1) nitrogen of HA. d and e, Structures of TS (d) and end minimum (e) of proton movement from the N(3) nitrogen of HA to the proton-acceptor site (site II).

TABLE 1

Selected structural parameters and total energies at the 4-31G level of theory of minima and TS structures (Fig. 3) of the activation process at the HA H_2 receptor and energies of activation and energies of reaction of the proton movement from site III toward N(1) (E_{act}^{PT1} and E_{react}^{PT1}) and from N(3) to site II (E_{act}^{PT2} and E_{react}^{PT2})

	H_3N-H	N(3)-H	Total energy
	Å	Å	hartrees
Minima			
AM^+/IM/FRM^-	1.075	1.035	−507.99728
AM/IM^+/FRM^-	2.081	1.050	−508.05068
AM/IM/FRM	2.088	1.954	−508.07700
TS			
AM/H^+/IM/FRM^-	1.413	1.040	−507.98552
AM/IM/H^+/FRM^-	2.084	1.376	−508.03586

Level of computation	E_{act}^{PT1}	E_{react}^{PT1}	E_{act}^{PT2}	E_{react}^{PT2}
	kcal/mol			
HF/4-31G//HF/4-31G	7.4	−33.5	9.3	−16.5
HF/4-31+G//HF/4-31G	7.5	−33.2	12.9	−9.6

constants. The major contribution to the corresponding eigenvector comes from the internal coordinate associated with stretching the H_3N-H bond. The energetics associated with such a proton transfer are shown in Table 1. The barrier to the movement, E_{act}^{PT1} is 7.4 kcal/mol, calculated with the 4-31G basis set. The process is found to be exothermic, with an energy of reaction, E_{react}^{PT1}, of −33.5 kcal/mol at the same level of theory. When the 4-31G basis set is augmented by diffuse functions on the heavy atoms of the formate group, yielding the 4-31 + G basis set, neither E_{act}^{PT1} nor E_{react}^{PT1} changes significantly. These results are very close to those calculated previously by Weinstein *et al.* (see lines VI and VII of Table 1 in Ref. 8) for HA monocation with the minimal basis set and a hydroxyl anion as proton acceptor in site II. The substitution, in the model, of the hydroxyl anion by a formate group as a negative proton acceptor in site II does not change the general shape of the transfer process from site III to N(1).

The other minimum on the potential energy surface (AM/IM/FRM) corresponds to the end products of the reaction in which the proton is bound to the formate anion. This minimum is reached after a TS structure (AM/IM/H^+/FRM^-), which corresponds to the proton being between N(3) and site II at a N(3)-H distance of 1.376 Å (Fig. 3d). The N(3)-H internal coordinate is the major component of the eigenvector of the hessian matrix with the negative eigenvalue. The results of calculations modeling the proton transfer from N(3) to the proton acceptor site of the receptor model are given in Table 1. Clearly, inclusion of the diffuse functions on the formate group affects the energy of activation and of reaction of the proton transfer. When results obtained with the 4-31G and 4-31 + G basis set are compared, E_{act}^{PT2} is found to change from 9.30 to 12.95 kcal/mol, and E_{react}^{PT2} increases considerably, from −16.52 to −9.63 Kcal/mol. Comparison of these results with the energetics obtained by Weinstein *et al.* (see line VIII of Table 1 in Ref. 8) for HA monocation and an ammonia as proton acceptor in site II shows that the presence of the formate group at site II has a profound effect on the proton release process from N(3) to site II. The energy of activation decreases and the proton transfer becomes exothermic.

Thus, the calculations show that both proton transfers are feasible from an energetical point of view. However, the proton movement from N(3) to site II has higher energy of activation

and, therefore, it becomes the rate-limiting step to trigger the biological response. This supports the conclusion that the pharmacological activity of some partial agonists (1) may be related to the ability of the compound to undergo the proton-relay process and especially the second proton transfer from the drug to the proton-acceptor site at the receptor.

NAGHA

Molecular determinants for recognition and activation. Previous calculations of the structures and energies of the ionic and tautomeric forms of NAGHA suggested that the N(3)-H tautomer of the monocationic form is recognized by the receptor (14). The interaction of the guanidinium side chain with the anchoring negative site is suggested as the first element in the molecular recognition of NAGHA. The structural properties of the guanidinium group allow NAGHA to interact with the receptor in two different modes. The first mode, denominated bind I, is based on the assumption that NAGHA and HA have the same conformational arrangement in their interaction at the HA H_2 receptor. Fig. 4a shows NAGHA placed inside the receptor model. The imidazole moiety of NAGHA is oriented between site II and site III, similarly to the imidazole ring of HA. This recognition mode of NAGHA has been considered above in the characterization of site I. The other mode of binding, bind II, is shown in Fig. 4b. NAGHA is recognized through the guanidinium-formate interaction and the hydrogen bond formed between the N(3) nitrogen of the imidazole ring and site II. Due to computational limitations, the semiempirical method AM-1 was used for the optimization of both systems. The structure of the receptor model and the linearity of the hydrogen bonds of the guanidinium-formate interaction alone were constrained.

The activation of the HA H_2 receptor to trigger a response is dependent on the successful proton movement from the proton-donor site (site III) to the proton-acceptor site (site II). It is, therefore, necessary to calculate the ability of NAGHA to activate the receptor through such a proton transfer. The potential energy curves for the proton transfer toward the N(1) nitrogen of the imidazole ring of NAGHA were considered for both modes of binding. The 5-methylimidazole molecule was used as a model of NAGHA, in order to perform *ab initio* calculations with the 4-31G split valence basis set. The choice of this basis set is based on the aforementioned results, which showed that inclusion of diffuse functions on the formate group does not change the general shape of the potential energy curve of the proton movement from site III to N(1). The results modeling the proton transfer are given in Table 2 and Fig. 5. The orientations of the imidazole ring in the truncated models were kept fixed at the position obtained from the optimization of NAGHA inside the receptor model (see Fig. 4). Although the models representing HA and NAGHA are composed of ammonium, 5-methylimidazole, and formate molecules, the total energies of the systems differ because of the relative orientation of the imidazole ring inside the receptor model (see Tables 1 and 2). The barrier to the movement, E_{act}^{PT1}, from site III to N(1) of NAGHA, in mode of binding bind I, is 15.8 kcal/mol, and the energy of reaction, E_{react}^{PT1}, is −34.3 kcal/mol. It was assumed, on the characterization of site I, that HA and NAGHA are recognized by the receptor with a similar orientation of the imidazole ring and side chain. Therefore, the proton transfer from site III to N(1) should have similar characteristics for both molecules (see Figs. 2 and 4a). The deviation observed in the calculated energies of activation of the proton-transfer process for HA and NAGHA is a consequence of the longer hydrogen bond distance between the nitrogens on ammonium and imidazole for NAGHA than for HA (3.26 Å versus 3.09 Å).

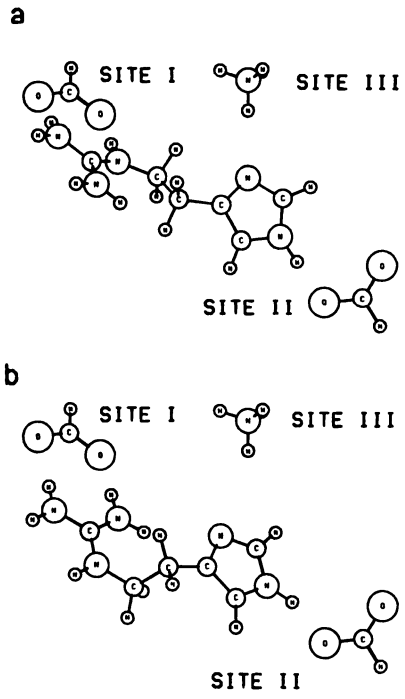

Fig. 4. NAGHA is placed in the proposed HA H_2 receptor model. The structural properties of the guanidinium group allow NAGHA to interact with the receptor in two different modes, denominated bind I (a) and bind II (b).

TABLE 2

Energy of activation and energy of reaction of the proton transfer from site III to N(1) (E_{act}^{PT1} and E_{react}^{PT1}) of the 5-methylimidazole model complex of NAGHA, calculated at the RHF/4-31G//RHF/4-31G level of theory

Mode of binding	E_{act}^{PT1}	E_{react}^{PT1}	Total energy[a]
	kcal/mol	kcal/mol	hartrees
Bind I	15.8	−34.3	−507.978933
Bind II	54.2	−56.0	−507.957768

[a] Values are for N(3)-H and H_3N-H distances of 1.0 Å.

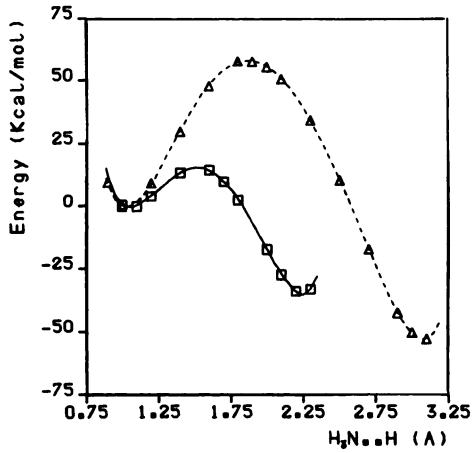

Fig. 5. Potential energy curves for proton transfer toward the N(1) nitrogen of the imidazole ring of NAGHA, calculated with the 4-31G basis set for mode of binding bind I (□) and mode of binding bind II (△).

Results in Table 2 show that the proton-transfer process, as a trigger of the biological response, is likely to occur only when NAGHA is interacting with the HA H_2 receptor in mode bind I, because of the high barrier obtained in the other mode, bind II (15.8 versus 54.2 kcal/mol). A way to rationalize this difference is by comparing the hydrogen bond distance and angle between the proton-donor site and the imidazole ring. The distances between the nitrogen of site III and N(1) are 3.26 and 5.54 Å for the modes of binding bind I and bind II, respectively. The other important difference between the two complexes is the hydrogen bond angle, which is linear in the mode of binding bind I, compared with 117.0° in the mode of binding bind II. The increase in the barrier is because of the larger distance between the two nitrogens and the nonlinear hydrogen bond of the mode bind II. Similar effects of the distance and angular dependence on the proton-transfer process have been reported previously (28).

These results support the conclusion that the pharmacological activity is obtained only when NAGHA is bound to the receptor in mode bind I, whereas, in the other mode of binding, bind II, NAGHA cannot lead to a response. Thus, the mode bind I of NAGHA will elicit properties of an agonist and mode bind II, those of an antagonist.

NAGHA as a weak antagonist. Antagonist activities were determined against HA stimulation (13), and the concentrations of HA needed to produce half-maximal responses in the absence and presence of different concentrations of antagonist were measured. The following equilibria take place

$$\begin{array}{ccc} \mathrm{NAGHA_{solv}} + \mathrm{R_{solv}} & \rightarrow & \mathrm{NAGHA\text{-}R}\ \Delta H_f^{\mathrm{NAGHA}} \\ \downarrow \Delta\Delta H_f & & \\ \mathrm{HA_{solv}} + \mathrm{R_{solv}} & \rightarrow & \mathrm{HA\text{-}R}\ \Delta H_f^{HA} \end{array}$$

where R is the HA H_2 receptor. These equilibria enhance other important elements in the simulation of the activation mechanism of the HA H_2 receptor, i.e., the stabilization of the complexes formed between the ligand molecules and the receptor model and the energy needed for solvating or desolvating the ligands. From a molecular point of view, once HA is added to the tissue it has to displace NAGHA from the receptor to give the pharmacological response. HA first has to be desolvated and NAGHA is solvated after the displacement. These values can be easily calculated with the proposed HA H_2 receptor model, and the difference of formation enthalpies between the complexes with HA and with NAGHA at mode bind II, $\Delta\Delta H_f$, can be determined theoretically as

$$\begin{aligned} \Delta\Delta H_f &= \Delta H_f^{\mathrm{HA}} - \Delta H_f^{\mathrm{NAGHA}} \\ &= E_{\mathrm{int}}{}^{\mathrm{HA}} - E_{\mathrm{solv}}{}^{\mathrm{HA}} - (E_{\mathrm{int}}{}^{\mathrm{NAGHA}} - E_{\mathrm{solv}}{}^{\mathrm{NAGHA}}) \end{aligned}$$

Table 3 shows E_{int} and E_{solv} for HA and NAGHA needed for the calculations. The system models used are shown in Figs. 2 and 4b. Table 3 shows the obtained value of $\Delta\Delta H_f$ for NAGHA compared with HA. The value of −1.39 kcal/mol indicates the ease of HA in displacing NAGHA from the receptor cavity simulated by our HA H_2 receptor model. This is interpreted as the reason why NAGHA acts as a weak antagonist at the HA H_2 receptor.

NAGHA as a partial agonist. NAGHA would elicit properties of a full agonist only in the mode of binding bind I, because in the proposed model the proton-relay process at the receptor that triggers the biological response can occur. A model of drug action was developed (29) for a drug that can bind in two modes to a given receptor, an agonist mode that elicits a response and an antagonist mode that does not. It was shown that the drug can lead to agonist, partial agonist, or antagonist behavior as a function of the relative affinities in each mode; a drug with much larger affinity in the agonist mode is a full agonist, a drug with much greater affinity in the antagonist mode is an antagonist, and a drug with comparable affinities in each mode is a partial agonist. The maximum fractional response of the system, R_{max}, is given by

$$R_{\mathrm{max}} = \frac{1}{1 + \dfrac{K_f^{\mathrm{bindII}}}{K_f^{\mathrm{bindI}}}}$$

where K_f^{bindI} and K_f^{bindII} are the formation constants of the drug-receptor complex for the drug in the agonist mode and in the antagonist mode, respectively. The formation constant, K_f, can be related to the formation entalphy of the reaction, ΔH_f, by the known equation

$$K_f = \frac{Q_{\mathrm{NAGHA\text{-}R}}}{Q_{\mathrm{NAGHA}} Q_{\mathrm{R}}} e^{\frac{-\Delta H_f}{\kappa T}}$$

where Q is the partition function, κ is the Boltzman constant, and T is the temperature. It is reasonable to assume that the partition functions of the NAGHA-receptor complexes in modes bind I and bind II will be similar. Thus, the relative ratio between the formation constants of NAGHA in modes bind I and bind II with the receptor will depend only on the difference of formation enthalpies, $\Delta\Delta H_f$. Therefore, the maximum fractional response is given by

$$R_{\mathrm{max}} = \frac{1}{1 + e^{\frac{-\Delta\Delta H_f}{\kappa T}}}$$

where $\Delta\Delta H_f$ is defined as

$$\Delta\Delta H_f = \Delta H_f^{\mathrm{bindII}} - \Delta H_f^{\mathrm{bindI}}$$

This can be evaluated from the difference in total energy

TABLE 3

E_{int} (energies of interaction), E_{solv} (energies of solvation), and $\Delta\Delta H_f$ (the difference in formation entalphy between two drug-receptor complexes) for HA and NAGHA

Ligand	E_{int}	E_{solv}	Total energy	
			Complex[a]	Ligand[a]
	kcal/mol	kcal/mol	hartrees	
HA	−116.60	−62.0	−0.10595	0.28433
NAGHA		−55.6		0.30346
Bind I	−109.26		−0.07513	
Bind II	−108.77		−0.07435	
	HA-NAGHA$^{\mathrm{bindII}}$		NAGHA$^{\mathrm{bindI}}$ − NAGHA$^{\mathrm{bindII}}$	
	kcal/mol		kcal/mol	
$\Delta\Delta H_f$	−1.39[b]		0.49[c]	

[a] Total energies of the ligand-receptor complex and the optimized isolated ligands calculated with the semiempirical method AM-1 (see Figs. 2 and 4). The energy of the HA H_2 receptor model is −0.204467 hartrees.

[b] Difference of formation enthalpy between the complex of the receptor with HA and with NAGHA, at mode of binding bind II, calculated as

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

[c] Difference of formation enthalpy between the complex of the receptor with NAGHA, at modes of binding bind I and bind II, defined as

$$\Delta\Delta H_f = \Delta H_f^{\mathrm{bindII}} - \Delta H_f^{\mathrm{bindI}}$$

between NAGHA bound to the receptor in modes bind I and bind II. Results in Table 3 show the total energies calculated for the complex of NAGHA, in both modes of binding, with the receptor model, using the AM-1 hamiltonian function. The geometries used for the calculation are shown in Fig. 4. In view of the results obtained, the attachment of NAGHA is likely to occur in both modes of binding, because both energies of interaction are similar, $\Delta\Delta H_f = 0.49$ kcal/mol (Table 3), which gives similar affinities for the receptor. The obtained theoretical value of R_{max} is 69% at a temperature of 37°. This value comes very close to the experimental estimates of R_{max}, 50–60% (13).

It can be concluded that the partial agonism of NAGHA is related to the ability of the molecule to interact with the HA H_2 receptor in agonist and antagonist modes of similar affinity.

Conclusions

The characterization of the different sites at the HA H_2 receptor has been determined by the molecular structures of two HA-related compounds, dimaprit and NAGHA. The complete model system proposed for the activation stage of the HA H_2 receptor is composed of the molecules of formate (anchoring site I), formate (proton acceptor, site II), and ammonium (proton donor, site III). Based on this model, a glutamate or aspartate residue is proposed as the HA cation anchoring to a negative receptor site and as a proton-acceptor site, and a lysine, arginine, or histidine residue is proposed as a proton-donor site.

Recently, the gene that encodes the HA H_2 receptor was cloned (30). Computer analysis of the amino acid sequence revealed extensive homology to other known GTP-binding protein-coupled receptors. Sequence alignments of the third transmembrane domain of the HA H_2 receptor show that Asp-98 is conserved among all the receptors that bind protonated amine ligands, including all the subtypes of the α- and β-adrenergic, dopaminergic, muscarinic, and serotonergic receptors (30). Therefore, it can be suggested that Asp-98 of the HA H_2 receptor is the proposed negative region at the receptor (site I) at which HA anchors its side chain. Gantz *et al.* (30) noted the absence of the two serine residues present in the fifth transmembrane region of the adrenergic and dopaminergic receptors. These serine residues are suggested to be sites of hydrogen bonding to the hydroxyl groups present in the catechol ring of adrenergic agonists (31). A nonconserved Asp-186 is found in helix 5 of the HA H_2 receptor at the position of the serine residue (30). This residue could be the proposed proton-acceptor site (site II) at which the N(3)-H of the imidazole ring of HA is hydrogen bonded. Further characterization of the chemical nature of the receptor sites in the authentic receptor can be used in subsequent studies to generate a three-dimensional macromolecular receptor model. In order to model receptor activation, it will be necessary to include the rest of the receptor structure, because the conformational changes induced in the macromolecule by the interaction of the recognition site with agonists are likely to be responsible for the propagation of the binding signal into a receptor-effector coupling that finally leads to the biological response (32).

The calculations associated with the proton movement from the proton-donor site (site III) to the proton-acceptor site (site II), proposed for the activation process at the HA H_2 receptor, reveal a sequential mechanism in the potential energy surface. Analysis of the energetics of such an activation mechanism, presented above, shows that proton transfers both from site III to the N(1) nitrogen of HA and from the N(3) nitrogen of HA to site II are feasible. The energies of activation and reaction corresponding to the second proton transfer are significantly higher. However, to obtain a realistic description of the energetics of the molecular mechanism of drug action, it becomes necessary to evaluate the energetics of the process in the electrostatic field generated by the various components of the polypeptide structure and water and to include these effects and the polarization of the macromolecule in the quantum mechanical descriptions.

Another important point of the present study is that the same HA H_2 receptor model has been used to elucidate the molecular determinants of molecules that act as full agonists, partial agonists, and antagonists. The partial agonism of NAGHA is related to the ability of the drug to bind with the receptor in agonist and antagonist modes of similar affinity. The present work also supports the hypothesis that the antagonists of the HA H_2 receptor act, at the same receptor sites as agonists, by blocking the access of agonist to trigger the proton-relay mechanism that causes the specific molecular change in the receptor that leads to the biological response. Our goal has been to explain the pharmacological properties of these compounds in terms of theoretical thermodynamic properties, calculated with the proposed receptor model. These molecular determinants of action, together with the receptor model, also provide a basis for a rational approach to the design of compounds with a given pharmacological activity.

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