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On the Mechanism of Histamine H2 Receptor Activation

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SUMMARY

The H2 receptor activation mechanism model proposed by Weinstein *et al.* [*Mol. Pharmacol.* 12:738–745 (1976)] has been considered for the set of H2 agonists. The energetics of the model proton transfer processes from a proton-donor site to the heterocycle ring of the agonist molecule and from this one to a proton acceptor site have been studied by using the semiempirical AM1 and MNDO quantum mechanical methods. The STO-3G *ab initio* molecular electrostatic potential for each compound in its active form has also been computed. Results show the mechanistic model to be satisfactory and lead to a qualitative and quantitative explanation of the H2 activity data.

It is well known that the pharmacological effects of histamine are mediated by two different receptors denoted as H1 and H2, first reported by Ash and Schild (1) and Black et al. (2), respectively. H1 receptors are responsible for smooth muscle contractions, for a strong depressive action and for some clinical patterns of allergic reactions. H2 receptors are involved in the stimulation of gastric acid secretion, inhibition of rat uterus contractions and stimulation of the spontaneous beating rate of the guinea pig right atrium.

In previous works Weinstein *et al.* (3, 4) suggested a possible mechanism model for activation of the H2-receptor. According to this model, the monocationic histamine approaches the receptor in its tautomeric form N3-H (Fig. 1). The protonated amine side chain is assumed to interact in an electrostatic way with a negatively charged group on the receptor. The interaction between N1 and a proton-donor site and of N3 with a proton-acceptor site provide the additional points of binding proposed in this model. Because of the neutralization of the cationic head of histamine, the electronic charge distribution is modified. This is reflected by a change in the tautomeric preference, the N1-H form being favored energetically under these conditions. Therefore, N1 should attract a proton from a proton-donor site, and N3 should also transfer a proton to a proton-acceptor one. In this way, this proton-relay system could trigger the biological response.

To test the validity of such a model, Weinstein *et al.* (5) conducted a theoretical study of the energetics involved in this proton-transfer process, using a receptor model and the methodology provided by modern quantum chemistry. This work dealt only with histamine and used a receptor model in that the negative group of the receptor was represented by an OH^- anion which binds the monocationic head of histamine. The proton donor and acceptor sites were simulated by the molecules of ammonium and ammonia, respectively. On the basis of energetic considerations about the proton-relay described above (obtained from *ab initio* model calculations), it was concluded that this activation process is energetically feasible, even though they emphasize the importance of the nature of the proton acceptor as well as the influence of the environment in the energetics.

Obviously, the acceptance of the validity of such a process as a possible H2 receptor activation mechanism considerably depends on the capability of that model to discriminate the different biological behaviors of agonists, partial agonists and certain antagonists and also on the possibility to use it to explain the range of activity of a set of agonists or antagonists.

According to the preceding discussion, a systematic study of the interactions arising from the use of that model has been performed for a series of agonists of histamine. The aim of the present study is then to show that even at the semiempirical level, the H2 receptor activation mechanism within the framework of Weinstein's model permits a quantitative classification of the family of agonists studied here. Such a study appears necessary before continuing with more sophisticated quantum chemical approaches.

The obtained results are then compared with available activity data and with typical structure-activity parameters such as the Molecular Electrostatic Potential, thus providing an explanation of the activity differences and also for the lack of a direct relationship between activity and MEP values (5a).

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ABBREVIATIONS: MEP, molecular electrostatic potential; MNDO, modified neglect of diatomic overlap; AM1, Austin model 1, SCF, self-consistent field.

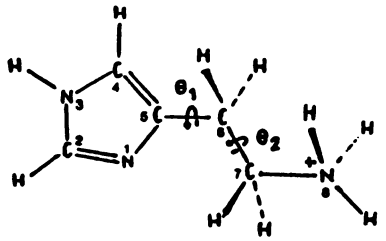

Fig. 1. H2 essential conformation (θ_1 = 180°, θ_2 = 180°) of the N3-H tautomeric form of monocationic histamine.

Modeling the Proton Transfer System

Experimental and theoretical considerations, at the semiempirical CNDO/2 level, suggest that histamine interacts with the receptor in its monocationic form as the N3-H tautomer (1, 6) and with a conformation (7) defined by $\theta_1 = 180°$, $\theta_2 = 180°$ (Fig. 1).

To study the proton-relay processes, each agonist compound in the active form indicated above is placed between the molecules of ammonium (as proton-donor site), ammonia (as proton acceptor site), and the OH^- anion, which neutralizes the positive charged ethylamine side chain nitrogen (see Fig. 1 in Ref. 5). The distances reported in Ref. 5 were always kept frozen in subsequent calculations.

Computational Methods

To determine the driving force and the energy barrier corresponding to the proton transfer processes between the heterocycle ring and the proton donor and acceptor sites for these H2-agonists, the MNDO and AM1 (8, 9) semiempirical all-valence electron molecular orbital methods of Dewar and co-workers were used (8, 9). These MNDO and AM1 calculations were performed with standard parameters using a locally modified version of the IBM/CMS MOPAC program package that includes the AM1 hamiltonian (10).[2] The use of these two different methods is justified since AM1 better describes the hydrogen bonds (9), whereas the performance of MNDO is better known. The choice of these computational methods stems from the fact that the main interest of this work lies in obtaining a relative value of activation and stabilization energy to establish a possible relationship with regard to the H2 activity of these compounds, rather than more accurate absolute values that will require the use of large basis sets and probably the inclusion of some electronic correlation effects because H bonds are formed and broken in the process.

A comparison between the proton transfer energetics for histamine obtained by using the AM1 molecular orbital method with and without geometry optimization of the portion ammonium/imidazol ring/ammonia has been performed at each point of the proton path from ammonium to N1 and in a second step from N3 to ammonia (the proton movement will be considered sequential in all cases, as reported in Ref. 5). As will be shown, the results make possible the study of this receptor activation mechanism at this level of approximation and with this construction of the receptor model without considering the expensive geometry optimization procedures.

Potential energy curves for the movement of the proton from the donor binding site to N1 and from N3 to the acceptor binding site were computed, at this level of the theory, by different SCF calculations at several selected points of the reaction coordinate. Once the potential energy curves were obtained, a fitting of the calculated values to fourth- or fifth-order polynomials was conducted to make a precise determination of both energy barrier and energy stabilization. The fitting was always performed with $r > 0.99$ and statistical significance. The polynomial regression was made by using the BMDP statistical program package (11).

Because it may be argued that the SCF approach may not be reliable along the entire potential energy curve, some points must be stressed. First, this is the approach used by Weinstein *et al.* (5) to test a possible mechanism model for the H2 receptor activity of histamine that this study tries to extend to a series of agonists. Yet the semiempirical methods used in this work were parametrized with respect to experimental values and are believed to include, through this parametrization, part of the correlation effects. I note again that the main purpose of the present work is to test the validity of Weinstein's model for a series of H2 receptor histamine agonists rather than to obtain accurate values for the energies involved in that mechanistic model.

Finally, the STO-3G *ab initio* molecular electrostatic potentials (12) for the N3-H tautomer of monocationic and neutral species in the conformation $\theta_1 = 180°$, $\theta_2 = 180°$ have been computed for these molecules to consider their relationship with the corresponding energy profile characteristics of the proton transfer from ammonium to the heterocycle ring and with the H2 activity data.

Although the use of different basis set would lead to different results, the MEP values are used only for comparative purposes, and hence only relative values are needed.

On the Geometry Optimization for Proton Transfer Process

Potential energy curves for the proton movement from ammonium to N1 and from N3 to ammonia for histamine placed at the receptor model were computed with the aim to understand the effect of geometry optimization on the characteristics of the energetics in such processes. Within the present computational framework this comparison was conducted by the AM1 molecular orbital computational method. First, the proton was released without geometry optimization and using crystallographic data (13) for histamine. In a second step, the transfer was made optimizing the ring geometry parameters at each position of the reaction path. With regard to this latter case, the ammonium/imidazole ring/ammonia portion was fully optimized with the only restrictions of the ring planarity and of N1-ammonium N and N3-ammonia N distances, whose corresponding values were kept frozen at 3.07 and 2.90 Å, respectively, as indicated in Ref. 5. The optimization of the starting histamine-simulated receptor geometry was also performed by using AM1, and the resulting distance between ammonium N and ammonia N was 7.71 Å, in good agreement with the one obtained by Weinstein *et al.* (5), which was of 7.91 Å. Potential energy curves for the two proton transfer processes were then calculated with (Figs. 2a and 3a) and without (Figs. 2b and 3b) geometry optimization.

From the results plotted in Fig. 2 for the first proton transfer process with and without performing the ring geometry optimizations, it is seen that both curves are practically parallel, as evidenced by the energy differences at each point of the reaction coordinate that lies in the 0.33–0.38 eV interval. For the second transfer (Figs. 3), the similarity between the two curves is only partial. The differences between the curves are in the range from 0.32 to 0.50 eV, the maximum difference corresponding to the situation in which the proton movement has been completed, i.e. the proton is now bonded to ammonia. The stabilization energy for the second proton transfer will thus be largely underestimated in the case of the curve obtained using crystallographic coordinates. Nevertheless, if we are only concerned with the part of the curve comprised between a coordinate path value of about 1.0 Å, at which the proton is bonded to N3, and of about 1.5 Å, corresponding to the point at which the activation energy is reached, the range of energy differences lies in the 0.32–0.40 eV interval. Therefore, a procedure that does not

[2] S. Olivella and J. M. Bofill, unpublished data.

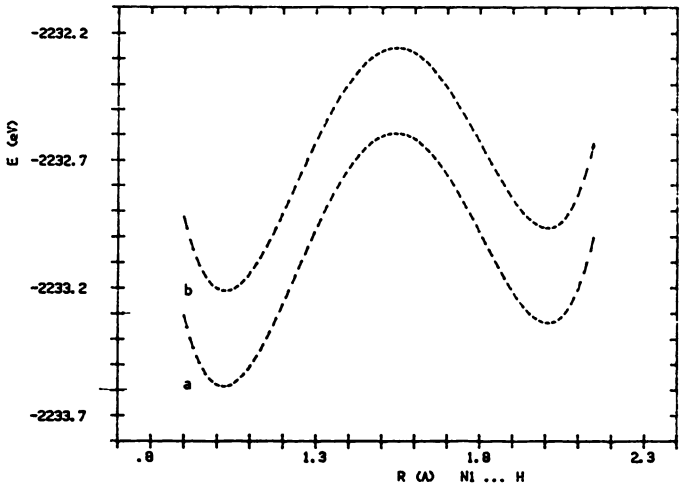

Fig. 2. Potential energy curves for the proton transfer process from ammonium to the imidazol ring (N1) obtained by using the AM1 method (*a*) with optimization of the ring geometrical parameters at each point of the reaction path and (*b*) without geometry optimization.

include ring geometry optimization at each point of the reaction path can be used if only activation energies have to be obtained (see Table 1). From the computational point of view, even at the semiempirical level, this fact is particularly interesting when the two proton transfer processes have to be studied for a series of molecules.

From this part of the present study, it can be concluded that to test the validity of Weinstein's mechanistic model of H2 agonist compounds within the semiempirical approaches used along this work it is enough to compute the proton transfer potential energy surfaces from crystallographic data without being forced to consider the effect of geometry optimization. This is possible since the methodology used seems to determine the energy barriers mostly from local effects. Whether this is an artifact of the semiempirical methods cannot be deduced from the present calculations since although the *ab initio* calculations of Topiol *et al.* (14) show differences between the optimized and unoptimized proton transfer paths their model system is not as rigid as the present one that contains an heterocycle ring.

MNDO versus AM1 Activation and Stabilization Energies

The activation and stabilization energies computed for each agonist of the MNDO and AM1 levels for both proton transfer processes are reported in Table 2.

Before discussing these energies, it is useful to compare our results for histamine with those arising from STO-3G *ab initio* calculations and reported in Ref. 5 (see Table 3). Concerning the activation energies, the AM1 results are closer to the *ab initio* ones than the MNDO values, as should be expected (10); a rather large difference appears in the case of the stabilization energies, and at least in part this could be explained as an effect of the differences in geometry used in our calculations (geometry obtained from crystallographic data) with regard to those in Ref. 5 (geometry optimized at the STO-3G level). Nevertheless, the interest of the present work lies more in the relative energies for a series of molecules than in the absolute values for a given agonist. In this context, it will be shown that AM1 and MNDO lead to the same conclusions.

For the series of agonists studied here, the analysis of the AM1 results indicates that a linear relationship between the activation and stabilization energies exists for the two proton transfer processes ($r > 0.99$ in both cases). Identical relationships are obtained when dealing with the MNDO method. Likewise, from the comparison of the MNDO and AM1 values for the activation and the stabilization energies, linear relationships appear with $r > 0.93$ for the first proton transfer and $r > 0.98$ for the second one, respectively.

Thus, although better activation energies could be expected from the use of AM1 owing to its increased ability to represent hydrogen bonds, the MNDO method can also be used if the main interest lies on the comparison of these energies for a series of analogue compounds.

Indeed, for the particular pharmacological case considered in this paper, this kind of analysis can also be conducted by using the stabilization energy data for the first and second proton transfer processes. However, for this latter case the calculation of the stabilization energy without geometry optimization is erroneous, as stated previously, but the error is nearly the same

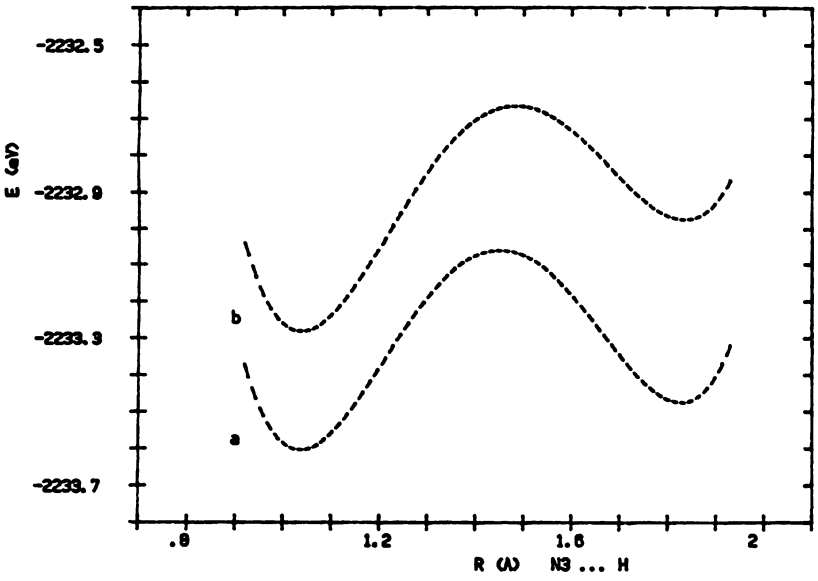

Fig. 3. Potential energy curves for the proton transfer process from the imidazole ring (N3) to ammonia obtained by using the AM1 method (*a*) with optimization of the ring geometrical parameters at each point of the reaction path and (*b*) without geometry optimization.

TABLE 1

Activation and stabilization energies for the proton transfer process from ammonium to the imidazol ring (N1) of histamine and from the imidazol ring (N3) to ammonia

Values obtained by optimization of the ring geometric parameters or by using crystalographic data.

Geometry	Proton Transfer			
	NH_4^+ to N1		N3 to NH_3	
	Activation	Stabilization	Activation	Stabilization
	kcal mol			
Optimized	17.11	−5.75	12.52	2.93
Crystallographic	16.35	−5.66	14.24	7.08

for all these H2 agonists, as shown by the relationships indicated above.

In the view of the preceding discussion only the AM1 activation energies will be used to study the validity of this H2 receptor activation mechanism model.

Comparative Analysis between *ab initio* Molecular Electrostatic Potential and the AM1 Activation Energy

It is well known that the molecular electrostatic potential (12) provides a measure of the interaction energy between a positive charge at a given space point and the molecule considered as a whole, since the molecular electrostatic potential (MEP) considers the repulsive effect of the nuclei together with the attractive one of the electronic charge density. Thus, a relationship between the MEP minima and the activation energies for the first proton transfer process will indicate what kind of interaction the MEP accounts for.

The *ab initio* (STO-3G) MEP values have been computed for each H2 agonist, in its active conformational and tautomeric form previously described for the monocationic species as well as for the neutral one. The two forms are considered since the first step in the H2 receptor activation model is the interaction between the side chain protonated amine and a negatively charged group at the receptor. However, the electronic charge distribution derived from the neutralization is in good agreement with that corresponding to the neutral species, as reported for the histamine in Ref. 3, and hence it just represents the molecular situation before the first proton transfer.

The MEP calculations at this methodological level for seven agonists of the total compounds studied in this work has been reported recently.[1] All show an MEP minimum near the ring heteroatom corresponding to the pyridine-like N of the histamine imidazole ring, mainly due to its own electron lone pair. With regard to the neutral forms, the MEP maps also present a common pattern for all molecules. In this case, an additional minimum for each compound appears near the side chain amine nitrogen, but this need not be considered because it is artificial in nature, arising from a simulation of the neutralized molecule by the use of the neutral one.

A comparison between the MEP minimum values for the monocationic and neutral species of each molecule (see Table 4) clearly reveals that the MEP minimum is strongly affected by the neutralization of the cationic head by an anion (OH^-). Consequently, the proton affinity is notably increased in that region of space, and this facilitates the molecular attraction of the proton from the proton-donor site. Although the MEP values reported here have been obtained by using a minimal STO-3G basis set and hence cannot be considered absolute accurate values, note that the increment in the MEP minimum value for the neutral form compared with the cationic one is practically constant for all agonists and for the histamine itself, being −78.1 ± 1.6 kcal/mol, except for 2-thiazolylethylamine, which presents an increase of −85.8 kcal/mol. This greater variation would be of significance since 4-nitrohistamine, having a lower activity than 2-thiazolylethylamine, presents an MEP minimum in the monocationic form that is deeper than the 2-thiazolyletylamine one, but this situation is reversed in the neutral forms, in correspondence with the activity data.

Concerning the AM1 activation energies from ammonium to N1 for these compounds, note the existence of a linear relationship ($r > 0.93$) between those energies and the *ab initio* STO-3G MEP minima in the neutral form (as well as in the monocationic one). This fact means that the activation energy for such a proton transfer is directly determined by the molec-

TABLE 2

Activation and stabilization energies for the proton movement from ammonium to the heterocycle ring and from the heterocycle ring to ammonia

Values computed by using AM1 and MNDO methods.

Compound	N	H2 activity	AM1				MNDO			
			NH_4^+ to ring		Ring to NH_3		NH_4^+ to ring		Ring to NH_3	
			Activation	Stabilization	Activation	Stabilization	Activation	Stabilization	Activation	Stabilization
			kcal/mol							
Histamine	I	100[a]	16.35	−5.66	14.24	7.08	25.71	−4.12	19.14	6.59
4-Nitrohistamine	II	0.6[a]	20.16	2.31	8.45	−3.77	29.93	4.61	12.42	−6.37
4-Chlorohistamine	III	11[a]	17.94	−2.36	11.98	3.00	27.26	−0.99	16.85	2.41
4-Methylhistamine	IV	43[a]	15.88	−6.85	15.29	8.72	25.39	−4.95	19.68	7.54
N8-Methylhistamine	V	74[a]	15.85	−6.67	14.89	8.18	25.26	−5.01	19.77	7.89
1,2,4-Triazol-3-ylethylamine	VI	6.8[a]	18.02	−1.94	7.47	−5.33	27.94	0.88	11.98	−6.27
2-Thiazolylethylamine	VII	2.2[a]					28.50	0.87	[c]	[c]
2-Methylhistamine	VIII	4.4[a]	15.43	−8.12	15.64	9.20	25.48	−5.11	19.82	7.45
N8,N′8-Dimethylhistamine	IX	51[a]	15.61	−7.18	15.25	8.80	25.03	−5.47	20.14	8.38
5-Methyl-1,2,4-triazol-3-ylethylamine	X	0.1[b]	17.00	−4.48	8.70	−3.13	27.55	−0.41	12.66	−5.41
5-Amino-1,2,4-triazol-3-ylethylamine	XI	16[b]	15.18	−8.69	11.01	1.19	25.35	−5.43	15.47	−0.10

[a] H2 activity determined on guinea pig right atrium (from Ref. 6).
[b] From Ref. 15.
[c] Proton transfer from the ring to ammonia for 2-thiazolylethylamine corresponds to a repulsive potential energy curve.

TABLE 3

Activation and stabilization energies for the proton transfer from ammonium to imidazol ring (N1) of histamine and from imidazol ring (N3) to ammonia

Values obtained by using AM1, MNDO, and STO-3G minimal basis *ab initio* methods.

	Proton Transfer			
	NH_4^+ to N1		N3 to NH_3	
	Activation	Stabilization	Activation	Stabilization
	kcal mol			
AM1[a]	16.3	−5.7	14.2	7.1
MNDO[a]	25.7	−4.1	19.1	6.6
STO-3G[b]	12.1	−18.7	16.2	14.8

[a] Geometry from crystallographic data.
[b] Geometry optimized at the STO-3G level (5).

TABLE 4

MEP values in the region near the ring heteroatom at which the proton will be transferred from ammonium for each molecule in its monocationic and neutral forms

Compound	N	H2 activity	Monocationic	Neutral
			kcal/mol	
Histamine[c]	I	100[a]	−22.04	−101.37
4-Nitrohistamine[c]	II	0.6[a]	−7.55	−86.36
4-Chlorohistamine[c]	III	11[a]	−12.84	−91.60
4-Methylhistamine[c]	IV	43[a]	−24.38	−103.01
N8-Methylhistamine[c]	V	74[a]	−23.82	−101.71
1,2,4-Triazol-3-ylethylamine[c]	VI	6.8[a]	−13.14	−92.84
2-Thiazolylethylamine[c]	VII	2.2[a]	−3.89	−89.68
2-Methylhistamine	VIII	4.4[a]	−25.79	−103.92
N8,N′8-Dimethylhistamine	IX	51[a]	−25.69	−102.26
5-Methyl-1,2,4-triazol-3-ylethylamine	X	<0.1[b]	−17.25	−96.15
5-Amino-1,2,4-triazol-3-ylethylamine	XI	16[b]	−22.41	−101.41

[a] H2 activity determined on guinea pig right atrium (6).
[b] From Ref. 15.
[c] From Ref. 5a.

ular proton affinity in the space region involved in the hydrogen bond between the histamine ring N1 (or its equivalent heteroatom for each agonist) and the ammonium molecule.

Considerations on the H2 Receptor Activation Mechanism

Recently (5a), the possible existence of a threshold value of the MEP minimum generated by the molecule in a region near the ring proton-acceptor heteroatom, according to which the agonists could be classified as strongly or weakly active, was suggested. This value would represent the critical proton affinity of the molecule in that region necessary to pick up with effectiveness the proton from the proton-donor subsite on the receptor. Results reported in this study, including some new compounds with respect to Ref. 6, add further arguments in favor of this hypothesis, although the explanation of the agonist activities based only in the MEP minima values is incomplete. Thus, there are two molecules, 2-methylhistamine and 5-amino-1,2,4-triazol-3-ylethylamine, with MEP minimum values higher than the threshold MEP minimum but with activities that are too low. Indeed, although this threshold value seems to discriminate between strong or weak agonists, it does not explain the activity variation among the strong agonists or among the weak ones.

However, a careful analysis of the AM1 activation energy data (see Table 2 and Fig. 4) for the two proton transfer processes makes it easy to understand the H2 agonist activity

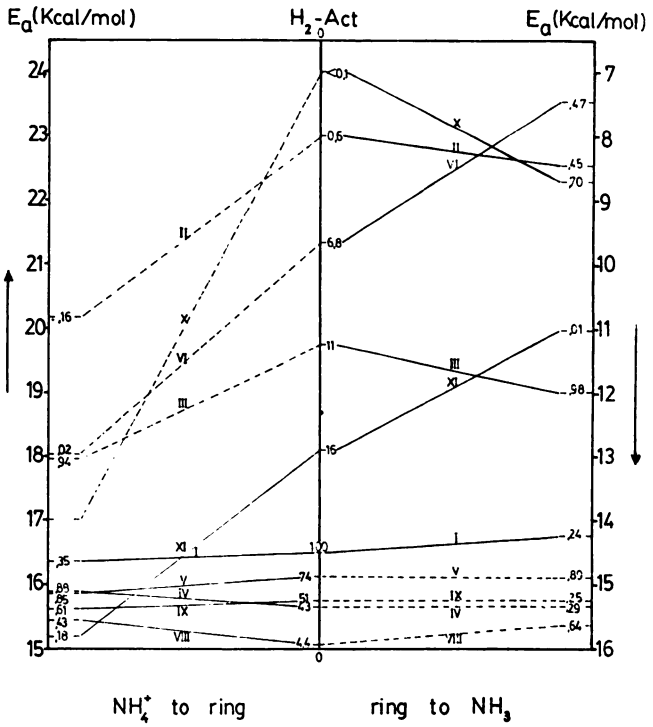

Fig. 4. Representation of the AM1 activation energy for the two proton transfer processes for each molecule studied. Values are given in kcal/mol. Those agonists having activation energy (E_a) lower or higher than that of histamine for the first or second proton transfer processes are represented by solid or *dashed lines*, respectively. The activity scale is in arbitrary units, whereas the activation energy scales are in inverse order to each other.

for most studied compounds. With respect to the first proton movement, histamine presents an intermediate position within all the molecules. 4-methylhistamine, N8-methylhistamine, NB,N′8-dimethylhistamine, and 2-methylhistamine have an energy barrier lower than that of the histamine decreasing in this order. However, 5-methyl-1,2,4-triazol-3-ylethylamine, 4-chlorohistamine, 1,2,4-triazol-3-ylethylamine, and 4-nitrohistamine have an activation energy higher than for histamine, increasing in this sense. With regard to the second proton transfer process, histamine is again placed in a middle position, its energy barrier showing little difference (approximately 2 kcal/mol) from that of the first proton transfer process. In this case it must be pointed out that compounds having a higher or lower activation barrier relative to the energy barrier of histamine are reversed with respect to the first proton movement. Thus, from Fig. 4 it can be seen that N3-methylhistamine, N8,N′8-dimethylhistamine, 4-methylhistamine and 2-methylhistamine are placed above histamine and having energy barriers increasing in this order, while 4-chlorohistamine, 5-methyl-1,2,4-triazol-3-ylethylamine, 4-nitrohistamine, and 1,2,4-triazol-3-ylethylamine are placed below histamine and also with this decreasing order of energy barriers. The only exception to this rearrangement is 5-amino-1,2,4-triazol-3-ylethylamine, which has an energy barrier for the two proton transfer processes lower than the ones corresponding to histamine. Thus, those compounds having the proton attraction process in a way easier than histamine have more difficulty than histamine to conduct the proton donation process, and conversely those compounds with a lower ability to attract the proton from a proton-donor present an easier proton donation.

The above considerations permit us to obtain not only a qualitative explanation of the H2-activity for the major part of these agonists but also to give a quantitative description of that biological process.

Thus, for 4-methylhistamine, N8-methylhistamine, N3,N′8-dimethylhistamine, and 2-methylhistamine, whose first proton transfer is energetically more feasible than for histamine, the release of the second proton is more difficult than for histamine and determines their H2-activity. In fact, the energy barriers for this second release are in good agreement with the H2-activity of each compound. So, a very good correspondence, even from the quantitative viewpoint, between the activation energies and H2-activities appears, although the activity of 2-methyl-histamine appears too low with regard to its activation energies.

Likewise, the H2-activity of 4-chlorohistamine, 1,2,4-triazol-3-ylethylamine, and 4-nitrohistamine, which release the second proton with energy barriers lower than the one corresponding to histamine, will be determined from the energy barrier of the first proton transfer. Again the existence of a good agreement between their activation energies and H2 activities can be verified.

This particular scheme, giving a satisfactory explanation for the H2-activity of these compounds, has a simple, direct justification from the viewpoint of the chemical intuition.

As the first step in this activation mechanism model is the neutralization of the agonist molecule in its monocationic form by a negative group on the receptor, the molecular electronic density will be modified and the proton affinity in the region close to the ring proton-acceptor heteroatom considerably increased. Then, those agonists containing electron-donating substituents attached to the heterocycle, i.e., 4-methylhistamine, 2-methylhistamine, or to the side chain ethylamine nitrogen, i.e., N8-methylhistamine, *N*8,*N*′8-dimethylhistamine, will be able to perform the proton transfer from the ammonium molecule in an easier way than histamine, because these substituents contribute to increase the electronic density on the ring or partially neutralize the positive charge on the ethylamine nitrogen, thus leading to a similar effect. For the same reasons, the second proton transfer for these compounds is more difficult with respect to the histamine, since the protonated-ring histamine molecule is less stabilized than the one of those molecules having electron-donating substituents stabilizing the positive charge on the ring. The second proton release from the ring is then more feasible for histamine than for these compounds, and it will be more difficult when the positive charge on the ring is more stabilized.

In turn, similar considerations can be used to understand the activity of 4-nitrohistamine, 4-chlorohistamine, and 1,2,4-triazol-3-ylethylamine. The nitro and chloro substituents are electron-withdrawing groups (nitro stronger than chloro in this sense). Likewise, although 1,2,4-triazol-3-ylethylamine has no substituent, it possesses a pyridine-like nitrogen in the position 4 of the histamine imidazol ring, which by the resonance effect diminishes the electronic density on the other ring pyridine-like nitrogen (the nitrogen that picks up the proton). In consequence, all these molecules will present the effect opposite to the one discussed above.

Despite this single explanation for the H2 activity of these molecules, two compounds: 5-amino- and 5-methyl-1,2,4-triazol-3-ylethylamine are not accommodated in this scheme. 5-amino-1,2,4-triazol-3-ylethylamine has energy barriers for the first and second proton transfer lower than those of the histamine. In consequence, both processes should be more feasible energetically and its activity higher than that of histamine, but it only presents an activity value of 16. In turn, 5-methyl-1,2,4-triazol-3-ylethylamine has an activation energy higher than the energy barrier of the histamine for the first proton movement and lower for the second one, but its activity is then too low ($P < 0.1$).

To interpret this apparent deficiency of the model note that the receptor site model at which the histamine binds is very simple and considers only those groups on the receptor directly involved in the activation mechanism. Note then that both compounds have a substituent attached to the equivalent position 2 of histamine. A possible explanation of why they do not follow the general trends predicted by the present model would be the existence of some kind of steric hindrance between the substituent bonded to that position and a region of the receptor close to it. With respect to this possibility, we previously reported that 2-methylhistamine has an activity (4.4) that is lower than the theoretical activity corresponding to its activation energy for the second proton transfer, and this fact would also be explained by the supposition of steric hindrance as an additional effect for those agonists having substituents on position 2. However, realize that apart from this limitation on the receptor model used there is also a limitation due to the approximate character of the semiempirical methodology used in this work. Accurate *ab initio* calculations will be necessary to distinguish between these two possibilities.

The only compound that does not fit the above explanation is 2-thiazolylethylamine. In that case, calculations have not been performed by AM1, because of the lack of AM1 parameters for the sulfur atom. The activation energies have been calculated for this molecule by using the MNDO method. Because the MNDO results relate to values obtained with AM1, it is possible to extrapolate the energy barriers for this compound (see Table 2) and consider it in the Discussion. The MNDO activation energy of 2-thiazolylethylamine for the first proton transfer places it between 1,2,4-triazol-3-ylethylamine and 4-nitrohistamine, in agreement with its H2-activity (2.2). Nevertheless, with regard to the second proton transfer, MNDO only predicts a repulsive potential energy curve, and hence the second proton transfer is not possible in this case, whereas the preceding analysis suggests that it is necessary. Apart from a possible drawback on the MNDO parametrization of sulfur, there is no obvious explanation for this compound in the framework of this simple model, which on the other hand gives a good and comprehensive explanation of the H2 activities of the remaining molecules at both qualitative and quantitative levels.

In view of the present results it is worth noting that it is more precise to talk about a given optimum MEP interval than about a minimum threshold value (see Footnote [1]). This fact may be understood since a deep minimum of MEP would also imply an enormous activation energy for the second proton transfer and consequently a poor H2 activity.

Conclusions

From the preceding discussion, it can be concluded that the H2 activity of an agonist depends on the modulation of the activation energy barriers for the first and second proton trans-

fer processes, which according to the Weinstein's model are responsible for the H2 biological activity.

This fact provides a criterion to anticipate the activity of a given agonist. For molecules with electron-donating substituents, the first proton transfer is facilitated although the second is more difficult with respect to the histamine; the proton transfer from the ring to the proton-acceptor is the step determining the H2 activity. Those molecules containing electron-withdrawing groups pick up the proton with more difficulty but they also release it more easily than histamine, and it is the transfer from the proton-donor subsite to the ring that modulates the H2 activity. In this context, histamine could be considered to represent the optimum for the overall relay system.

However, note that all agonists with substituents on position 2 exhibit an H2 activity lower than the one predicted by this simple model. A possible explanation for these molecules would be the existence of some kind of steric hindrance in the receptor, which is not reflected in the present receptor model.

In summary, the Weinstein H2 receptor model together with simple quantum chemical calculations permit a rather accurate description of the H2 activity.

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