

## Electrostatic medium effects upon histamine H<sub>2</sub> receptor activation

Juan S. Gómez-Jeria<sup>a,1</sup>, Julia Parrá-Mouchet<sup>a</sup> and Demian Morales-Lagos<sup>b,1</sup>

<sup>a</sup>*Universidad de Chile, Facultad de Ciencias, Departamento de Química, Casilla 653 Santiago (Chile)*

<sup>b</sup>*Universidad Austral de Chile, Facultad de Ciencias, Instituto de Química, Casilla 567 Valdivia (Chile)*

(Received 1 February 1991; in final form 7 March 1991)

### Abstract

In order to examine environmental effects, the interaction of histamine (HA) with a model of the H<sub>2</sub> receptor was analyzed using the self-consistent reaction field theory at the CNDO/2 level, in vacuo, and in the presence of a polarizable continuum medium. The main findings reported in this work are: (a) a concerted proton movement is not possible in vacuo or in the presence of a polarizable medium; (b) the most stable form in all the systems and environments analyzed is the one in which there is a proton transference from the model site to HA; (c) it seems necessary to consider other possibilities in the receptor modeling in order to sustain the HA receptor activation hypothesis.

### INTRODUCTION

Histamine (HA), in addition to its role in allergic conditions and regulating acid secretion in the stomach, is a neurotransmitter in the brain. HA seems to participate in sleep and wakefulness, neuroendocrine regulation, emesis, thermal and cardiovascular regulation, drinking and feeding behavior [1,2]. The pharmacological effects of HA are mediated by at least three receptors, denoted H<sub>1</sub>, H<sub>2</sub> and H<sub>3</sub>. Receptor H<sub>1</sub> is involved in allergic reactions and contraction of the smooth muscles, receptor H<sub>2</sub> in gastric secretion, contractions of the guinea pig atrium, and H<sub>3</sub> autoinhibits HA release in the brain.

Histamine possesses two tautomers in both the free base and cationic (protonated ethylamine side chain) forms. Experimental and theoretical results show the preference of the N3-H tautomeric form over the N1-H one when the side chain is protonated, and the inverse situation with neutral HA (see Fig. 1 for nomenclature) [3-7].

On this basis, a model for the activation of the H<sub>2</sub> receptor has been proposed by Weinstein and co-workers [5,7,8]. It is suggested that the first step corresponds to the approach of protonated HA in its N3-H tautomeric form to the H<sub>2</sub> receptor. After the neutralization of the ethylamine side chain by a

<sup>1</sup>Authors to whom correspondence should be addressed.

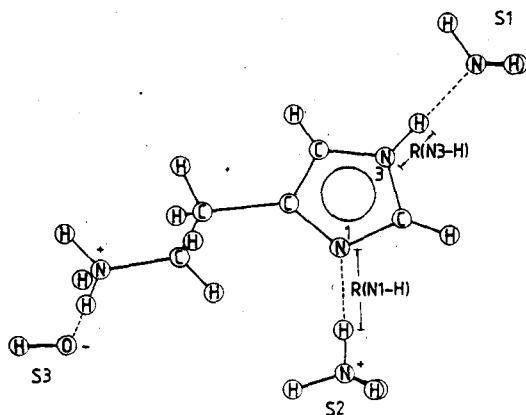


Fig. 1. Proton relay system. S1, S2 and S3 are histamine H2 receptor sites.

receptor's negative site (S3 in Fig. 1), a modification of the electronic distribution occurs. This causes the N1-H tautomer to become energetically favored. In these conditions, N1 could attract a proton from a proton-donor site (S2 in Fig. 1), and N3 could transfer another to an acceptor site (S3 in Fig. 1). It is proposed that this proton-relay system triggers the biological responses. Similar results were obtained for HA and some of its derivatives by using semiempirical quantum mechanical methods [9]. Within this model we may explain the low agonist activity of betazole not as caused by a lack of proton transfer from N3 to N1 but because of a non-optimum relative location of the proton donor and receptor sites within it [10].

An important conclusion of these works is that the environment could play a fundamental role in the energetics of this proton-relay system [7-9]. Environmental effects may be due to the chemical nature of the receptor sites or to long range electrostatic effects induced in the system by the molecular surroundings. From the work of Weinstein and co-workers [5,7,8] it is clear that the first effect may be determinant for the values of activation and stabilization energies, but no calculations have been done to analyze the influence of the last effects.

For this reason, we present here results obtained by applying the self consistent reaction field (SCRF) theory at the CNDO/2 level, including electrostatic continuum solvent effects (SCRF-CNDO/2), to the model of Weinstein and co-workers [5,7,8].

#### METHODS, MODELS AND CALCULATIONS

The electrostatic environmental effects are incorporated by using an extended version of the generalized Born formula, which also includes steric hindrance effects upon desolvation of the atomic centers of the molecular sys-

tem [11,12]. For details of the theory, we refer the reader to the literature [11-15]. Within this approach, and in the framework of the CNDO/2 methodology, the diagonal elements of the effective Fock operator  $F(\epsilon, P)$  are given by

$$[F(\epsilon, P)]_{\mu\mu} = [F(1, P)]_{\mu\mu} + \left[1 - \frac{1}{\epsilon}\right] \sum_B Q(P)_B [1 - F_{AB}] \Gamma_{AB} \quad (1)$$

where  $\epsilon$ ,  $Q_B$ ,  $F_{AB}$  and  $\Gamma_{AB}$  are, respectively, the medium's dielectric constant, the net charge of atom B, a parameter characterizing specific neighborhood effects upon solvation and the solute-solvent interaction integrals [11-15].  $F(1, P)$  is the Fock operator of the isolated solute. The non-diagonal Fock elements remain constant.

We have considered the following two situations proposed by Weinstein and co-workers [5,7,8] (see Fig. 1):

(a) One HA molecule with a protonated ethylamine side chain interacting with two sites: S1 ( $\text{NH}_3$ ) and S2 ( $\text{NH}_4^+$ ). This model will be referred to as System I.

(b) The same system as above to which we added a new site, S3 ( $\text{OH}^-$ ). This is called System II and physically corresponds to a situation in which the ethylamine side chain is neutralized.

For these two systems we calculated the whole proton transfer surface (PTS) for the processes  $\text{N3-H} \rightarrow \text{S1}$  and  $(\text{S2}) \text{NH}_3\text{-H}^+ \rightarrow \text{N1}$ . At each calculation step we consider proton displacements of 0.1 Å. This process was carried out for the isolated system (i.e. in vacuo) and in the presence of a strong polarizable medium (i.e.  $\epsilon=80$ ).

CNDO/2 methodology was used because previous studies of several H-bonded systems have shown that the shape of the proton transfer potential curves and the relative stability of these minima show similar trends to those of ab initio STO-3G calculations. This, and more recent calculations, indicate that when emphasis is placed in the qualitative trend and not in absolute magnitudes, the methodology employed here is appropriate [15-20]. Therefore, a comparative analysis in related systems, by employing standard geometries or Weinstein and co-workers [5,7,8] geometry, may be carried out without reaching the absolute minimum of the free-energy surface. It is also necessary to stress that the continuum representation of electrostatic solvent effects coupled to the CNDO/2 methodology is still a valid and useful tool if employed with the appropriate cautions [21,22].

## RESULTS AND DISCUSSION

Figures 2 and 3 display the PTS for System I in vacuo ( $\epsilon=1$ ), and in the presence of a polarizable medium ( $\epsilon=80$ ). Figures 4 and 5 show an analogous situation for System II. In Tables 1 and 2 we show the stabilization and activation energies respectively.

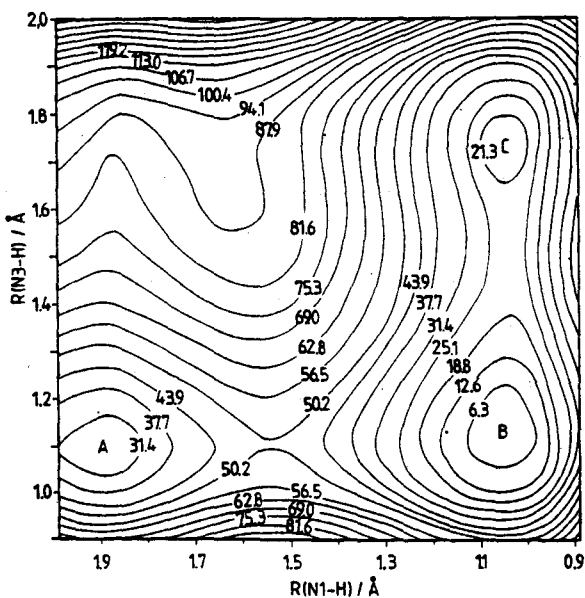


Fig. 2. Proton transfer surface for System I and  $\epsilon = 1.0$ .

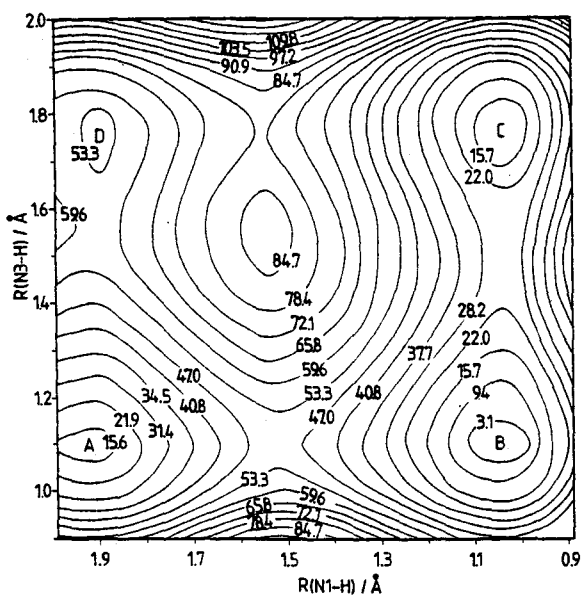


Fig. 3. Proton transfer surface for System I and  $\epsilon = 80.0$ .

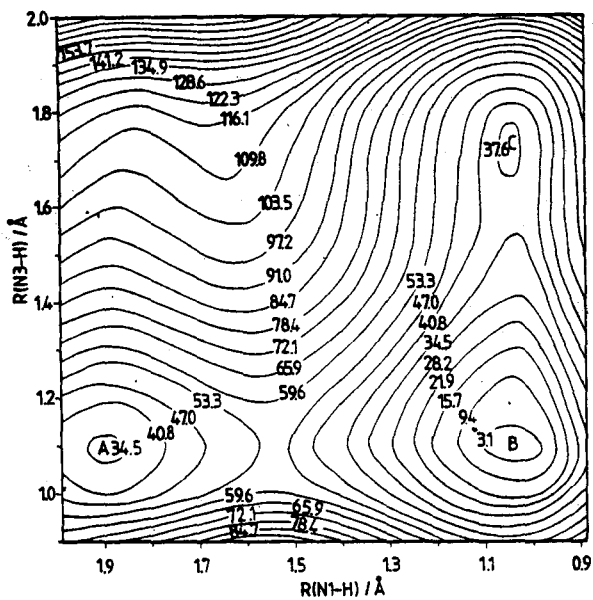


Fig. 4. Proton transfer surface for System II and  $\epsilon = 1.0$ .

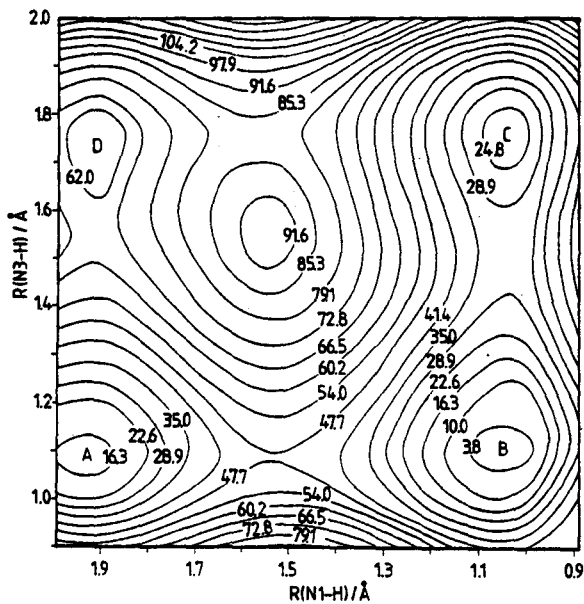


Fig. 5. Proton transfer surface for System II and  $\epsilon = 80.0$ .

TABLE 1

Stabilization energies (kcal mol<sup>-1</sup>)

Form		$\epsilon=1.0$		$\epsilon=80.0$	
		I	II	I	II
A	[NH <sub>3</sub> -HA-NH <sub>4</sub> <sup>+</sup> ]	25.73	32.63	13.18	13.80
B	[NH <sub>3</sub> -HAH <sup>+</sup> -NH <sub>3</sub> ]	0.0	0.0	0.0	0.0
C	[NH <sub>4</sub> <sup>+</sup> -AH-NH <sub>3</sub> ]	18.83	36.40	14.43	23.20
D	[NH <sub>4</sub> <sup>+</sup> -A-NH <sub>4</sub> <sup>+</sup> ]	-	-	51.46	60.90

TABLE 2

Activation energies (kcal mol<sup>-1</sup>)

System	$\epsilon$	A→B	B→A	B→C	C→B	C→D	D→C	D→A	A→D
I	1.0	23	49	22	4	-	-	-	-
	80.0	37	50	26	11.5	65.5	28.5	4.5	43
II	1.0	22.5	55	38	1.5	-	-	-	-
	80.0	32	46	32	9	59	21	2	49

In each of the figures the starting situation is marked A and is symbolically denoted by [NH<sub>3</sub>-HA-NH<sub>4</sub><sup>+</sup>]≡[S1-HA-S2].

Also, the following stable forms are possible: [NH<sub>3</sub>-HAH<sup>+</sup>-NH<sub>3</sub>], [NH<sub>4</sub><sup>+</sup>-AH-NH<sub>3</sub>] and [NH<sub>4</sub><sup>+</sup>-A-NH<sub>4</sub><sup>+</sup>]. In the following they will be referred to as B, C and D, respectively.

In the PTS for System I in vacuo we may observe that the most stable form is the one in which N1 is protonated (B, Fig. 2), and that form D is not allowed. The order of relative stabilities is B > C > A (Table 1). The molecule arrives at the receptor in form A and it is expected to reach form C. Our calculations indicate that A needs to overcome a barrier of 23 kcal mol<sup>-1</sup> to reach form B, and that B has an activation energy of 22 kcal mol<sup>-1</sup> to transform itself into C (Table 2).

The influence of a polarizable medium upon the proton relay process in System I is shown in Fig. 3 ( $\epsilon=80$ ). Again, it is observed that form B is the most stable, but a strong stabilization of form A (about 12 kcal mol<sup>-1</sup>) is evident with respect to the situation in vacuo (Table 1). However, a destabilization of minimum C with respect to minimum A occurs. Form D is highly unstable. In relation to activation energies, it is observed that the influence of a polarizable medium is mainly an increase of the activation energy for the A→B (about 13 kcal mol<sup>-1</sup>) and C→B (about 7 kcal mol<sup>-1</sup>) steps (Table 2).

The results of the analysis of the PTS of System II can be summarized as follows:

(1) In vacuo (Fig. 4) form B is still the most stable, but forms A and C have similar stabilization energies in contrast to System I in vacuo. Form D is also absent (Table 1). Neutralization also destabilizes forms A and C over form B.

(2) With respect to the activation energies, neutralization in vacuo induces a considerable increase of the barrier for the B→C step, a moderate one for the B→A step, and a lowering of the activation energy for the C→B step (Table 2). The activation energy for the A→B step remains almost constant.

(3) The influence of a polarizable medium consists mainly of the following factors (Fig. 5): (a) A strong stabilization of forms A and C with respect to form B, and an increase of the instability of form C with respect to form A. (b) An increase of the activation energy for the A→B step, and a lowering of the B→A and B→C activation energies. (c) Comparison of the medium's effects between Systems I and II shows that form C, which is expected to exist at the receptor's level, is more favored in System I. Therefore, a first conclusion is that neutralization of the side chain within this model does not explain the formation of C. This is supported by the fact that, in spite of a lowering of the activation energy for the B→C step in System II and an increase of the same energy for System I, System I still has a lower activation energy.

In relation to the formation of form C from A, Weinstein et al. [8] concluded their work by suggesting that, "under these circumstances the entire proton relay mechanism may change from sequential to concerted". Our results, including non-specific medium effects clearly suggest that, within this model, concerted proton movement is not possible even in the presence of a strong polarizable medium. Assuming that semiconcertation may occur and that form B is never reached, we may propose the following steps leading to form C: first, the proton belonging to site S1 begins to move towards N1 until an N1-H distance of about 1.5 Å is reached. At this moment, the activation energy for the A→B step is reached. Then, the N3-H proton begins to move towards S2 in a concerted way with the first proton, in such a way that the whole system will move along an isoenergetic pathway to reach form C. Considering that System II favors the A→B step and System I the B→C step, it seems not too far reaching to suggest that the first proton movement could activate a conformational change in the receptor, in such a way that the negative site S3 moves away from the ethylamine side chain, facilitating the energetics of the process.

However, it seems evident that the nature of the receptor sites employed in these kinds of models is fundamental. The actual modeling of the S1 site with an ammonia molecule is not able to explain the passage from B to C. Also, the high barrier for the A→B step suggests that the ammonium group is not sufficiently acidic to release its proton easily.

In Table 3 we show some activation and stabilization energies for System II in vacuo, calculated by different quantum chemical methods. Taking the ab initio STO-3G results as the standard ones, the following remarks are pertinent:

TABLE 3

Activation and stabilization energies for System II in vacuo (kcal mol<sup>-1</sup>)

Method	Activation energy		Stabilization energy <sup>a</sup>	
	A→B	B→C	A	C
CNDO/2	22.5	38	32.6	36.4
STO-3G <sup>b</sup>	12.1	(16.2) <sup>c</sup>	18.7	(14.8) <sup>c</sup>
MNDO <sup>d</sup>	25.7	19.1	4.1	6.6
AM1 <sup>d</sup>	16.3	14.2	5.7	7.1

<sup>a</sup>With respect to B form. <sup>b</sup>Ref. 8. <sup>c</sup>Data for neutral HA with geometry optimization. <sup>d</sup>Ref. 9.

(a) With respect to the activation energies for the A→B and B→C steps, STO-3G and CNDO/2 results give the same trends, while MNDO and AM1 calculations predict opposite trends. It must be stressed that the B→C step is obtained at the STO-3G level with geometry optimization for neutral HA (i.e. without protonation of the side chain), whose energetics are similar to those of neutralized HA [9].

(b) CNDO/2 calculations predict that form A will be more stable than form C by about 4 kcal mol<sup>-1</sup>. STO-3G results, with geometry optimization of only form C predict the opposite. A complete comparison with our results is not possible, but we may note that MNDO and AM1 results also favor form A. Furthermore, these last calculation methods predict very low stabilization energies that are very different from the STO-3G results.

#### ACKNOWLEDGMENTS

This work has received financial support from FONDECYT (Project 1111-88). Also, some funds from Universidad Austral de Chile (Project DIC S-88-23) and Universidad de Chile (Project Q-2442) are gratefully acknowledged.

#### REFERENCES

- 1 L.B. Houch and J.P. Green, *Handb. Neurochem.* 6 (1984) 145.
- 2 J.P. Green in H.Y. Meltzer (Ed.), *Psychopharmacology: The Third Generation of Progress*, Raven Press, New York, 1987, pp. 273-279.
- 3 J.J. Bonnet and J.A. Ibers, *J. Am. Chem. Soc.*, 95 (1973) 4829.
- 4 K. Proud, S. Critchley and C.R. Ganellin, *Acta Crystallogr., Sect. B*, 30 (1974) 2884.
- 5 H. Weinstein, E. Chou, C.L. Johnson, S. Kang and J.P. Green, *Mol. Pharmacol.*, 12 (1976) 738.
- 6 W.G. Richards, J. Wallis and C.R. Ganellin, *Eur. J. Med. Chem.*, 14 (1979) 9.



- 7 S. Topiol, H. Weinstein and R. Osman, *J. Med. Chem.*, 27 (1984) 1531.
- 8 H. Weinstein, A.P. Masurek, R. Osman and S. Topiol, *Mol. Pharmacol.*, 29 (1986) 28.
- 9 F.J. Luque, F. Illas and R. Poulana, *Mol. Pharmacol.*, 32 (1987) 557.
- 10 Y.G. Smeyers and C. Muñoz-Caro, *Eur. J. Med. Chem.*, 24 (1989) 411.
- 11 R. Constanciel and R. Contreras, *Theor. Chim. Acta*, 65 (1984) 1.
- 12 R. Contreras and J.S. Gómez-Jeria, *J. Phys. Chem.*, 88 (1984) 1905.
- 13 R. Contreras and J.S. Gómez-Jeria, *Acta Sud Am. Quím.*, 6 (1986) 9.
- 14 J.S. Gómez-Jeria and R. Contreras, *Int. J. Quantum Chem.*, 30 (1986) 581.
- 15 J. Parra-Mouchet, R. Contreras and A. Aizman, *Int. J. Quantum Chem.*, 33 (1988) 41.
- 16 P. Schuster, *Int. J. Quant. Chem.*, 3 (1969) 851.
- 17 S. Nakawa and H. Umeyama, *J. Am. Chem. Soc.*, 100 (1978) 7716.
- 18 R. Contreras and A. Aizman, *Int. J. Quantum Chem.*, 27 (1984) 293.
- 19 S.N. Mohammad and A.J. Hopfinger, *Int. J. Quantum Chem.*, 22 (1982) 1189.
- 20 R. Contreras, L. Padilla, J.S. Gómez-Jeria and A. Aizman, *J. Mol. Struct. (Theochem)*, 210 (1990) 147.
- 21 J.S. Gómez-Jeria and D. Morales-Lagos, *J. Phys. Chem.*, 94 (1990) 3790.
- 22 D. Morales-Lagos and J.S. Gómez-Jeria, *J. Phys. Chem.*, 95 (1991) 5308.