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The histamine H_1-receptor antagonist binding site. Part I: Active conformation of cyproheptadine

Marc J. van Drooge, Gabriëlle M. Donné-op den Kelder* and Hendrik Timmerman

Department of Pharmacochemistry, Faculty of Chemistry, Vrije Universiteit, de Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

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SUMMARY

The active conformation of several histamine H_1-antagonists is investigated. As a template molecule we used the antagonist cyproheptadine, which consists of a piperidylene ring connected to a tricyclic system. The piperidylene moiety is shown to be flexible. The global minimum is a chair conformation but, additionally, a second chair and various boat conformations have to be considered, as their energies are less than 5 kcal/mol above the energy of the global minimum. Two semi-rigid histamine H_1-antagonists, phenindamine and triprolidine, were fitted onto the various conformations of cyproheptadine in order to derive the pharmacologically active conformation of cyproheptadine. At the same time, the active conformation of both phenindamine and triprolidine was derived. It is demonstrated that, within the receptor-bound conformation of cyproheptadine, the piperidylene ring most probably exists in a boat form.

INTRODUCTION

Histamine H_1-antagonists are important agents in the treatment of allergic disorders. Although many H_1-antagonists are known and used, they all have some disadvantages in therapy, sedation being one of them. In recent years some antagonists were marketed devoid of this side effect; currently, new antagonists are still being developed. However, this development is in most cases not based on a model for the binding site of the histamine H_1-receptor but on a rather classical way of molecular manipulation. We therefore initiated the current programme. In this study we present results from modeling studies on the histamine H_1-receptor, the active conformation of a semi-rigid H_1-antagonists cyproheptadine, currently used as a preliminary model of the H_1-receptor antagonist binding site.

H_1-antagonists can structurally be divided into two classes, the classic and non-classic. The

* To whom correspondence should be addressed.

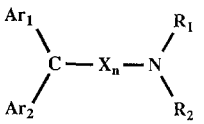

Fig. 1. Basic chemical structure of classic H_1-antagonists: Ar_1 and Ar_2 are aromatic groups, NR_1R_2 is a tertiary amine, X_n is a short chain linking the amine and the aromatic groups.

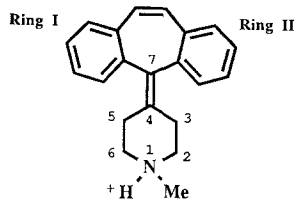

Fig. 2. Cyproheptadine. Numbering used for the piperidylene ring is indicated.

classic antagonists all share the same basic chemical structure (Fig. 1). They all contain two aromatic groups and a nitrogen function connected through a short chain, with a length of 3–4 carbon atoms. The nitrogen function usually is a tertiary amine, but quaternized compounds can also be highly active [1]. Removing one aromatic group reduces activity, while removing both aromatic groups leads to inactivity [2]. The nitrogen atom is important, as can be concluded from the optimal length of the connecting chain [3], and the fact that in this class of compounds no structures without this nitrogen are reported to have important H_1-antagonistic activity.

Non-classic antagonists are structurally more diverse. Generally, they have more than one aromatic group and a basic nitrogen atom. However, there are very active compounds known which either lack a strong basic function (e.g. temelastine [4]) or have only one aromatic group (e.g. benzimidazole derivatives [5]). In the former case, polar groups probably fulfil the same function as the basic nitrogen atom, whereas in the latter case an additional hydrophobic chain is necessary for high activity.

Very little is known about the structure of the histamine H_1-receptor. Although recently the H_1-receptor has been expressed in Xenopus Oocytes [6], the receptor has not been isolated yet and the DNA sequence is not known. Information on the binding site can then be obtained by comparing the structures of the ligands acting on the receptor. Until now, only a few studies have been published [7,8]. In these investigations the potent ($pA_2 = 9$ [9]) classic H_1-antagonist cyproheptadine (Fig. 2) was used as a reference molecule to which other antagonists were compared. The reason for choosing cyproheptadine as a reference molecule most probably was that this compound was considered the most rigid H_1-antagonist known at the moment. Therefore, the receptor-bound conformation of cyproheptadine might be very similar to the conformation as present in solution or in crystallized form. An essential assumption in the two literature studies [7,8] on the H_1-antagonist binding site was indeed that the receptor-bound conformation of cyproheptadine and the X-ray structure are similar.

In Fig. 2 the structural formula of cyproheptadine is shown. It consists of a cycloheptatriene ring fused to two benzenes and connected via a double bond to an N-methyl piperidylene group. Because of its cyclic nature and double bonds, cyproheptadine seems to be very rigid, and therefore the assumption that the crystal conformation mimics the receptor-bound conformation seems reasonable. However, recently a conformational analysis study on cyproheptadine was published [10], and from NMR studies in $CDCl_3$ the authors concluded that cyproheptadine can assume at least two different conformations.

In the current study we aim to establish the biologically active conformation of cyproheptadine

acting on the histamine H_1-receptor. We first want to point out that conformations other than the two chair conformations of the piperidylene ring found in solution and in crystallized form should be considered. These conformations will be of importance only if their energy is relatively low compared to the global minimum of the molecule. The reason that conformations with a higher energy in solution or in vacuo can still be the active conformation is that the receptor, by creating an environment different from either solution or vacuo can stabilize these conformations. Because this receptor environment is not known, all low-energy conformations should be considered with an energy within 5 kcal/mol from the calculated global minimum.

By fitting (superimposing) other semi-rigid ligands onto cyproheptadine, one of the possible conformations of cyproheptadine can be shown to be the biologically active conformation acting on the H_1-receptor. The results can be used for building a model of the H_1-receptor binding site.

STRATEGY

The strategy we followed in order to model the H_1-receptor was to check the literature as to whether cyproheptadine was still the most rigid H_1-antagonist known with a high H_1-antagonistic activity. No other compound was found. We investigated the conformational flexibility of this molecule. Different low-energy conformations were considered as possible biologically active conformations. The three-dimensional (3D) arrangement of functional groups within the active conformation of cyproheptadine and other H_1-antagonists will be similar. Therefore, we looked for other semi-rigid antagonists in literature and fitted these (phenindamine and triprolidine, pA_2-values between 9 and 10 [11,12], Fig. 3) on the various conformations of cyproheptadine. The conformation of cyproheptadine on which both other antagonists can be fitted can be considered to be the active conformation of cyproheptadine.

To evaluate the quality of a fit, first the quality of the superimposition, and secondly the energy of the fitted conformation relative to its global minimum energy, have to be established.

METHODS

General

All calculations on cyproheptadine, phenindamine and triprolidine were performed on the protonated species. Initial geometries for cyproheptadine and triprolidine were taken from the Cam-

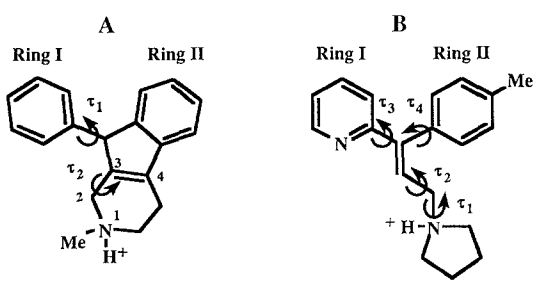

Fig. 3. Structures of phenindamine (A) and triprolidine (B). Assignment of ring I and II and the dihedral angles varied within the conformational analysis studies and flexible fitting procedures.

bridge Structural Database [13,14], while phenindamine was built with the modeling package Chem-X* using crystal structure fragments.

The molecular mechanics program MMX** was used for conformational analysis studies on triprolidine and phenindamine. Within MMX the charge-charge electrostatic interaction term was used with a default dielectric constant of 1.5.

The semiempirical quantum mechanical method MNDO implemented in MOPAC version 4.0 [15] was used for a final optimization of all structures. MNDO optimizations were performed on internal coordinates using the default optimiser until Herbert's test was satisfied (using the keyword 'PRECISE') or, if not possible, until all gradients were smaller than 1 kcal/mol/Å or 1 kcal/mol/rad.

The molecular modeling program Chem-X was used for rigid and flexible fitting. The flexible fitting minimiser uses the Chem-X non-bonded energy force field. This consists of a Van der Waals term (Buckinham term (exp-6) [16,17]) and an electrostatic Coulomb term [17] using charges from an MNDO Mulliken population analysis. The flexible fitting minimizations were carried out using a quadratic gradient minimiser until the sum of gradients was smaller than 5 [17].

Centroids of and planes through aromatic rings were defined by the corresponding six-ring atoms.

Flexibility of cyproheptadine

In this study, the flexibility of the tricyclic ring system of cyproheptadine was not examined. This system is considered to be rigid because inversions of similar tricyclic ring systems are known to have a high energy barrier [10,18]. Assuming that the flexibility of cyproheptadine resides in the piperidylene ring this flexibility can be accessed by a conformational analysis. However, a complete conformational analysis of ring structures is rather complicated, mainly because many different energy minima are present and it is difficult to locate them all [19]. Therefore, we chose a simpler approach and generated different ring conformations in analogy to cyclohexane. Recently, an extensive conformational analysis of cyproheptadine has been performed (reaction-coordinate approach) by Sadek et al. [10], which indeed indicates the existence of a multitude of possible transition states and local minima.

Using the molecular modeling package Chem-X, we constructed the possible chair and boat conformations for the piperidylene ring of cyproheptadine (Fig. 4). One chair and three boat conformations are possible. As each ring conformation can be incorporated into cyproheptadine in two ways, this results in two conformations with a chair piperidylene ring conformation and six conformations with a boat piperidylene ring conformation (henceforth, 'boat' or 'chair' will refer to the conformation of the complete molecule cyproheptadine, and not only to the conformation of the piperidylene ring). The six boat conformations contain two pairs of conformations which are mirror images. In this study, we compared cyproheptadine with triprolidine and phenindamine. As triprolidine is a non-chiral molecule, it is impossible to use triprolidine to distinguish between two mirror images. For the chiral molecule phenindamine, the absolute configuration of the active isomer is unknown. Therefore, we considered only one of the two mirror images, and

* Chemical Design Ltd., Oxford, U.K.

** Version 88, derived from MM2(77) (QCPE 395) and MMP1 Pi (QCPE 318).

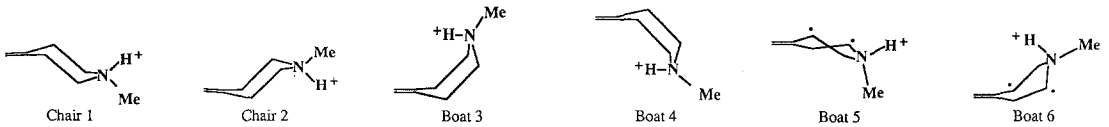

Fig. 4. Schematic drawings of the six conformations of cyproheptadine chosen to be minimized. Only the N-methyl piperidylene ring is shown; the orientation of the tricyclic ring system remains constant. In boat 5 and boat 6, flagpole ring atoms are indicated by a star.

thus four boat conformations remained. The two chair and four boat conformations (chair 1, 2 and boat 3 to 6) were optimized using the semiempirical method MNDO.

It has to be noted that a large number of alternative twisted conformations is also possible. However, as we found it impossible to investigate all these conformations whereas the position of the nitrogen atom with respect to the aromatic rings will not vary much among several of these conformations, we chose to investigate only four boat conformations. Boat 3 and 4 were chosen, as they represent (before geometry optimization) conformations with the position of the nitrogen atom maximally different from that in the chair conformations. Boats 5 and 6 represent conformations with the position of the nitrogen atom intermediate between those of the chair and boat 3 and 4 conformations.

The position of the N-methyl group is assumed not to be important for fitting, as both tertiary amines in their protonated form as well as several quaternary ammonium ions are known to be active compounds [1]. Therefore, in the two chair conformations, the N-methyl group was placed equatorially. Axial conformations were not investigated, as this conformation generally has a higher energy, and the position of the methyl group probably only has a minor effect on the conformation of the remainder of the molecule. Also, in the boat conformations only one position for the N-methyl was considered (Fig. 4).

Conformational analysis of phenindamine and triprolidine

The conformational analyses of phenindamine and triprolidine were performed using the dihedral driver of the molecular mechanics program MMX. The dihedral driver systematically drives selected dihedral angles with user-defined increments; each generated conformation is optimized, keeping only the chosen dihedral angle(s) fixed.

In the case of phenindamine, the structure was first pre-optimized with MMX, whereafter the exocyclic dihedral angle τ_1 (Fig. 3) was rotated with increments of 15°. Subsequently, all low-energy conformations were fully optimized (including driven dihedral) within MMX and the lowest-energy conformation finally optimized within MNDO to give the global minimum conformation and its energy.

The conformational analysis of triprolidine was performed sequentially and for the E-isomer only (Z-isomer is 1000-fold less active [22]). First, dihedral angles τ_1 and τ_2 were examined, then τ_2 and τ_3, and finally τ_3 and τ_4 (Fig. 3). This seems reasonable, since τ_1 influences τ_4 only slightly. First triprolidine was pre-optimized with MMX and the result used as input for a conformational analysis study on τ_1 and τ_2 with increments of 60° (the reason for this rather large increment was the limited availability of disk space). Next, dihedral angles τ_2 and τ_3 were varied. Input for this second conformational-analysis study were the lowest-energy conformations with different τ_1-val-

ues resulting from the previous analysis. Correspondingly, input for the third conformational analysis on angles τ_3 and τ_4 were the lowest-energy conformations with varying τ_2 values generated by the second conformational analysis. All energetically allowed conformations resulting from the third analysis were optimized with MMX, and the lowest-energy conformation was further optimized with MNDO to give the global minimum.

As molecular mechanics methods such as MMX are known to be less well suitable for highly conjugated systems [21] such as cyproheptadine, it was optimized with MNDO. As phenindamine and triprolidine were going to be fitted onto the MNDO-optimized structure of cyproheptadine, these structures were also finally optimized with MNDO. The rational basis for this approach is that different geometry optimization methods may give different values for optimal bond lengths and bond angles, thereby influencing the results of the fits. The combination of two optimization methods (MMX, MNDO) may introduce only small errors in the localization of the global minima.

In the fitting procedures described in the next section, initially only exocyclic dihedral angles were varied. In triprolidine the flexibility of the molecule can almost completely be described by these angles. However, in case of phenindamine this is not completely true. As the distance between the basic nitrogen atom and the aromatic plane, which are both part of a tricyclic system, might be important for fitting, we generated some non-minimum-energy conformations by varying endocyclic dihedral angle N_1–C_2–C_3–C_4 (τ_2) inside the tetrahydropyridine ring of phenindamine (Fig. 3). This angle is mainly responsible for variations in the distance between the basic nitrogen atom and the aromatic plane. Starting with the global minimum of phenindamine (τ_2 is 19°) and using the dihedral driver of MMX τ_2 was driven from 90 to −90 degrees with increments of 5 degrees. The resulting low-energy conformations were subsequently optimized with MNDO keeping τ_2 fixed. These structures were also used for fitting at a later stage.

Fitting

The H_1-antagonists phenindamine and triprolidine were fitted on the template cyproheptadine. The groups selected to be fitted were the basic nitrogen atom and the two aromatic rings, as it is known that these groups are important for binding to the receptor (see introduction). The aromatic rings were represented by two dummy atoms 1.8 Å above and below the centroids of the ring (Fig. 5). When these dummy atoms are fitted, not only the centroids of the aromatic rings tend to be superimposed but also the aromatic planes are positioned in parallel. The advantage of fitting dummy atoms instead of, for example, three carbon atoms of the aromatic rings, is that rotation around the axis perpendicular to the aromatic plane is not restrained.

Compounds were fitted by first using a rigid fitting procedure, followed by a flexible fitting procedure. Within the first procedure, the global minimum conformation of phenindamine and triprolidine was fitted onto cyproheptadine only allowing global rotations and translations. Subsequently, in the flexible fitting procedure also user-defined exocyclic dihedral angles were minimized with respect to restraints set by the user (see below) on the one hand, and the nonbonded energy of the fitted molecule on the other hand. For phenindamine, dihedral angle τ_1 was minimized and for triprolidine, angles τ_2, τ_3 and τ_4 (Fig. 3). The fitted structures were subsequently optimized in MNDO, optimizing all internal coordinates except for the dihedral angles adjusted in the flexible fitting procedure. Finally, these optimized structures were superimposed on cyproheptadine again, and distances between fitted groups and angles between planes determined.

Within the fitting procedures, a restraint constant of 100 kcal/mol/Å^2 was used for the nitrogen

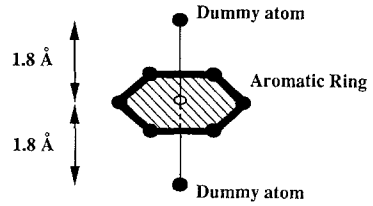

Fig. 5. Dummy atoms assigned for fitting aromatic rings. The dummy atoms are positioned on the axis perpendicular to the plane defined by the ring atoms, passing through the centroid of the same ring, and 1.8 Å above and below the plane.

atoms to be matched, and a constant of 10 kcal/mol/Å^2 for the dummies representing the aromatic rings. The reason for not giving the nitrogen and dummy atoms equivalent restraint constants is that the balance between fitting the nitrogen atom and the aromatic rings is not only determined by this constant, but also by the distance chosen between the dummy atom and the centroid of the corresponding ring (i.e. 1.8 Å). We observed that, with these restraints, the nitrogen atom and the aromatic rings could be fitted well.

To quantify the quality of the fit for the nitrogen atoms, we considered their distance when superimposed. The fit of the aromatic groups was quantified by the angle between the fitted planes (Pla I and Pla II) as well as the distance between the centroids of the rings (Cen I and Cen II). Ideally, these distances and angles should be zero. The energy of the fitted conformation should be within 5 kcal/mol of the global minimum energy.

RESULTS

Flexibility of cyproheptadine

In Fig. 4, the six conformations of cyproheptadine chosen to be optimized by MNDO are presented. In Table 1, the energies of the two chair and four boat conformations of cyproheptadine after optimization with MNDO are shown. It can be seen that the chair 1 conformation of cyproheptadine has the lowest energy. This chair conformation is also found in the crystal structure [13]. The energy difference between the two chair conformations is only 0.6 kcal/mol. The boat conformations have energies about 1.5 to 3.6 kcal/mol above the lowest-energy conformation. Only boat 5 has a real boat conformation in that bonds N_1–C_2 and C_4–C_5 run almost parallel (angle is 1°). The other three boat conformations are more or less twisted, as can be seen in Table 2 from the differences in the distances between the opposing piperidylene ring atoms.

Table 2 further demonstrates that the nitrogen atom lies in all six conformations approximately

TABLE 1
MNDO RESULTS FOR 6 LOW-ENERGY CONFORMATIONS OF CYPROHEPTADINE

	Chair 1	Chair 2	Boat 3	Boat 4	Boat 5	Boat 6
ΔH_f(kcal/mol)[a]	240.6	241.2	243.3	242.1	244.2	242.1
$\Delta\Delta H_f$(kcal/mol)[b]	—	0.6	2.7	1.5	3.6	1.5

[a] Heat of formation of the two chair and four boat conformations of cyproheptadine.
[b] Heat of formation relative to chair 1.

TABLE 2
GEOMETRIC PARAMETERS OF THE SIX LOW-ENERGY CONFORMATIONS OF CYPROHEPTADINE AFTER MNDO OPTIMIZATION

	Chair 1	Chair 2	Boat 3	Boat 4	Boat 5	Boat 6
N - Cen I (Å)[a]	6.444	6.428	5.998	6.110	6.459	6.043
N - Cen II (Å)[b]	6.427	6.412	6.061	6.052	6.432	6.109
N - Pla I (Å)[c]	3.508	3.126	2.429	3.361	3.157	3.448
N - Pla II (Å)[d]	3.587	3.152	2.350	3.449	3.162	3.348
Cen I - Cen II (Å)[e]	4.760	4.786	4.788	4.774	4.771	4.772
Pla I - Pla II (deg)[f]	66.06	64.20	64.10	65.18	64.90	65.30
N1 - C4 (Å)[g]	2.995	2.988	2.793	2.841	2.999	2.840
C2 - C5 (Å)[h]	2.972	2.969	2.911	3.036	2.990	2.850
C3 - C6 (Å)[i]	2.971	2.968	3.017	2.851	2.806	3.036

[a-b] Distance between basic nitrogen atom and the centroids of ring I or II resp. (Cen I, Cen II).
[c-d] Distance between basic nitrogen atom and the planes through ring I or II resp. (Pla I, Pla II).
[e] Distance between Cen I and Cen II.
[f] Angle between Pla I and Pla II.
[g-i] Distances between opposing atoms of the piperidylene ring.

on the plane of symmetry of the tricyclic system, as the distance between the nitrogen and both planes or centroids is approximately equal (compare rows 1 and 2, and 3 and 4 in Table 2).

In Fig. 6, the six conformations of cyproheptadine are superimposed (tricyclic systems are matched). Figure 6 reveals the two chair conformations to be different especially with respect to the position of the nitrogen atoms (distance is 1.6 Å, Table 3). Examining Table 3, it is evident that chair 2 and boat 5 are identical with respect to the position of the basic nitrogen atom (distance is only 0.08 Å). Assuming that only the relative positions of the aromatic groups in the tricyclic system and the basic nitrogen atom of cyproheptadine are important for H_1-antagonistic activity, and not the exact conformation of the piperidylene ring, boat 5 most probably will not be the active conformation of cyproheptadine: its conformational energy is 3.6 kcal/mol higher than that of chair 2, whereas they both have similar relative positions of the functional groups.

However, in boat 3, 4 and 6 the position of the nitrogen atom relative to the tricyclic system is very different from those in the chair conformations, and they also differ mutually. Boats 4 and 6 were generated from different conformations, but after optimizing with MNDO they appeared as approximate mirror images, as can be seen from the same energies and equivalent geometric parameters (Table 2). The nitrogen atom in either boat 4 or 6 has distances of about 1.0 and 2.5 Å to the corresponding nitrogens in the chair 1 and 2 conformations, respectively; the nitrogen atom in boat 3 has distances of 2.5 and 1.0 Å, respectively.

Summarizing, five different conformations of cyproheptadine were generated of which four (chair 1, 2 and boat 3, 4) have widely different positions for the basic nitrogen atom. Because the energies of all conformations are within 5 kcal/mol from the lowest-energy conformation (chair 1), they are all possible candidates for the biologically active conformation of cyproheptadine acting on the H_1-receptor.

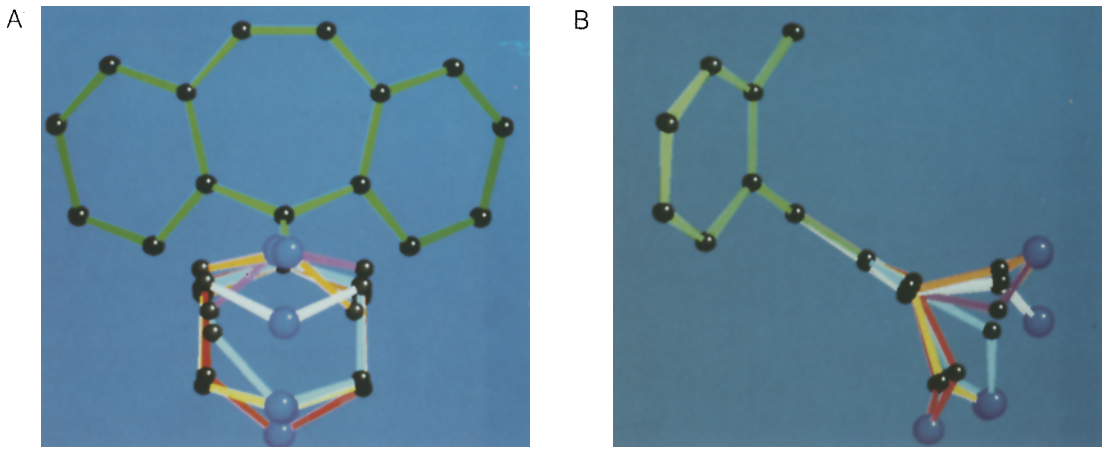

Fig. 6. Superimposition of the six MNDO optimized chair and boat conformations of cyproheptadine. All 15 carbon atoms of the tricyclic ring system were fitted onto each other. Front (A) and side view (B). The tricyclic ring system of all conformations is coloured green. The piperidylene ring of chair 1 is coloured white, chair 2 yellow, boat 3 red, boat 4 orange, boat 5 blue and boat 6 purple. For reasons of clarity, the methyl groups are not shown. The basic nitrogen atom (blue) occupies widely different positions in the different conformations.

Fitting

In Table 4, the results of fitting phenindamine and triprolidine on cyproheptadine are shown. Only the fits on both chair conformations and one boat conformation (boat 3) are quantified in this table. The fits on boat 4 (or 6) are significantly worse and therefore have been omitted.

Examining the fits of both phenindamine and triprolidine on the two chair conformations of cyproheptadine (Table 4, Fig. 7), the fit on chair 2 appears to be slightly better. However, compared to the fits on boat 3 the fits on chair 1 and 2 are much worse.

If we closely examine the fits of phenindamine and triprolidine on boat 3 and the two chairs (Table 4, Fig. 7), it can be seen that both the nitrogen atoms and the two aromatic rings of both compounds can be fitted considerably better on boat 3 than on the two chairs. Especially, the fit of Ring I and II of phenindamine and of ring II of triprolidine is much better. The energies of the conformations fitted on boat 3 relative to their global minimum energy are 4.0 and 1.8 kcal/mol, respectively.

One of the reasons that phenindamine cannot be fitted correctly on the chair conformations of

TABLE 3
DISTANCES BETWEEN THE BASIC NITROGEN ATOMS OF THE TWO CHAIR AND FOUR BOAT CONFORMATIONS OF CYPROHEPTADINE[a]

	Chair 1	Chair 2	Boat 3	Boat 4	Boat 5	Boat 6
Chair 1	—	1.562'	2.504	1.014	1.493	1.039
Chair 2	1.562	—	1.063	2.462	0.079	2.483

[a] Distances (Å) after superimposing the 15 carbon atoms of the tricyclic ring system.

cyproheptadine might be that in the fitting procedures the endocyclic angle τ_2 (Fig. 3) was not varied, keeping the position of the nitrogen atom relative to aromatic ring II fixed. To examine the effect of changing τ_2, conformations with various τ_2 values were generated (see Methods), and all low-energy conformations fitted on chair 2. The quality of these fits appeared to be approximately equal to the fit of the unstrained conformation of phenindamine (Table 5 shows the fit of the most strained conformation, having an energy less than 5 kcal/mol above the energy of the global minimum after fitting). Therefore, even taking into account the flexibility of the tetrahydropyridine ring of phenindamine, the compound still cannot be fitted correctly on the chair 2 conformation of cyproheptadine.

We further investigated the effect of decreasing the restrain constant on the distance between the nitrogen atoms from 100 to 40 kcal/mol/Å^2 (see Methods). The results are shown in Table 5. The aromatic groups are now fitted somewhat better, whereas the nitrogen is fitted worse. However, the fit on chair 2 is still not acceptable. Therefore, we conclude that phenindamine cannot be fitted correctly on either of the two chair conformations of cyproheptadine.

Because only a limited number of low-energy conformations of cyproheptadine was investigated, it is in principal possible that also other conformations bind to the H_1-receptor. However, as in the case of phenindamine the position of the basic nitrogen atom with respect to the aromatic ring II is nearly fixed (distance to its centroid is approx. 5.5 Å, distance to its plane is approx. 0.4 Å), it can only be fitted reasonably onto a conformation of cyproheptadine, which has a small distance between the plane through its aromatic rings and the nitrogen atom. As a conformation

TABLE 4
QUALITY OF THE FITS OF PHENINDAMINE AND TRIPROLIDINE ON THE CHAIR 1 AND 2 AND BOAT 3 CONFORMATIONS OF CYPROHEPTADINE

	Chair 1[b]	Chair 2[b]	Boat 3[b]
Phenindamine[a]			
$\Delta\Delta H_f$ (kcal/mol)[c]	0.7	1.3	1.8
N - N (Å)[d]	0.248	0.266	0.166
Angle Ring I (deg)[e]	1.0	4.6	8.8
Distance Ring I (Å)[f]	0.638	0.537	0.280
Angle Ring II (deg)[g]	42.1	31.3	23.3
Distance Ring II (Å)[h]	0.710	0.685	0.308
Triprolidine[a]			
$\Delta\Delta H_f$ (kcal/mol)[c]	1.6	3.0	4.0
N - N (Å)[d]	0.191	0.178	0.083
Angle Ring I (deg)[e]	7.4	6.1	5.8
Distance Ring I (Å)[f]	0.318	0.280	0.213
Angle Ring II (deg)[g]	7.3	9.2	13.6
Distance Ring II (Å)[h]	0.669	0.638	0.318

[a] Fitted compound.
[b] Conformation of cyproheptadine.
[c] Heat of formation of fitted compound relative to its global minimum.
[d] Distance between the fitted nitrogen atoms.
[e] Angle between the fitted Rings I.
[f] Distance between the centroids of the fitted Rings I.
[g] Angle between the fitted Rings II.
[h] Distance between the centroids of the fitted Rings II.

with a distance much smaller than in boat 3 does not exist, other possibly active conformations of cyproheptadine will at best give results similar to those of the boat 3 conformation. This strongly suggests that boat 3 is the active conformation on the H_1-receptor.

DISCUSSION

Flexibility of cyproheptadine

From the minimization of the different chair and boat conformations (of cyproheptadine) it is evident that the two chair conformations have the lowest energy. Chair 1, which is similar to the crystal structure [13], has an energy 0.6 kcal/mol lower than chair 2 (Table 1). A similar result was found by Sadek et al. [10], using the semiempirical method AM1 (1.1 kcal/mol difference; protonated species; N-Methyl equatorial). In the present study, the position of the N-methyl group in the chair conformations was kept equatorial as this position is assumed to be favoured above the axial position. Sadek et al. [10] calculated the energies of the chair conformations both with the methyl group in equatorial and in axial position, and the results confirmed our assumption. From these results, the chair 1 conformation can be concluded to be the global minimum conformation of cyproheptadine.

In addition to the chair conformations, we also generated the low-energy boat 3, 4, 5 and 6 conformations. These conformations have an energy 1.5 to 3.6 kcal/mol above the global minimum of cyproheptadine (Table 1). These differences are small compared to the differences found in cyclohexane between chair, and twisted-boat or boat conformations (5 to 6.5 kcal/mol [22]). However, it is known that in six-membered rings other than cyclohexane these energy differences can be smaller [22]. In case of cyproheptadine an interaction between the protonated nitrogen and the π-electrons of the double bond C_4–C_7 (Fig. 2) may stabilize boat conformations relative to the chairs. Also the presence of an sp^2 carbon atom (C_4) in the ring may stabilize boat conformations because of the reduced torsional barrier of the sp^2–sp^3 bonds [22]. As the boat conformations of cyclohexane are more than 5 kcal/mol higher in energy than the chair conformations, in modeling

TABLE 5
QUALITY OF THE FITS OF PHENINDAMINE ($\tau_2 = 45°$) ON THE CHAIR 2 CONFORMATION OF CYPROHEPTADINE

Restrain constant[a]	100	40
$\Delta\Delta H_f$ (kcal/mol)[b]	3.7	3.7
N - N (Å)[c]	0.245	0.444
Angle Ring I (deg)[d]	29.1	28.2
Distance Ring I (Å)[e]	0.759	0.572
Angle Ring II (deg)[f]	5.2	6.2
Distance Ring II (Å)[g]	0.561	0.449

[a] Restrain value for the distance between the nitrogen atoms (kcal/mol/Å^2): normal (100) or reduced (40).
[b] Heat of formation of fitted compound relative to its global minimum.
[c] Distance between the fitted nitrogen atoms.
[d] Angle between the fitted Rings I.
[e] Distance between the centroids of the fitted Rings I.
[f] Angle between the fitted Rings II.
[g] Distance between the centroids of the fitted Rings II.

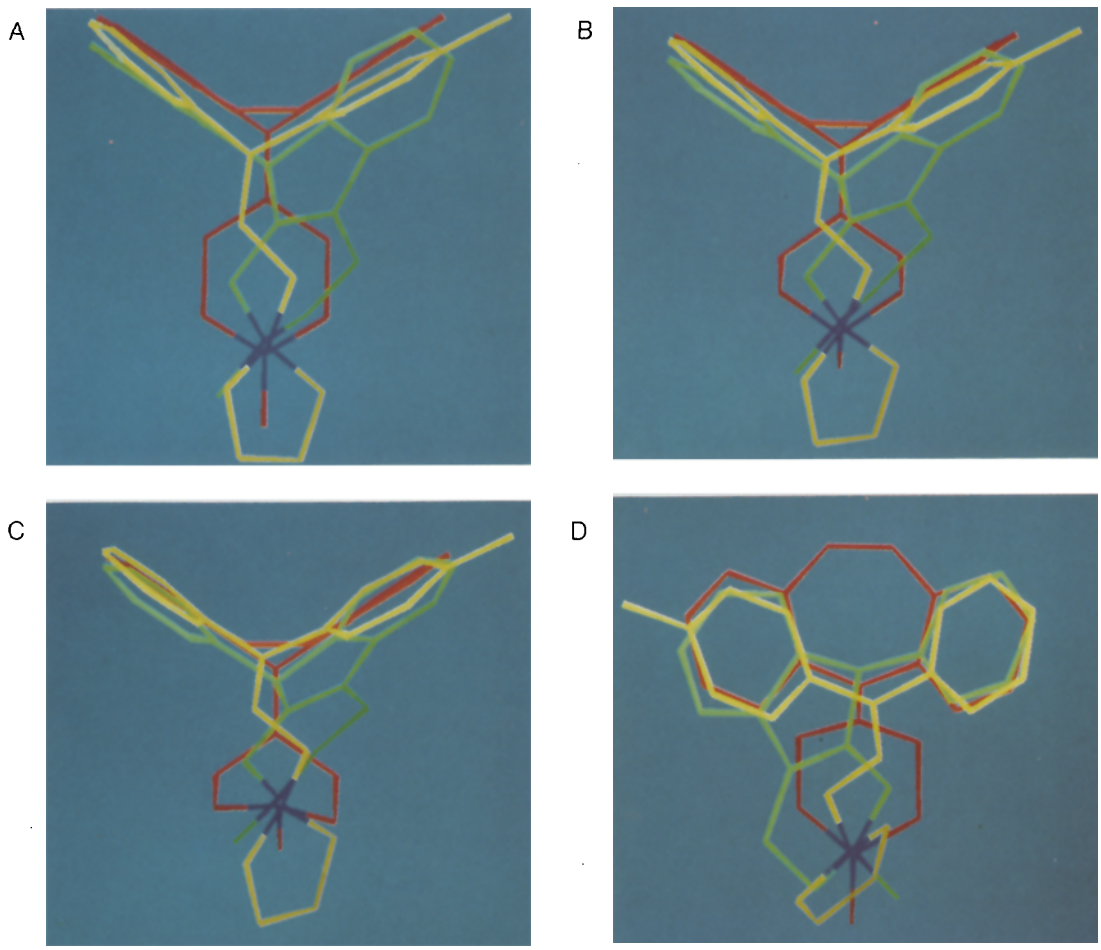

Fig. 7. Fits of phenindamine (green) and triprolidine (yellow) on cyproheptadine (red) in chair 1 (A), chair 2 (B) and boat 3 (C,D) conformations (see also Table 4). The fit on boat 3 gives the best results.

studies on six-membered rings usually only the chair conformations are considered. However, from the present study it can be concluded that the energies of the boat conformations are sufficiently low to be energetically accessible, and have to be considered as possible active conformations. Indeed, our results point to boat 3 as the active conformation on the H_1-receptor.

Beside being an H_1-antagonist, cyproheptadine also is an antagonist at other receptors, e.g. serotonine-S_2 and acetylcholine M-receptors [23]. Until recently, cyproheptadine was assumed to be rigid and to bind to all these receptors in a conformation similar to the crystal structure [7,8,24]. However, Sadek et al. [10] already pointed out that two chair conformations of cyproheptadine are present in solution, and therefore both have to be taken into account when considering the active conformation of cyproheptadine. Our study indicates that other conformations of cyproheptadine also have to be considered. Therefore, the assumption that the three functional groups of cyproheptadine (two aromatic rings and a basic nitrogen atom) are arranged similarly after binding to either of the different receptors is not necessarily valid, and the active conformation of cyproheptadine has to be derived separately for each receptor.

Histamine H_1-receptor antagonist binding site

By fitting the functional groups of H_1-antagonists on cyproheptadine we determined that the boat 3 conformation of cyproheptadine is its active conformation on the H_1-receptor antagonist binding site. The rationale behind superimposing functional groups is that these groups bind to the same receptor locations, implying that the antagonists themselves bind to the same receptor site.

It is experimentally difficult, even impossible, to verify whether antagonists bind to the same or to different receptor sites. For example, most H_1-antagonists are competitive, but some compounds display non-competitive antagonism towards the H_1-receptor (e.g. cyproheptadine [9]). However, these data do not give a decisive answer to the question whether these different antagonists bind to the same receptor site. In the case of cyproheptadine, its non-competitive nature [9] can be explained by its very slow dissociation rate from the H_1-receptor ($t_{1/2} >> 90$ min [25]). However, in some cases modeling studies can be helpful. As the semi-rigid compounds investigated in this study can be fitted onto each other, this suggests that these antagonists bind to the same site.

So far only a few modeling studies have been carried out on the H_1-receptor. In 1986, Borea et al. [7] compared the crystal structures of 14 H_1-antagonists and calculated the distance between the centroid of one of the aromatic rings and the basic nitrogen atom to be 6.2 ± 0.2 Å. Also, after optimizing the crystal structures this distance did not change. The authors concluded that, for a high H_1-antagonistic activity, the distance between the basic nitrogen atom and the centroid should be close to the above value of 6.2 Å.

In 1985, Naruto et al. [8] defined a more detailed model of the H_1-binding site by fitting seven potent H_1-antagonists and histamine itself onto each other. In their study, the centroids of two aromatic rings and the protonated nitrogen were fitted. All compounds (including cyproheptadine) were minimized simultaneously with additional restraints for the atoms to be fitted. Within this procedure the crystal structure of cyproheptadine was minimized. Therefore, it is not surprising that in the final model the conformation of cyproheptadine is similar to this X-ray structure, as in a minimization procedure usually no high energy barriers can be taken. Furthermore, it appears to be difficult to evaluate the quality of the study: although all compounds could be fitted in a low-energy conformation, no data on the quality of the fits were presented.

The main drawback of the above studies is the fact that the authors assume the crystal structure conformation (or a similar conformation) of cyproheptadine to be the active conformation. A second drawback is that the aromatic groups were considered to be one-dimensional points (centroids). Obviously, when the aromatic planes of different antagonists interact with the receptor, these planes will be oriented in a similar way (coplanar), which cannot be achieved by only using centroids. Therefore, in the present study not only the distance between fitted centroids was considered but also the angle between fitted aromatic planes.

Another improvement of this study is that three semi-rigid antagonists, cyproheptadine, phenindamine and triprolidine, were investigated. Although in the above-mentioned modeling studies on the H_1-receptor, more antagonists were taken into account, the only semi-rigid antagonists considered were cyproheptadine and triprolidine. Because of the high flexibility of the remaining antagonists, it is very probable that these will also fit into the model we present, but probably also on any other postulated receptor model. Preliminary studies on some of these compounds (such as diphenhydramines, di-aryl-aminopropanes) have already shown that these can be fitted in our model. The results of these studies will be published in the near future.

CONCLUSIONS

This study strongly suggests that the crystal structure conformation of cyproheptadine (chair 1), in which the piperidylene group has a chair conformation, is not likely to be the active conformation of cyproheptadine acting on the H_1-receptor. Instead, a conformation in which the piperidylene group has a boat-like structure (boat 3) is proposed to be the active conformation. The energy of this conformation calculated with MNDO is 2.7 kcal/mol higher than the energy of the global minimum chair 1 conformation.

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