A COMPUTER MODELLING STUDY OF HYDROGEN BONDS IN LIGAND--\(\beta\)-ADRENOCEPTOR COMPLEXES: ITS IMPLICATIONS IN THE DEDUCTION OF A RECEPTOR MAP*

MARCEL R. LINSCHOTEN, GERT W. KLEIN KRANENBARG, SJEF J. DE KIMPE, JAAP WILTING** and LAMBERT H.M. JANSEN
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Utrecht University, Catharijnesingel 60, 3511 GH Utrecht (The Netherlands)
JOOP H. VAN LENTHE
Department of Theoretical Chemistry, Faculty of Chemistry, Utrecht University, Padualaan 14, 3584 CH Utrecht (The Netherlands)
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ABSTRACT

Recent experimental evidence indicates that the side chain carboxylate group of an aspartic acid residue located at position 113 (Asp\(^{113}\)) in the pharmacologically important \(\beta\)-adrenergic receptor protein is directly involved in the binding of \(\beta\)-adrenergics, most of which are analogues of phenylethanolamines or phenoxypropanolamines. The binding species is known to be the aminic monocation. This has led to the hypothesis that a direct interaction takes place between the carboxylate group at the receptor and the protonated amino function of the ligand.

In the present study, a quantum-mechanical conformational analysis of the ethanolamine-formate complex is presented. This work has enabled us to construct a \(\beta\)-adrenoceptor map which accounts for the binding of some major classes of \(\beta\)-adrenergic ligands. The results of this study suggest that hydrogen bonding plays a role in the interaction between these ligands and the \(\beta\)-adrenoceptor.

INTRODUCTION

In recent years, the primary structures of a number of pharmacologically important members of the G-protein-coupled receptor family have been determined using gene cloning and sequencing techniques. Included are subtypes of the \(\alpha\)-adrenergic [1], \(\beta\)-adrenergic [2], muscarinic cholinergic [3] and serotonergic receptors [4]. The structure of one of the members of this family, the rhodopsin receptor, has been studied in some detail using electron diffraction [5] and electron microscopic techniques [6]. These studies suggest that this
protein consists of seven membrane-spanning α-helical regions connected by loops. Together, these α-helices make up a cylindrical or spiral-like structure with a hole in the middle. Both theoretical approaches and experimental findings strongly suggest a similar structure for the other members of this family [7].

A number of studies on the β-adrenoceptor subtypes has established that the ligand binding site of the β-adrenoceptor is located within the membrane-spanning regions, probably in a pocket which is formed by juxtaposed α-helical regions [8]. Site-directed mutagenesis studies have identified a number of amino acid sequences or individual residues which are crucial for ligand binding [9]. One of these residues was found to be the aspartic acid residue Asp\textsubscript{113} located in the third membrane-spanning region. Replacement of the CH\textsubscript{2}−COO\textsuperscript{−} side chain of Asp\textsubscript{113} of the native β-adrenoceptor by CH\textsubscript{2}−CONH\textsubscript{2} (asparagine [Asn\textsubscript{113}]-mutant) resulted in an 8000–40 000-fold decrease in the affinity of a series of agonists, evidencing a crucial role for the carboxylate group. Furthermore, replacement of the Asp\textsubscript{113} side chain by CH\textsubscript{3}−CH\textsubscript{2}−COO\textsuperscript{−} (glutamate [Glu\textsubscript{113}]-mutant) resulted in a smaller decrease (300–1500-fold) of the affinity. The latter finding suggests that the carboxylate group of the glutamate residue Glu\textsubscript{113} contributes to the binding of agonists, although its positioning in the receptor site is clearly not ideal. As the ratios of the affinities for a number of agonists are the same for both the original and mutated receptor proteins, it seems likely that Asp\textsubscript{113} is directly involved in ligand binding [10]. On the basis of experimental evidence, IJzerman et al. showed that the active species of the β-adrenergics is the amine monocation [11]. Taken together, these data suggest that the side chain amino group which is present in all classes of β-adrenergics (Fig. 1) directly interacts with the carboxylate moiety of Asp\textsubscript{113}.

Hadzi and co-workers published a number of studies on the interaction of the ethanolamine moiety of β-adrenoceptor ligands with an anionic carboxylate-type site several years before the involvement of a carboxylate group in the binding of β-adrenergics was proved [12–14]. Interestingly, the most stable hydrogen-bonded ethanolamine–carboxylate complex found by Hadzi’s group was of the chelate type in which both the β-OH group and the protonated amino moiety form hydrogen bonds towards either oxygen of the carboxylate moiety [13].

Although the exact three-dimensional structure of the binding sites of the β-adrenoceptor subtypes has yet to be determined, the first modelling studies have already appeared in the literature [15]. These studies may help in the design of new mutant proteins to clarify which residues are involved in the active site. Awaiting more detailed information on the three-dimensional structural of the binding site, any modelling study aiming at rational drug design seems to be highly speculative. However, the combination of both the (limited) direct structural information on the binding site and indirect infor-
Fig. 1. Structures of representative β-adrenergic phenylethanolamines (a), 2-amino-tetralins (b), phenoxypropanolamines (c) and tetrahydroisoquinolines (d).

mation derived from the three-dimensional structure of the β-adrenergic ligands may give important clues to aid in a rational drug design process.

The experimental evidence for the involvement of the carboxylate group of Asp113 in the β-adrenoceptor binding site triggered us to perform a receptor mapping analysis which includes a carboxylate moiety. In the underlying analysis, an attempt is made to rationalize the binding of some major classes of β-adrenergics. As will be shown, hydrogen bonding seems to play a key role in these ligand-β-adrenoceptor interactions.

METHODS

The derivation of a receptor model from the three-dimensional shape and physical chemical properties of ligands alone is called receptor mapping [16]. In this method, it is assumed that the ligands generate electrostatic and steric fields which are complementary to the fields generated by the receptor surface. The deduction of common features of structurally diverse ligands may yield information on the basic structural properties necessary for ligand binding.

A typical receptor mapping procedure consists of the following steps.

(1) Deduction of the binding pharmacophore. The pharmacophore of a ligand is defined as the three-dimensional pattern of functional groups or electrostatic fields induced by these groups necessary for both recognition and binding to the receptor site.

Additional interaction points at the hypothesized receptor site are often necessary to explain the binding of various classes of ligands (see, for example, the deduction of a dopamine receptor map by Marshall’s group [17]).

(2) Conformational analysis of a series of structurally dissimilar compounds all exerting affinity towards the receptor. Conformational flexibility of ligands
poses a major problem in a receptor mapping study as it is by no means certain that a ligand binds towards the receptor in its absolute minimum energy conformation. The energy which is released during the formation of a drug–receptor complex may easily overcome an unfavourable conformational change of the ligand. It is common practice in receptor mapping studies to include all ligand conformers having energies up to 3 kcal mol$^{-1}$ above the absolute energetic minimum.

As an initial step, a rigid analogue displaying high affinity towards the receptor, can be taken as a template molecule to which all other compounds are to be fitted. In a rigorous receptor mapping study, however, a full conformational analysis of all ligands should be performed.

(3) Automated fitting procedure. In this procedure, the distances between the analogous pharmacophoric and site points of each of the compound classes are minimized by checking fits of each of the energetically allowed conformers.

(4) Conformational analysis of closely related inactive compounds. This may provide clues on the steric extent of the receptor pocket.

It should be noted that it is implicitly assumed in the purely geometric procedure that the physicochemical properties of corresponding pharmacophoric points of different classes of ligand are analogous. This need not necessarily be the case. Recently, a procedure has been developed which allows for simultaneous minimization of the differences of the steric and electrostatic fields of a series of compounds (comparative molecular field analysis (CoMFA) [18]).

(1) Deduction of pharmacophore and definition of site points

Extensive synthesis programmes in the field of $\beta$-adrenergics have established the importance of a primary or secondary aliphatic amino moiety, a hydroxyl group at the $\beta$-position and an aromatic nucleus. Representative classes of $\beta$-adrenergics include the phenylethanolamines (PEAs), related ring-closed PEAs (tetralins), the aryloxypropanolamines (AOPAs) and the tetrahydroisoquinolines (THIs) (Fig. 1). Although the $\beta$-OH group enhances affinity in most cases, its presence is not always necessary. For a review on the SAR of $\beta$-adrenergics, see ref. 19.

(2) Conformational analysis of the ethanolamine–formate complex

As the free carboxylate group of the side chain of the Asp$^{113}$ residue is clearly involved in the receptor binding of $\beta$-adrenergics, it seems worthwhile to include this group in the receptor mapping analysis.

Hadzi and co-workers carried out a number of quantum-chemical analyses of the ethanolamine–formate model complex. These studies indicate that the chelate-type complex in which both the OH and the $\text{NH}_3^+$ groups of the ethanolamine side chain interact with an oxygen atom of the carboxylate group is
more stable than complexes in which only one hydrogen bond is formed [13]. However, these authors did not perform a conformational analysis of the ethanolamine–formate complex.

The torsional angle $\tau_{OCCN}$ defines the relative positions of the pharmacophoric OH and NH$_3^+$ groups in the ligand (Fig. 1). Therefore, we studied the complex formation energy as a function of $\tau_{OCCN}$ to derive all conformers within 3 kcal mol$^{-1}$ above the absolute minimum.

The interaction between the $\beta$-adrenergic ligands and the carboxylate moiety of Asp$^{113}$ was modelled by a simple ethanolamine–formate complex as described by Hadzi and co-workers [12–14].

As shown in Fig. 2, the formate can be positioned on either side of the ethanolamine as viewed along the two carbon atoms. These two families of conformers will be referred to hereafter as family A and family B conformers.

Family A conformers displaying negative $\tau_{OCCN}$ angles are stereoisomers of family B conformers having identical positive $\tau_{OCCN}$ values. A similar stereoisomeric relationship exists between family B conformers having $\tau_{OCCN} < 0$ and family A conformers having $\tau_{OCCN} > 0$. Therefore, only conformers satisfying $\tau_{OCCN} > 0$ were actually considered in our calculations. Complexes in which $\tau_{OCCN} < 0$ will be called reversed type A or B conformers in our studies.

**Computational method**

In the calculation of the complex energies, we used the English version of the GAMESS programme [20] (HF-SCF method) at the STO-3G level for the
geometry optimizations and at the 4-31G level for the evaluation of the interaction energy (Cray version v2.1). The calculations were run on the Cray X-MP/24 of Cray Research Inc. at Bracknell, Gt. Britain.

We used ab initio methods since Koller et al. have shown that CNDO, MNDO and MNDO/H calculations yield unreliable energies and geometries on the methyamine–formate complex [21].

**Computational procedure**

(1) The starting structure of the ethanolamine–formate complex was derived by combining the relevant coordinates from crystal structures of formic acid [22] and ephedrine monohydrogen phosphate [23]. In the latter crystal structure a similar chelate-type hydrogen bonding occurs between two oxygen atoms of the phosphate and the charged ethanolamine side chain of ephedrine. A good set of initial coordinates was thus obtained.

(2) The geometry of the complex was then fully optimized at the STO 3G level keeping \( \tau_{OCCN} \) fixed to its original value of \(-57.3^\circ\).

(3) \( \tau_{OCCN} \) was subsequently fixed at \(-62.3^\circ \) and \(-52.3^\circ \), respectively. These two conformers of the complex were then optimized using the coordinates obtained in step (2). The variables in the geometry optimizations were selected on the basis of their sensitivity to the change of \( \tau_{OCCN} \) (Table 1, see Fig. 3 for atom definitions).

(4) The geometries obtained in step (3) were used as starting points for two adjacent values of \( \tau_{OCCN} \). Each optimization step yielded the starting coordinates for two adjacent values of \( \tau_{OCCN} \). The process was repeated until disruption of one of the hydrogen bonds occurred. Both A- and B-type complexes were evaluated.

The calculation of the interaction energy is based on the model

\[
L^+ (\text{conf.} x) + P^- (\text{conf.} y) \rightarrow PL (\text{conf.} z)
\]

where \( L^+ \) and \( P^- \) denote the charged ethanolamine (ligand) and formate (as-

### Table 1

**Variables used in the ab initio geometry optimizations**

<table>
<thead>
<tr>
<th>Distance</th>
<th>Angle</th>
<th>Dihedral angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>H(5)–O(4)</td>
<td>O(6)–H(5)–O(4)</td>
<td>H(5)–O(4)–C(1)–C(2)</td>
</tr>
<tr>
<td>O(6)–H(5)</td>
<td>C(7)–H(6)–O(5)</td>
<td>O(6)–H(5)–O(4)–C(1)</td>
</tr>
<tr>
<td>N(3)–H(9)</td>
<td></td>
<td>C(7)–O(6)–H(5)–O(4)</td>
</tr>
</tbody>
</table>

See Fig. 3 for the atom definitions.
partate side chain of the Asp_{13} residue at the receptor protein), respectively. Conf.x and conf.y are the minimum energy conformers of the isolated charged species, whereas conf.z relates to the minimum energy conformation of the complex.

It should be noted that the absolute value of the interaction energy is of no importance in this study since only the energy differences between conformers are employed.

Although the exact environmental conditions at the receptor site remain to be determined, Hadzi's group showed that, in the presence of water, the ionic complex form is favoured over the neutral species [24]. The latter is favoured under in vacuo conditions as evidenced by a proton transfer from the amine cation to the formate in the course of the above reaction. Thus, the interaction energy is defined as

\[ E_{\text{int}} = E_{\text{PL}} - E_{L^+} - E_{P^-} \]

(3) Automated flexible fitting procedure

The performed quantum-mechanical conformational analysis of the ethanolamine-formate complex was used as a starting point to rationalize the binding of some major series of \( \beta \)-adrenergic compounds. Referring to Fig. 1, it is interesting to note that \( \beta \)-adrenergic activity is contained both in compounds having the side chain amino group in a cisoid relationship to the aromatic nucleus [tetrahydroisoquinolines (THIs)] and in compounds in which a transoid relationship between these groups exists (tetralin derivatives). The presence of a \( \beta \)-OH function seems to be detrimental for \( \beta \)-adrenergic activity in analogues of the THI series [25], suggesting that the THIs have a different mode of binding towards the \( \beta \)-adrenoceptor.

The presence of a carboxylate anion in the binding pocket may help in the rationalization of the binding modes of the various \( \beta \)-adrenergics. As an ex-
Fig. 4. A fit of the tetrahydroisoquinoline trimetoquinol (TMQ) on the \([\text{tetralin}]\,[\text{formate}]\) complex.

Fig. 5. The \([\text{tetralin}]\,[\text{formate}]\) complex (left) and the position of its associated ligand points and carboxylate site point (right).

ample, a fit of representatives of these two classes is shown in Fig. 4. In this model, the amino group of the THIs takes the position of the \(\beta\)-OH group of the tetralin derivatives. This is a purely geometrical fit: a geometry optimization of the THI-formate complex and a full receptor mapping analysis comprising all major classes of adrenergics is outside the scope of this article and will be published elsewhere [26].

In the preliminary study described here, we limited ourselves to a receptor mapping analysis comprising the 2-aminotetralins [Fig. 1(b)] and the phenoxyproanolamines [Fig. 1(c)].

The \([1R,2R-1,5,6\text{-tri hydroxy-2-aminotetralin}]\,[\text{formate}]\) complex (Fig. 5) was used as a template molecule for the receptor mapping procedure. The co-
ordinates of the tetralin moiety were taken from a published crystal structure [27]. In this particular structure \( \tau_{\text{OC}CN} \) is equal to \(-73^\circ\). In computro, we then attached the formate group to the tetralin structure using the coordinates of the optimized ethanolamine-formate complex (family A, \( \tau_{\text{OC}CN} = -72.3^\circ \)).

The essential ligand points used in the fitting procedure were selected as follows.

(1) Two ligand points defined on the normal through the centre of mass of the aromatic ring located at 3.5 Å on either side of the ring.

(2) Two ligand points located on the oxygen atoms of the \( m\)-OH and \( p\)-OH substituents at the aromatic ring.

(3) One ligand point located on the central carbon atom of the carboxylate moiety.

The distance matrix of the ligand points of the template tetralin structure is given in Table 2.

The rationalisation of the fact that phenylethanolamines [PEAs, Fig. 1(a)] and aryloxypropanolamines [AOPAs, Fig. 1(c)] share a common binding site has received considerable attention in the literature [28,29]. As a result of the presence of an oxymethylene bridge between the aromatic nucleus and the ethanolamine side chain, a similar spatial relationship between the essential groups (which are known to be the aromatic nuclei, the \( \beta\)-hydroxyl group and the amino function) is impossible. It is therefore assumed in most studies that the aromatic nucleus of the AOPAs occupies a region at the receptor site different from that of the PEAs. The strong \( \beta\)-adrenergic activity of catechol-type AOPAs such as RO 363 [Fig. 1(c), \( R = \text{homoveratryl} \)], on the other hand, suggests that the catechol nuclei occupy identical regions at the receptor surface.

In order to check the latter possibility, the formate complex of the compound depicted in Fig. 1(c) (\( R = H \)) was included in this study. The ligand points for this AOPA-type compound were selected in a similar fashion to that described

**TABLE 2**

Distance matrix of the pharmacophoric points and carboxylate site point in the template [(1R,2R)-1,5,6-trihydroxy-2-aminotetralin] [formate] complex

<table>
<thead>
<tr>
<th></th>
<th>C30</th>
<th>D34</th>
<th>O13</th>
<th>O14</th>
<th>D33</th>
</tr>
</thead>
<tbody>
<tr>
<td>C30</td>
<td>0.00</td>
<td>6.25</td>
<td>8.78</td>
<td>8.27</td>
<td>7.81</td>
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<td>D34</td>
<td>0.00</td>
<td>4.48</td>
<td>4.44</td>
<td>7.00</td>
<td></td>
</tr>
<tr>
<td>O13</td>
<td>0.00</td>
<td>0.00</td>
<td>2.69</td>
<td>4.44</td>
<td></td>
</tr>
<tr>
<td>O14</td>
<td>0.00</td>
<td>0.00</td>
<td>4.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D33</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For the location of the ligand points and the site point, see Figs. 5 and 6.
Fig. 6. The [phenoxypropanolamine] [formate] complex and its associated ligand points and carboxylate site point. The torsion angles are defined as follows: \( \tau_1 \), C(1)–C(2)–O(3)–C(4); \( \tau_2 \), C(2)–O(3)–C(4)–C(5); \( \tau_3 \), O(3)–C(4)–C(5)–C(6).

for the tetralin template molecule (Fig. 6). As the conformational freedom of the ethanolamine part of the AOPA side chain is restricted by the complex formation with the formate, the number of freely rotatable bonds in the AOPA structure is limited to three. The restricted conformational freedom of \( \tau_{OCCN} \) was modelled by setting \( \tau_{OCCN} \) to discrete values for which ethanolamine–formate complex geometry optimizations had been carried out and simply attaching the formate to the AOPA using the coordinate data obtained for the ethanolamine–formate complexes. In this way a series of AOPA–formate complexes was obtained. The preliminary analysis presented here is restricted to a conformational analysis of only two family A complexes (i.e. \( \tau_{OCCN} \) values of \(-72.3^\circ\) and \(-62.3^\circ\)). These values were selected as they are close to the absolute minimum energy conformations of the ethanolamine–formate complex. A full analysis, including all possible complex conformers, will be presented elsewhere [26]. Each of these two complexes was then subjected to a further conformational analysis by varying each of the remaining torsional angles in steps of \( 10^\circ \). Using a procedure described elsewhere [29], the distance matrix of each calculated AOPA conformer was checked against the distance matrix of the template molecule, allowing a maximum distance deviation of 0.2 Å. Using an energy rejection criterion of 40 kcal mol\(^{-1}\), only the energetically favourable compatible conformations were saved to disk.

RESULTS AND DISCUSSION

Conformational analysis of ethanolamine–formate complex

The results of the ab initio STO-3G geometry optimization of ethanolamine, format and the ethanolamine–formate complex may be summarized as follows.
(a) Two hydrogen bridges are formed \(d_{\text{O(4)-O(6)}} = 2.6 \text{ Å}, d_{\text{N(3)-O(8)}} = 2.7 \text{ Å}\).
(b) A proton is transferred from the \(\text{NH}_3^+\) group to the formate.
(c) The optimum \(\tau_{\text{OCCN}}\) of the complex is \(-57.3^\circ\).

These results are in line with the results of previous investigations by Solmajer et al. [13]. For the sake of comparison, the total energy of the complex was re-evaluated at the STO 4G level for \(\tau_{\text{OCCN}} = -64^\circ\) and found to be \(-395.490063 \text{ H (Hartree)}, \) which is 0.026 H (ca. 16 kcal mol\(^{-1}\)) lower than the value found by Solmajer. The difference is due to the fact that Solmajer performed a less complete geometry optimization.

Some X-ray studies provide direct experimental evidence for the existence of chelate-type hydrogen bonds in substituted 2-hydroxyethylamines. Gorman et al. [30] found this type of hydrogen bonding in the crystal structure of the [ephedrine] \([N\text{-benzyloxy carbonyl-L-leucine}]\) complex. An investigation of a number of ethanolamine salts in the Cambridge Structural Database reveals similar hydrogen-bonding behaviour in the crystal structure of ephedrine mono-hydrogenphosphate [23]. A very recent study by Tintelnot and Andrews discusses, among other things, the surroundings of carboxylic groups in proteins. Chelate-type binding with arginine residues occurs quite often [31].

The dependency of the interaction energy on \(\tau_{\text{OCCN}}\) is shown in Fig. 7.

The following conclusions can be drawn.

1. Both the A and B family of complex conformers have absolute minima around \(\tau_{\text{OCCN}} = -50^\circ\).

Fig. 7. Differences in the interaction energy of each of the calculated conformers with respect to the absolute minimum energy conformation (Family A, \(\tau_{\text{OCCN}} = -52.3^\circ\); \(E_{\text{int}} = -146.1 \text{ kcal mol}^{-1}\)).
Hearn et al. evaluated $\tau_{OCCN}$ for a number of crystal structures of $\beta$-adrenergic ethanolamines and found values varying from $-40^\circ$ to $-74^\circ$ [23].

(2) Family A conformers show consistently higher interaction energies between the ethanolamine and the formate moieties. The difference is, of course, nil at $\tau_{OCCN}=0^\circ$, which is the borderline between family A and family B conformers. The largest difference is found at the minimum energy conformers (4 kcal mol$^{-1}$).

(3) The form of the curves can be explained by considering the following factors. (a) The strength of hydrogen bonds (largest at $\tau_{OCCN}=0^\circ$). Enlargement of $\tau_{OCCN}$ to values greater than $140^\circ$ (B-family conformers) leads to a disruption of the O(4)–H(5)⋯O(6) hydrogen bond and, consequently, a lowering of the interaction energy. (At $\tau_{OCCN}=132^\circ$ the H(5)–O(6) distance is 1.9 Å, whereas this distance grows to 2.7 Å at $\tau_{OCCN}=160^\circ$). (b) Unfavourable Van der Waals contact between H(11) and O(8) in type-A conformers for values of $\tau_{OCCN}< -80^\circ$. (c) Unfavourable Van der Waals contact between

TABLE 3

Compatible [phenoxypropanolamine][formate] conformers (family A, $\tau_{OCCN}$ values of $-72.3^\circ$ and $-62.3^\circ$)

<table>
<thead>
<tr>
<th>$\tau_3$</th>
<th>$\tau_2$</th>
<th>$\tau_1$</th>
<th>$E$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\tau_{OCCN} = -72.3^\circ$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-170.0</td>
<td>75.0</td>
<td>80.0</td>
</tr>
<tr>
<td>2</td>
<td>100.0</td>
<td>115.0</td>
<td>80.0</td>
</tr>
<tr>
<td>3</td>
<td>110.0</td>
<td>-95.0</td>
<td>-110.0</td>
</tr>
<tr>
<td>4</td>
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<td>115.0</td>
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</tr>
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<td>5</td>
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<td>115.0</td>
<td>70.0</td>
</tr>
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<td>-110.0</td>
</tr>
<tr>
<td>7</td>
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<td>105.0</td>
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<td>80.0</td>
</tr>
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</table>

For the definitions of $\tau_1$, $\tau_2$ and $\tau_3$, see Fig. 6. The energy of the conformers was calculated using simple Lennard-Jones and torsional terms, see ref. 29.
Fig. 8. Representatives of each of the two clusters of compatible [phenoxypropanolamine][formate] conformers found. The distance matrices of the template [tetralin][formate] complex and the [phenoxypropanolamine][formate] complex overlap within the tolerance. Left, conformation 13; right, conformation 14 (see Table 3).

H(13) and O(6) in type-B conformers. The maximum steric repulsion occurs at \( \tau_{\text{OCCN}} = -97^\circ \). The H(13)-O(6) distance increases again at either side of this \( \tau_{\text{OCCN}} \) value. (d) Energy differences between eclipsed and gauche conformers of the ethanolamine itself.

On the basis of the relatively small lowering of the interaction energy at \( \tau_{\text{OCCN}} < 140^\circ \), it may be concluded that the strength of the O(4)-H(5)\( \cdots \)O(6) hydrogen bond is small as compared with the N(3)-H(9)\( \cdots \)O(8) hydrogen bond. This is to be expected as the latter hydrogen bond has an ionic nature. We performed some model calculations on the ethylamine-formate complex to evaluate this. It was found that the O(4)-H(5)\( \cdots \)O(6) hydrogen bond contributes only ca. 6% to the total binding energy. On the basis of these results, it may be safely concluded that the N(3)-H(9)\( \cdots \)O(8) hydrogen bond is the major determinant of the total interaction energy.

**Automatic flexible fitting procedure**

The resulting conformations of the phenoxypropanolamines compatible with the tetralin-formate complexes as generated by our automatic flexible fitting procedure are given in Table 3. Analysis of the results shows that the compatible conformations can be divided in two clusters: one cluster is characterized by negative values of \( \tau_1 \) and \( \tau_2 \) (Fig. 6) whereas the other cluster displays positive values for these torsion angles. The resulting fits of two representatives of these clusters are depicted in Fig. 8.
CONCLUSION

The incorporation of a carboxylate moiety in a receptor mapping study on the \( \beta \)-adrenoceptor clearly offers opportunities in the rationalization of the binding behaviour of some major classes of \( \beta \)-adrenergics. This study suggests an important role of hydrogen bonds in the interaction of \( \beta \)-adrenergics with the \( \beta \)-adrenoceptor.

This modelling study accounts for the following experimental findings.

(a) On the basis of the relatively small contribution of the \( \beta \)-OH group to the total interaction energy of the side chain, a rationalization can be given for the fact that compounds missing the \( \beta \)-OH group still show a considerable affinity, whereas compounds missing the amino function do not bind to the \( \beta \)-adrenoceptor.

Compounds in which the amino and \( \beta \)-OH functions are exchanged still show affinity, albeit less pronounced [32].

(b) A rationalization can be given for the fact that both cisoid and transoid side chain conformers can interact with the receptor.

(c) The oxymethylene bridge in the AOPA-type compounds does not exclude a binding mode of the aromatic nucleus in which it interacts with the same receptor subsite as the aromatic nucleus of the PEA-type compounds.

The deduction of the final receptor map has very recently been completed at our laboratories. These results will be published elsewhere [26].

Our approach can be considered as a hybrid of a docking study and a receptor mapping study. This hybrid approach might be useful in other cases in which preliminary data on the structure of receptor proteins are available.

ADDITIONAL MATERIAL

Upon request, the authors can provide coordinates of the optimized complexes.

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