Journal of Computer-Aided Molecular Design, **13:** 57–68, 1999.
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A computational model of the nicotinic acetylcholine binding site

Enrique Gálvez-Ruano[a,*], Isabel Iriepa-Canalda[a], Antonio Morreale[a] & Kenny B. Lipkowitz[b]
[a]*Departamento de Química Orgánica, Universidad de Alcalá, E-28871 Alcalá de Henares, Spain;* [b]*Department of Chemistry, Indiana University-Purdue University at Indianapolis, 402 North Blackford Street, Indianapolis, IN 46202, U.S.A.*

Received 17 July 1998; Accepted 13 August 1998

Key words: agonists, antagonists, minireceptor, molecular dynamics

Summary

We have derived a model of the nicotinic acetylcholine binding site. This was accomplished by using three known agonists (acetylcholine, nicotine and epibatidine) as templates around which polypeptide side chains, found to be part of the receptor cavity from published molecular biology studies, are allowed to flow freely in molecular dynamics simulations and mold themselves around these templates. The resulting supramolecular complex should thus be a complement, both in terms of steric effects as well as electronic effects, to the agonists and it should be a good estimation of the true receptor cavity structure. The shapes of those minireceptor cavities equilibrated rapidly on the simulation time scale and their structural congruence is very high, implying that a satisfactory model of the nicotinic acetylcholine binding site has been achieved. The computational methodology was internally tested against two rigid and specific antagonists (dihydro-β-erytroidine and erysoidine), that are expected to give rise to a somewhat differently shaped binding site compared to that derived from the agonists. Using these antagonists as templates there were structural reorganizations of the initial receptor cavities leading to distinctly different cavities compared to agonists. This indicates that adequate times and temperatures were used in our computational protocols to achieve equilibrium structures for the agonists. Overall, both minireceptor geometries for agonists and antagonists are similar with the exception of one amino acid (ARG209).

Introduction

Different families of receptors acting at the central nervous system (CNS) can be defined. Among them, the nicotinic acetylcholine receptor (nAChR) is an important one due to its role in several cognitive disorders such as Alzheimer's and Parkinson's diseases. The nAChR belongs to a super-family of ligand-gated channels and is cation selective (Na^+). Its functional repertoire consists of, at first glance, several simple actions: first, binding a specific neurotransmitter; second, opening a gate; third, conducting Na^+ ions across the membrane and fourth, desensitizing.

nAChR is a large protein (MW ca. 290 000) to which a small neurotransmitter molecule, acetylcholine (ACh) binds. Due to the fact that the binding site is in the extracellular domain and the gate is close to the cytoplasmatic end of the channel, when ACh binds, the channel opens via propagated conformational changes throughout the polypeptide.

Understanding how the receptor interacts with ACh from the structural point of view means knowing which residues (amino acids) are most important as well as knowing how they are organized in the binding site. This information can help us to elucidate the type of interactions that occur between ACh and the receptor cavity. To address these issues the following four items must be considered:
(1) *Amino acid sequence.* Using *Torpedo californica* (used by neurochemists as a model for homologous receptor for higher vertebrates because of its conserved structure through evolution), the primary structure for the five component subunits of nAChR has been elucidated [1–3]. We also mention here the paper of Maricq et al. [4] in which they showed the sequence identity

*To whom correspondence should be addressed.

between nAChR and the other members of this family (GABA, glycine and $5HT_3$ receptors).
(2) *Three-dimensional structure.* The overall structure of the receptor, in the absence [5] and in the presence [6] of ACh, was determined by Unwin using electron microscopy. Other features of the tertiary and even the secondary structure were also determined.
(3) *Three loop theory.* Using several photoaffinity labeling probes, Devillers-Thiery et al. [7] proposed a model of the binding site for agonists and competitive antagonists. From their studies several amino acids located within the domain of the nAChR have been identified: loop A, TRP86, ASP89 and TYR93; loop B, TRP149 and TYR151; loop C, TYR190, TYR198 and ARG209.
(4) *Mutagenesis studies.* The same group carried out mutagenesis studies and showed that the functionality and/or activity of the channel changes after mutation of the aforementioned amino acids. Accordingly, those amino acids are considered to be most important in the interaction with ACh.

With respect to the type of interaction taking place between neurotransmitter and receptor cavity, Dougherty and Stauffer [8] proposed a model in 1990 called 'cation-π interaction' as a novel type of interaction for ACh with aromatic amino acids at biological binding sites. This model is relevant to our studies. In our situation the binding site is composed mostly of aromatic amino acids and the ACh is a cationic molecule, as are nicotine and epibatidine (at physiological pH the amino groups are protonated). Moreover, this model is in good agreement with the type of interactions that we have proposed earlier [9]. Molecular modeling approachs to elucidate the nAChR structure are in progress from other laboratories. The work of Sansom [10], using simulated annealing via restrained molecular dynamics is an example where good results compared with experimental data are obtained.

In this paper we address the following question: what is the structure of the nicotinic receptor binding site? To answer that question we determine what polypeptide residues comprise the binding site and then use molecular dynamics simulations to allow those residues to mold themselves around known potent agonists thereby forming a binding pocket.

Materials and methods

Description of the model. Our model is based on three fundamental assumptions:

- The receptor is complementary to the conformation in which the neurotransmitter binds.
- The ACh receptor cavity is composed of the amino acids that, to date, are described in the work of Deviller-Thiery.
- The *arginine* theory.

A preliminary account of our model can be found in a previous paper [9]. However we think it is important to specify and clarify these assumptions.
I. The conformation in which the ACh binds to the nAChR is not known experimentally. However, we propose in Figure 1 (see Results) the bioactive conformation of ACh. We rationalize this conformation as follows: The two *rigid* antagonists, which are *specific* to nAChR at the CNS, are superimposed by ACh. Moreover, due to the specificity of these antagonists on displacing ACh from the binding pocket, it is reasonable to assume structural and electronic congruency between these two molecules and ACh (see Results).
II. From the biological studies performed on the nAChR [7], we know the functional amino acids making up the receptor cavity. These amino acids are SER82, ASP83, TRP86, ASP89, TYR93, TYR190, TYR198 and ARG209. The way in which we arranged these amino acids initially around the neurotransmitter is based on the following stereoelectronic criteria: (a) SER82, ASP83, TRP86, ASP89 and TYR93 (region in the receptor with enhanced electron density) are associated with the cationic part of ACh (electron-deficient). (b) ARG209, TYR190 and TYR198 (region in the receptor with deficient electron density) are associated with the ester end of ACh (planar and rich in electron density). (c) Hydrogen bonding interaction, van der Waals forces and the formation of a charge-transfer complex, are also taken into account.
III. Once ACh binds to the recognition site, ARG209 is positioned near a physiological location that triggers conformational changes and induces the channel to open. The location of ARG209 for other agonists like nicotine or epibatidine quite similar to that of ACh (vide infra). The contrary is observed when the antagonists are used, and thus, the channel remains closed. In other words, the location of ARG209 is critical to channel opening, and this location is a criterion we can use to classify molecules as agonists or antagonists. Our molecular dynamics simulations confirm this hypothesis (vide infra).

Computational tools. Molecular mechanics and molecular dynamics calculations were done us-

ing QUANTA/CHARMm, a commercially available molecular modeling program from Molecular Simulations, Inc. Molecular structures were built using templates having standard bond lengths and bond angles that are geometry optimized. Atomic charges were assigned using the CHARMm Param 23.2 parameter set [11,12] and molecular mechanics calculations were done using the CHARMm force field [11]. Geometry optimizations were carried out with an Adopted-Based Newton Raphson optimization algorithm [11]. Structures were considered fully optimized when the energy changes between iterations were less than 0.01 Kcal/mol. For all calculations described herein the dielectric of the medium was set to unity, the electrostatic cutoff distances were set to 14 Å and the Lennard-Jones nonbonded cutoff distances were set to 14 Å.

Molecular dynamics calculations were done using the same force field, parameter set, dielectric constant and cutoffs as in the molecular mechanics calculations. A velocity verlet algorithm was used to integrate Newton's equations of motion using 1 fs timesteps. The SHAKE algorithm [13] was used to constrain bond lengths and bond angles to their equilibrium values with a relative tolerance of 10^{-9} thus enabling a time step of 1 fs to be used for the integrations of the equations of motion. The simulation protocol involved adding kinetic energy to the system slowly over a time period of 25 ps, and then bringing the temperature of the system to body temperature (310 K). The system's kinetic and potential energies were allowed to fully equilibrate over a time period of 50 ps. Following this a full simulation, hereafter called the production run, was carried out over a time period of 1000 ps. During this time period molecular structures were uniformly sampled along the system's trajectory and stored on disk for post-simulation processing.

Water molecules used in the molecular dynamics simulations are the TIP3 waters of Jorgensen [14]. Molecular structure comparison was done using a least squares fitting facility in QUANTA. Rigid body fittings were carried out to determine the structural similarity or difference between pairs of structures. The (dis)similarity of two structures is given as a root mean square (rms) deviation in Å. The more similar two structures are, the smaller this value becomes, converging ultimately to zero when the same molecule is superimposed on itself. The electrostatic similarity was carried out using Carbo's method [15] as implemented in SPARTAN [16]. In this case the (dis)similarity of two structures is given by a dimensionless parameter, Carbo's index (C.I.), between 0 and 1 where the former indicates completely different structures while the later is indicative of a perfect matching.

Modeling strategy. The strategy we adopt here is to assume there exists a complementary relationship between known agonist molecules and the acetylcholine receptor cavity. This complementarity should reflect both steric and electronic features of the system. Hence we use the agonist molecules as templates around which pertinent amino acids side chains of the polypeptide are molded. In this regard, then, we consider the agonist as a 'hand' and the receptor, whose structure is as yet unknown but which will be determined (in part) here, as a 'glove'. This glove is formed conceptually by selecting it's major components (the amino acid side chains) and allowing those components to flow around the agonist molecule until they reach a thermodynamic, equilibrium structure. This equilibrium structure is a host-guest complex that reflects the electronic and steric complementarity between the agonist and the receptor.

To accomplish this we use the above mentioned conformation for ACh. Initially we position the amino acid side chains (see above) around this conformation of agonist such that hydrophobic groups of the receptor are near hydrophobic patches of the agonist, negative receptor residues are near oppositely charged, positive sites of agonist and positively charged receptor residues are adjacent to negatively charged atoms on the agonist. This initial structure is immersed in a 15 Å radius sphere of TIP3 water molecules and geometry optimized with molecular mechanics to alleviate any bad van der Waals contacts between water and solute (the receptor surrounding the agonist).

At this stage we have an initial 'guess' as to what the receptor cavity looks like. However, even though we have geometry optimized the entire system it is clear that individual water molecules, both inside as well as on the exterior of the minireceptor-encapsulated agonist molecule, are only in local energy minima. More important, though, is that we have not allowed the system to fully equilibrate in a way that amino acid groups can move freely (albeit within an aqueous environment) to their preferred locations around the agonist (or antagonist) molecule.

The initial guess structure is allowed to evolve to its thermodynamically most probable structure, presented here as the system's final resting state, over a long simulation time of 1 ns. This thermodynamically

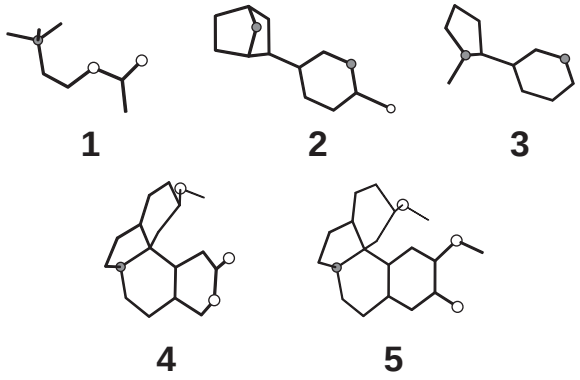

Figure 1. The five molecules under study. **1**, acetylcholine; **2**, epibatidine; **3**, nicotine; **4**, dihydro-β-erytroidine; **5**, erysoidine. Open circles represent oxygen, except in molecule **2** that represents Cl, shaded circles stand for nitrogen and the rest are carbon atoms. Hydrogen atoms are omitted for clarity.

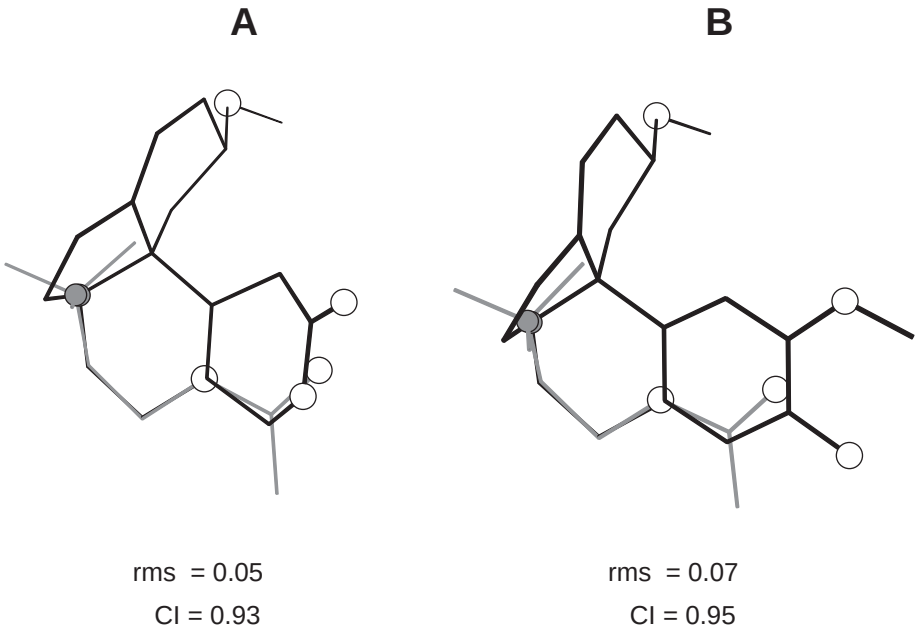

Figure 2. (A) Least squares fitting of acetylcholine with dihydro-β-erytroidine. (B) Least squares fitting of acetylcholine with erysoidine. Atom designations are as in Figure 1.

derived 'minireceptor', so named because we only use the unlinked salient amino acid side chains constituting the receptor cavity rather than the entire polypeptide, should be similar in shape for similar guest molecules, i.e., the agonists. But, the shape of the cavity should be different for dissimilar guest molecules, i.e., the antagonists. This similarity/dissimilarity is an internal test, then, of the computational strategy we use in our study here. The results of our simulations provide us with an initial glimpse as to the true shape of the nAChR cavity. Using this knowledge, further studies to better elucidate the structure of the receptor can take place along with *de novo* drug design strategies to predict novel drug candidates for the nAChR.

Several pertinent questions can be raised at this point. First, how far will the side chains migrate in such a simulation? It will be shown below that for the agonists little migration occurs while for the antagonists an argenine side chain migrates substantially (see also the section on validation of the computational protocol). Next, what information is to be derived from such a crude model? For us, deriving even a semblance of what the real receptor cavity looks like

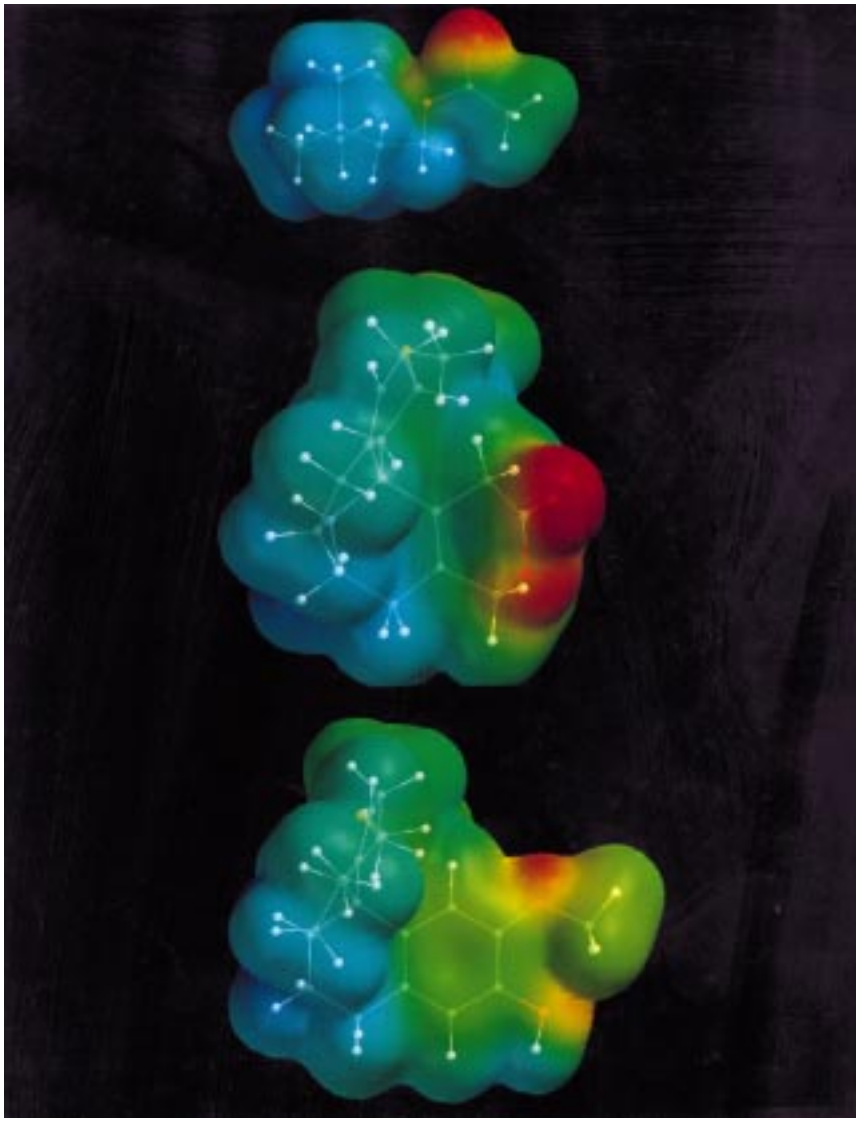

Figure 3. Molecular electrostatic potential surfaces for **1** (top), **4** (middle) and **5** (bottom). Derived using the AM1 Hamiltonian and continuum solvent model.

is especially useful because it allows us to consider alternative receptor structures that might alternatively be possible in light of known binding affinities and biological activities for other agonists/antagonists, but it also allows us to begin our ultimate goal of *de novo* design, synthesis and pharmacological testings of these new molecules. Next, is the global minimum hoped to be found from the MD simulations more meaningful than a local minimum for such a model? There is no unambiguous answer to this question; we base our construction of the receptor binding site on the premise that receptor-substrate binding complexes are at (or near) their thermodynamic equilibrium geometries, a point proved later where we show our thermodynamically derived structures are retained for five known complexes. However this is simply a working assumption that we are cognizant of and which my not be true for the nAChR. Finally, in this regard, using 'flying' amino acid residues in a water bath could be problematic for describing receptors that discriminate between different agonists and antaginist molecules by means of steric interactions.

Results

The agonists: acetylcholine, **1**, epibatidine, **2**, and nicotine, **3**; and the antagonists: dihydro-β-erytroidine, **4**, and erysoidine, **5**, are presented in Figure 1.

Above, we assumed structural and electronic congruence between the ACh, **1**, and **4** and **5** . Here we justify quantitatively that assumption. The structural similarities between **1** and the antagonists are depicted in Figure 2, where a rigid least-squares fit has been performed between **1** and **4** and between **1** and **5**.

The similarity between acetylcholine and both antagonists can be quantified; the rms fit between **1** and **4** is 0.05 Å and between 1 and **5** is 0.07 Å.

To assess the electronic congruence between these molecules we have calculated their electrostatic potential surfaces (EPS) and performed a similarity analysis in the same way as before (**1** with **4** and **1** with **5**). In Figure 3 we can see the EPS, at the AM1 level of theory with solvation included.

Using Carbo's method to calculate the (dis)similarity between two molecules, from the EPS point of view, we find excellent electrostatic conguence: **1** with **4** 0.93 and **1** with **5** 0.95.

Based on the above conformation for ACh, which is consistent with the rigid antagonists, the other two agonists, **2** and **3**, were superimposed onto ACh. We use here the same procedure as before, and present those fits in Figure 4. The rms agreement between **1** and **2** is 0.12 Å and between **1** and **3** is 0.15 Å, again indicating structural congruence. The EPS for **1**, **2** and **3** are presented in Figure 5. All three agonists are likewise congruent in an electrostatic sense.

Following the computational strategy outlined in the Methods section above, each molecule is surrounded by the receptor's amino acid side chains, geometry optimized with molecular mechanics, and then placed in a 15 Å radius sphere of TIP3 water molecules. We find 393 water molecules in and around the acetylcholine complex and 391 water molecules for the nicotine, epibatidine, dihydro-β-erytroidine and erysoidine complexes. These solvated systems are further geometry optimized, heated, equilibrated and then a 1 ns simulation is carried out. To reiterate, the computational methodology we adopt is to use the ligands (**1**–**5**) as templates around which the receptor side chains could flow freely, molding themselves into a thermodynamically acceptable supramolecular shape that is a complement to the ligands. Initially we thought to hold fixed the ligands in their aforementioned shapes throughout the simulation. However, we allowed the ligands to adopt any conformer they wanted during the simulation. In other words, no constraints of any type were put on the ligands during the simulation. As it turns out, the time-averaged shape of acetylcholine over the entire simulation time period is very similar to it's initial conformation. Likewise, the average shapes of nicotine, epibatidine, dihydro-β-erytroidine and erysoidine are similar to their proposed bioactive conformations. The measure of agreement between time-averaged structures (averaged over the entire simulation time period) compared to their corresponding initial conformations are: for **1** 0.04 Å; for **2** 0.05 Å; for **3** 0.06 Å; for **4** 0.09 Å and for **5** 0.07 Å.

The thermodynamically averaged nAChR derived from our simulations is presented in Figure 6. For each simulation the atomic coordinates for all amino acid side chains are averaged. Hence one obtains an average shape of the minireceptor as it is molded around each of the agonists and each of the antagonists (note that there is no physical basis for doing this averaging; rather we show such an averaged structure for convenience only). Individual molecular graphics views for each minireceptor could be presented. Instead, however, we note that the minireceptor formed for all three agonists are very similar in shape to one another (in the natural system the receptor will modify its shape slightly to accomodate these molecules) so we average the atomic coordinates for the three minireceptors derived for each of the three agonists. This provides an average, composite view of what the receptor should look like for nAChR binding to the agonists in general which is the problem we are attempting to solve in this study.

The same kind of strategy was then used for the antagonists. The thermodynamically averaged nAChR in this case is different, as expected. We will comment about this in a following section.

Validation of the computational protocol

The computational approach developed here is in essence a thermodynamic annealing strategy. Using amino acids from the polypeptide chain that are known (from molecular biology studies) to interact with the neurotransmitters we first judiciously place those amino acids around each neurotransmitter and then heat the solvated system using a Newtonian dynamics technique until the system has been equilibrated.

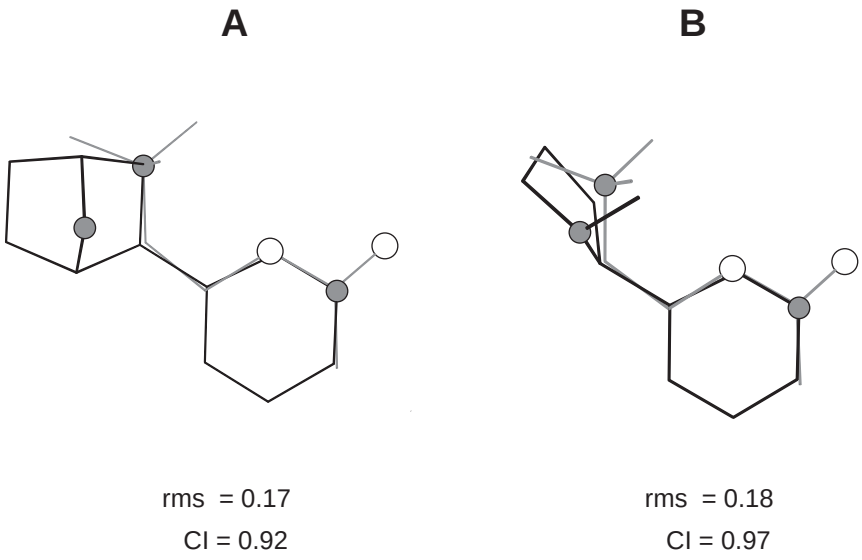

Figure 4. (A) Least squares fitting of acetylcholine with epibatidine. (B) Least squares fitting of acetylcholine with nicotine. Atom designations are as in Figure 1.

As expressed above we find that the interacting amino acids move to and then fluctuate about their equilibrium positions. In some ways the approach presented is similar to the mini- and pseudoreceptor methodologies described by Vedani and Snyder [17] but we select the interacting amino acids to be placed around the neurotransmitter based on molecular biology experiments rather than using a probabilistic amino acid selection method [18].

At this point we need to ask how valid the methodology is. Unfortunately we can not compare our predicted receptor structure to experiment because no experimental structure exists. However, we can use this computational methodology on other known receptor systems to evaluate its merits and this has been done. We selected five different ligand-receptor structures that have been co-crystallized and whose X-ray crystallographic structures are known (see below). The substrates, and the interacting amino acid residues comprising the receptor cavity, were generated from each crystal structure, energy minimized, solvated with TIP3 waters and taken through the simulation protocols as described above. To generate the minireceptor a five Angstrom radius region from the substrate's center of mass was excised from the PDB crystal structure so that whole residues were taken. Missing valences needed for the empirical force field-based MD calculations were then filled with hydrogens. This generated the initial guess of amino acid side chains (that in these instances are known in contrast to our nAChR problem). Of particular concern in this computational methodology was whether or not the five (known) solvated 'receptors' containing their bound guests would maintain their structural integrity during the simulation or whether they would dissociate or rearrange; a situation that would cast doubt on the method we used because, presumably, these co-crystalized systems are at or near their equilibrium structures.

Three acetylcholinesterase inhibitors were studied first: tacrine [20] (PDB ref. code 1acj); edrophonium [20] (PDB ref. code 2ack); and huperzine [21] (PDB ref. code 1vot). We also considered two adenosine deaminase inhibitors: 1-deaza-adenosine [22] (PDB ref. code 1add) and 6-hydroxyl-1,6-dihydropurine ribonucleoside [22] (PDB ref. code 2ada) to serve as a representative and diverse set of receptor-substrate systems to ensure the generality of our method. To monitor the structural integrity of the receptor-substrate complexes we evaluated the root mean squared (rms) differences between the solvated MD structures to the initial, X-ray derived structures. The rms differences for each system, averaged over the simulation time periods, are as follows: 1acj (2.56); 1ack (2.53); 1vot (1.80); 1add (2.51); 2add (1.43). These values correspond to the heavy (non-hydrogen) atom positions. In no examples throughout their trajectories did the rms difference exceed 3.5 Å, and in two examples the greatest difference between simulation structure and known crystal structure was <2 Å.

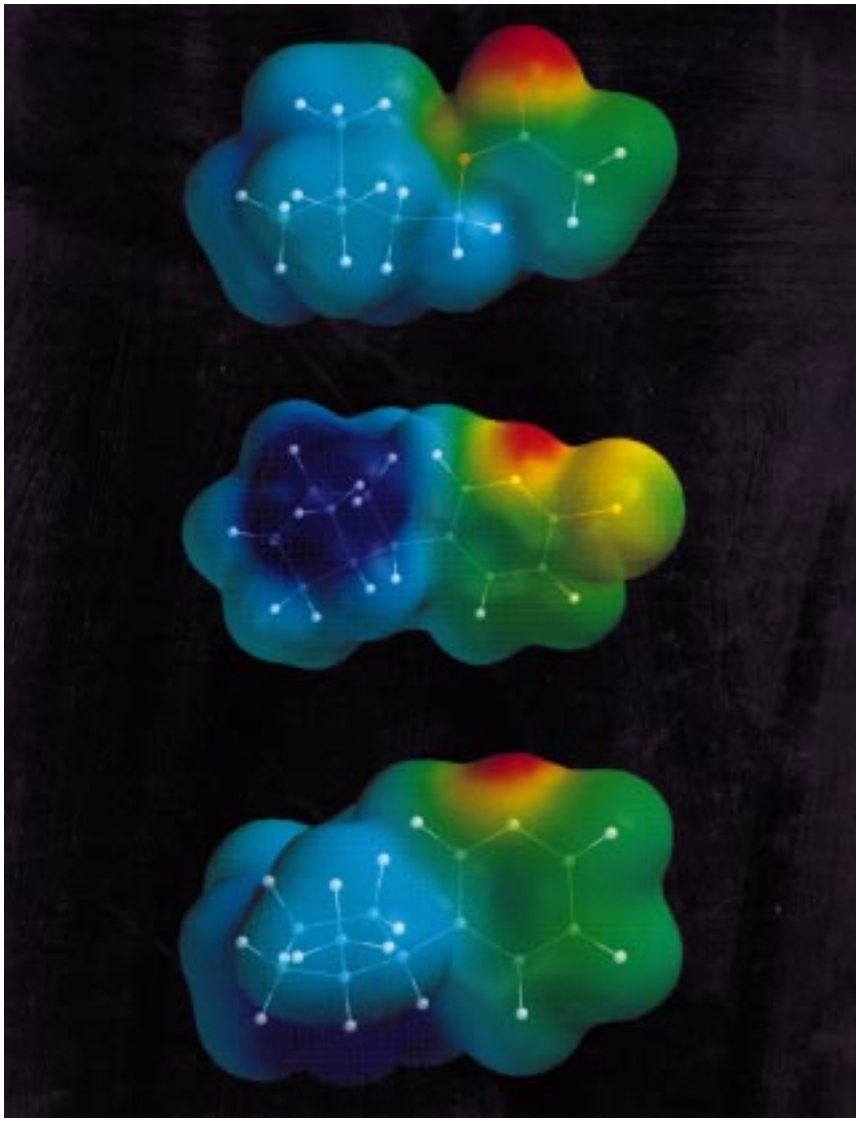

Figure 5. Molecular electrostatic potential surfaces for **1** (top), **2** (middle) and **3** (bottom). Derived using the AM1 Hamiltonian and continuum solvent model.

Finally, another 1 ns was run in the 1acj example to see how stable the structure would be after doubling the time period of our methodology. For that simulation the rms difference is 2.9 Å which is a further indication that the system has equilibrated and is stable.

Hence for the five systems whose receptor structures are known, we find that we can maintain those experimental receptor cavities using our methodology. Moreover, for the unknown nAChR we find three of our predicted receptor structures to be equivalent for the natural neurotransmitter and two of its agonists as one would anticipate, but, we find they adopt a different structure for the nAChR when antagonists are bound also as anticipated. These results, taken *in toto*, validate the computational methodology we have adopted for this study.

Discussion

Bioactive conformation for ACh at the nAChR binding site. It is well known that the bioactive conformation frequently is not the global minimum energy conformation and as much as 30 kcal/mol between both can, in principle, exist [23]. Also, there must be a steric

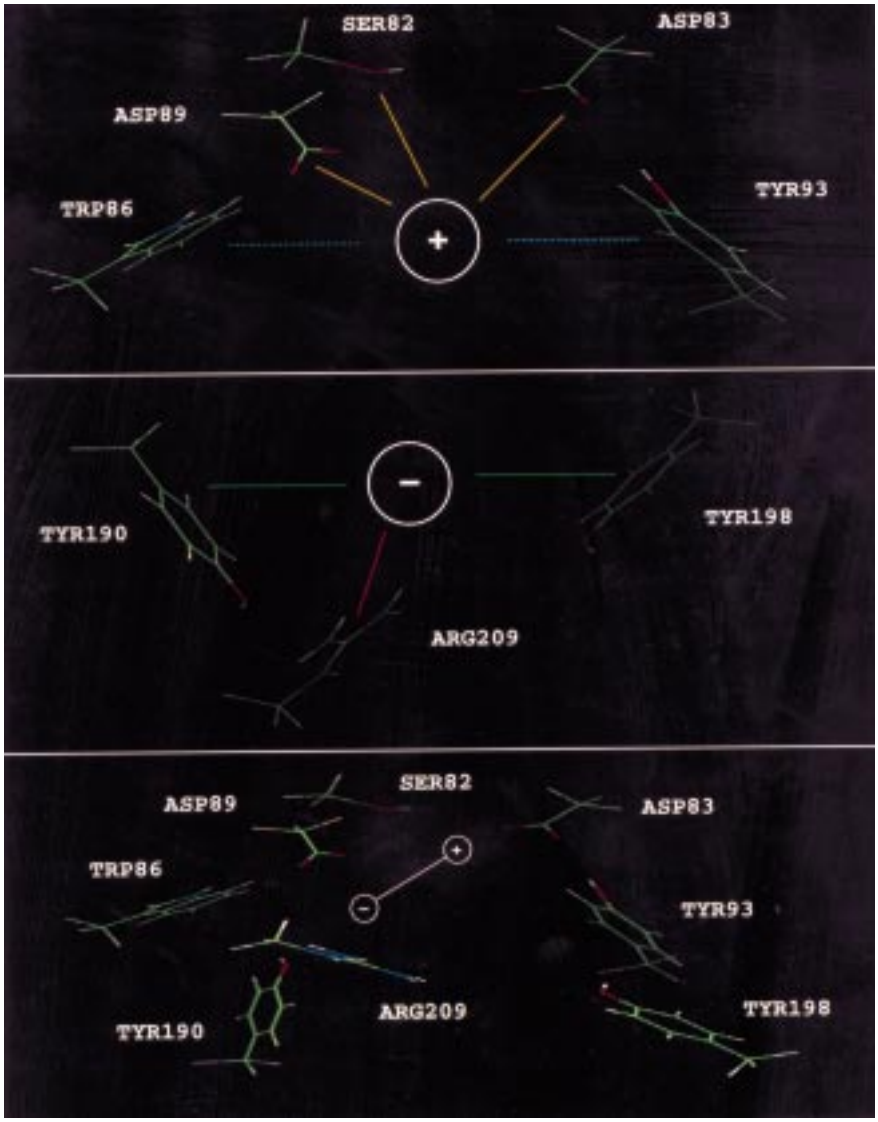

Figure 6. Thermodynamically averaged nAChR binding pocket. The top view represents the interaction between the cationic part of the agonists and the negative part of the binding pocket. The middle view represents the interaction between the anionic part of the agonists and the positive part of the binding pocket. The bottom view is the whole representation of the interaction between the agonists (represented as a dipole) and the receptor cavity derived from molecular simulations in a water bath. Water molecules are omitted for clarity. Color coded lines in this figure are described in the text.

and electronic complementarity between the bioactive conformation and the receptor binding site. This leads to two questions regarding our proposed bioactive conformation for ACh: How far are we, energetically, from the global minimum conformation, and, what information can we deduce about the steric and electronic requirements of the nAChR? With respect to the first question, the bioactive conformation we propose for ACh was obtained with a fully geometry optimization procedure using the AM1 Hamiltonian and a continuum solvent model. A conformational analysis was undertaken using the CHARMm force field and implementing a standard grid search method. The global minimum so obtained, was then fully geometry optimized in the same way as the proposed bioactive conformation and found to be 5 kcal/mol more stable than the bioactive conformer. To address the second question, we have the rigid (and specific) antagonists. This rigidity dictates the conformation and electronic complementarity these molecules must have to bind to

Table 1. Average distances for the nAChR binding pocket (agonists)

Negative end		Positive end	
d(−)-ARG209	3.4 Å	d(+)-SER82	5.6 Å
d(−)-TYR190	6.9 Å	d(+)-ASP83	6.0 Å
d(−)-TYR198	4.6 Å	d(+)-TRP86	5.4 Å
		d(+)-ASP89	5.5 Å
		d(+)-TYR93	5.3 Å
Average	5.0 Å	Average	5.6 Å

Table 2. Average distances for the nAChR binding pocket (antagonists)

Negative end		Positive end	
d(−)-ARG209	4.1 Å	d(+)-SER82	4.4 Å
d(−)-TYR190	5.2 Å	d(+)-ASP83	4.5 Å
d(−)-TYR198	6.6 Å	d(+)-TRP86	6.4 Å
		d(+)-ASP89	8.1 Å
		d(+)-TYR93	4.8 Å
Average	5.3 Å	Average	5.6 Å

the receptor. This complementarity is well addressed based on the results of the last section, as well as the energetic one through the energy comparison made before. As a conclusion, we can say that our proposed bioactive conformation is consistent based on the above criteria.

Time-averaged structure for the nAChR binding site. The bottom part of Figure 6 depicts the overall time-averaged positions of the receptor cavity side chains obtained for the three agonists studied in this paper. To appreciate the dimension of the cavity, we present in Figure 7 a diagram characterizing the relative position of the amino acids side chains and relative distances between them. The distances correspond to centroids located on the various residues. These centroids were defined as follows: for TRP and TYR all the atoms in the ring; for ASP the carboxyl unit (the carbon and the two oxygens); for SER just the hydroxylic hydrogen; and for ARG209, the two quaternary nitrogens (with their hydrogens) and the carbon directly attached to these nitrogens.

To better appreciate the different spatial positions in the cavity of the agonists and antagonists, the distances between the positive end and negative end of the ligands to their neighboring complementary amino acids in the cavity were computed. These values are summarized in Table 1 (agonists) and Table 2 (antagonists).

In these tables the descriptor d(−)-ARG209 means, for example, the distance from the negative end of the ligand to the ARG209 residue. These distances, as above, refer to the separation of centroids. For the ligands, the centroids were defined as follows: for **1** the three methyl groups and the carbon attached to them (positive end) and the O-C-O unit (negative end); for **2** the quaternary N with it two hydrogens (positive end) and the unit N-Cl-N (negative end); for **3** the quaternary N with it methyl group (positive end) and the pyridinic N (negative end); for **4** the quaternary N with it hydrogen (positive end) and the unit O-C-O (negative end) and for **5** the quaternary N with it N (positive end) and the unit CH_3O-C-C-OH (negative end). For the amino acid side chains the centroids are the same as above. The descriptor d(+)-ARG209, for example, means the same but for the positive end of the ligands.

Comparing both tables we can see that the interactions arising from the ligand's positive end produce almost the same effect in positioning the surrounding amino acids for both agonists and antagonists. In contrast, there is a difference arising from the ligand's negative end, where the ARG209 is located.

Types of interactions between ACh and nAChR binding site. In the top panel of Figure 6 we represent the interactions between the positive (cationic) end of the agonists with the negatively charged region of the binding site. The yellow lines denote the conventional ion pair type of interactions while the blue line indicates cation-π interactions with the aromatic residues. In the middle panel the negative (anionic) end of the agonists interacting with the positively charged region of the binding site is shown. Here the red line shows hydrogen-bond type of interactions with the guanidinium group of ARG209 while the green ones represent the formation of a charge-transfer complex. The bottom panel is the overall picture of what the complete nAChR looks like. In this composite view, the agonists are represented by a dipole, to emphasize the different kinds of interactions taking place in the receptor cavity.

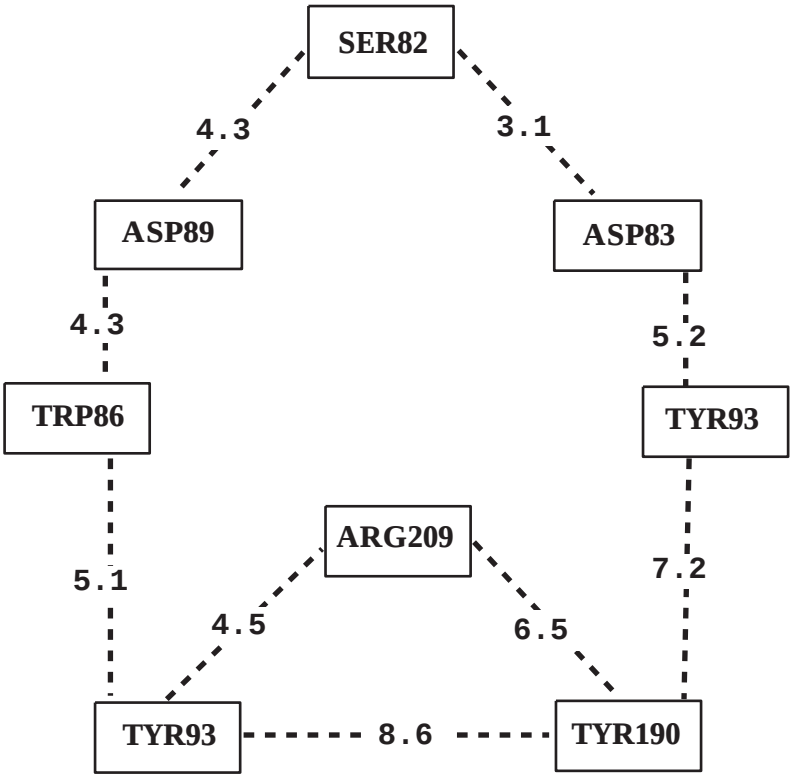

Figure 7. Relative positions and distances (in Å) of the agonist's thermodynamically averaged nAChR binding pocket.

Conclusions

The goal of this study was to establish the shape of the as yet unknown nicotinic acetylcholine binding site of the nAChR. To accomplish this task we first selected the critical amino acid residues found to comprise that binding site from published molecular biology studies. The side chains of those residues were then placed around three agonists and then around two antagonists in a way where complementary binding was maximized. That crude guess of what the binding site looks like was the starting point for molecular dynamics simulations.

The purpose of the molecular simulations was to allow those amino acid side chains to flow around the ligands until they reached an equilibrium state. That final state should correspond to the most favorable positions of the polypeptide residues making up the cavity, and that final structure should be a good representation of the actual receptor binding site. This simulation was done in a condensed phase where the water molecules were treated explicitly. Beginning with three different agonists, MD simulations were carried out giving rise to three very similar placements of side chains in that water bath. Moreover, all three systems rapidly converged to their final resting states suggesting those final structures, which we posit here are good representations of the actual receptor cavity, are thermodynamically stable states (at least on the time scale used here). In contrast, the two antagonist structures converged more slowly to a different minireceptor geometry. This is a result that we anticipated and it is one that suggests the timescales of the simulations used for the agonists were adequate to derive a meaningful structure.

The three similar minireceptor structures were combined to create a composite structure that is simply the average shape of the receptor site for all three simulations (for the three agonists) once they have been equilibrated (the coordinates of each individual structure are available from the author in PDB format). The structural features of that cavity were described, the distances between positive and negative ends of both agonists and antagonists with that cavity were tabulated, and the types of interactions between charged portions of the ligands and the minireceptor were discussed. It is apparent that we now have a reasonable working model of the nicotinic acetylcholine receptor

binding site, and, we have some understanding of the types of interactions between agonist and antagonist ligands with that receptor. Work is now in progress to (1) generate the remainder of the polypeptide backbone and (2) carry out computer-aided *de novo* design of new ligands capable of binding to this site.

Acknowledgements

The authors acknowledge support from the IUPUI Chemical and Biomolecular Computational Facility for computer time. Daniel Robertson is also acknowledged for assisting with the figures. We thank the Spanish 'Comisión Interministerial de Ciencia y Tecnología' (Grant SAF95-0639) for support of this research.

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