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Identification of multiple allosteric sites on the M_1 muscarinic acetylcholine receptor

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Abstract Staurosporine and four staurosporine derivatives were docked on the rhodopsin-based homology model of the M_1 muscarinic acetylcholine receptor in order to localize the possible allosteric sites of this receptor. It was found that there were three major allosteric sites, two of which are located at the extracellular face of the receptor, and one in the intracellular domain of the receptor. In the present study, the localization of these binding sites is described for the first time. The present study confirms the existence of multiple allosteric sites on the M_1 muscarinic receptor, and lays the ground for further experimental and computational analysis to better understand how muscarinic receptors are modulated via their allosteric sites. These findings will also help to design and develop novel drugs acting as allosteric modulators of the M_1 receptor, which can be used in the treatment of the Alzheimer's disease.

Keywords: M_1 muscarinic acetylcholine receptor; G-protein coupling receptors; staurosporine; Allosteric sites; Docking simulations; Molecular dynamics; Alzheimer's disease

1. Introduction

Muscarinic acetylcholine receptors are members of the superfamily of G-protein coupling receptors. Within this family of receptors, there are five distinct subtypes falling into two main groups: The first group contains the M_1, M_3 and M_5 subtypes that preferentially couple to G_q/G_{11} G-proteins class; and the second group consists of the M_2 and M_4 subtypes, which couple to G_i/G_o subclass [1].

Recently, the M_1 subtype has received more attention due to its important role in cognitive processing relevant to the Alzheimer's disease, particularly in short-term memory. It has been hypothesized that the M_1 subtype could be a promising target for the design and development of drugs that improve cognitive abilities [2]. It was suggested that M_1 muscarinic agonists might offer an advantage in treating the Alzheimer's disease, by activating post-synaptic M_1 receptors [2–5]. This strategy is presumably less limited than using acetylcholinesterase inhibitors, because it does not require the production and release of acetylcholine from presynaptic terminals.

However, M_1 agonists lack selectivity, which limits their clinical use. Alternatively, recent studies have shown the possibility of developing selective M_1 allosteric modulators able to induce a conformational change in the receptor, to increase the affinity of the natural agonist (in this case, acetylcholine), and to improve the G-proteins coupling to the receptor [6,7]. It has also been hypothesized that muscarinic receptors possess multiple allosteric sites that mediate the effects of various agents on the binding of ligands to the acetylcholine-binding site [8]. Most of the studied allosteric agents, such as gallamine, strychnine, brucine, alcuronium, tubocurarine and others, are consistent with the ternary complex allosteric model, in which the primary and allosteric ligands bind simultaneously to the receptor, modifying each other's affinity.

Until now, the location of the allosteric sites of the M_1 receptor has been unknown, which has limited the possibility of understanding the mechanisms in which this type of receptor is allostericaly modulated. In this study, we performed docking simulations of staurosporine and four indolocarbazoles (Chart 1) on the rhodopsin-based homology model of the M_1 receptor. It has been experimentally shown that these compounds have a complex allosteric effect on M_1–M_4 muscarinic receptors, involving more than one allosteric site [9]. In this contribution, we show the existence of three allosteric sites involved not only in the positive cooperativity with the orthosteric site, but also in the activation of a possible acetylcholine-independent G-protein coupling to the receptor.

2. Materials and Methods

2.1. Protein setup

The rhodopsin-based homology model of the M_1 muscarinic receptor reported by Hulme and co-workers was employed in present study ignoring the 129-amino acid deletion in the third intracellular loop (i3). This deletion did not modify ligand binding activity and shows good signaling activity [10–12]. The accuracy of the structure and the protonation states were analyzed with the program WHAT IF [13]. A disulfide bond between residues C98 and C178 was assigned. The protein was embedded in a TIP3P water box ($100 \times 80 \times 100$ Å). 30

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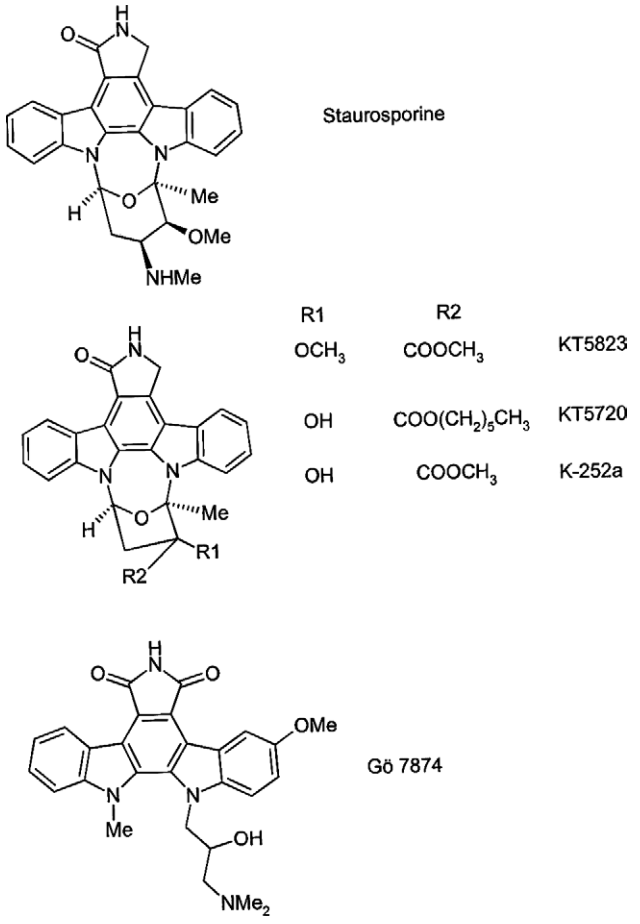

Chart 1. Chemical structures of the five allosteric modulators employed in the present study.

Chlorine and 13 sodium ions were added to produce a neutral charge on the system, and to closely mimic the physiological ionic strength. Protein and ions were modeled with the CHARMM 27 force field.

2.2. Molecular dynamics simulation of the M_1 receptor

In order to relax the homology model (e.g., to assign random positions to the atoms in the model), a short molecular dynamics simulation was performed using the program NAMD 2.6 [14]. For the simulation, periodic boundary conditions and the particle mesh Ewald were employed. The non-bonded cutoff, switching distance and non-bonded pair-list distance were set to 9, 8 and 11 Å, respectively. The SHAKE algorithm applied to all bonds to hydrogen atoms allowed a 2-fs time step. NPT ensemble was maintained with a Langevin thermostat and a Langevin piston barostat. The system was minimized for 1000 steps using the conjugate gradient algorithm with restraints to all protein atoms. 1000 Additional steps were used to minimize the whole system with no restraints. The solvent and protein were equilibrated, and the whole system was warmed up and relaxed for 200 ps.

2.3. Ligands setup

Ligands were constructed and optimized using the program MOE [15]. The optimization was carried out at semi-empirical level using the AM1 method, and partial charges on atoms were assigned using the Gasteiger–Marsili method [16]. At the end, the dihedrals allowed to rotate were assigned with the aid of the program AUTOTORS.

2.4. Docking simulations

Docking simulations were performed with the AutoDock program (v. 3.0.5) [17]. This program uses the efficient Lamarckian Genetic algorithm and its scoring function comprised by van der Waals, Coulomb potential electrostatics, hydrogen bonding, a volume-based solvation term and an estimation of the entropic cost of binding through a weighted sum or torsional degrees of freedom terms. Additionally, one does not need to specify the possible binding site, since the algorithm allows an efficient searching of the entire surface of the target.

Grid maps representing the protein were calculated with the aid of AUTOGRID. A cubic box of $126 \times 126 \times 126$ points, with a spacing of 0.6 Å between the grid points and centered on the geometric center of the protein, was calculated. The dimensions of the box were big enough to cover the entire surface of the receptor. Docking simulations were carried out using the Lamarckian Genetic Algorithm, with an initial population of 500 individuals, a maximum number of 50 000 000 energy evaluations, a maximum number of 27 000 generations, a translation step of 2 Å, a quartenion step of 50° and a torsion step of 50°. For the local search, the pseudo-Solis and Wets algorithm was applied using a maximum number of 300 iterations per local search. Docking simulations consisted of 100 independent runs. Resulting orientations lying within 2.0 Å in the RMSD were clustered together and represented by the orientation with the most favorable free energy of binding.

3. Results

Docking simulations of the five allosteric modulators on the homology model of the M_1 muscarinic receptor revealed the existence of three principal allosteric binding sites along the protein surface. Best docking results of each compound were located in a similar position on the three binding sites. We labeled these binding sites as follows: (1) EXC (located close to the entrance of the binding site), (2) ENT (at the entrance of the binding site), and (3) INC (located in the intracellular domain, close to the intracellular loop). The three binding sites are shown in Fig. 1. The computed free energies of binding to each site on the free receptor are summarized in Table 1. For each allosteric modulator, the results are described as follows.

3.1. Staurosporine

This molecule constitutes the base structure of the allosteric modulators studied here. AutoDock placed this molecule on the three binding sites mentioned above. The best-ranked cluster, containing 31 out of 100 independent runs, docked staurosporine in the INC site. Sixteen independent runs docked this modulator in the ENT site. Finally, only 5 out of 100 runs docked it in the EXC allosteric site. The best docking orientations of staurosporine on the M_1 muscarinic receptor are shown in Fig. 2.

3.1.1. Staurosporine binding to the INC site. The indolocarbazol moiety of staurosporine makes contact with residues S126, A135, T354, F355, S356, V358, K359, E360 and N422. The rest of the molecule interacts with residues T58, V59, N60, I119, D122, R123 and K136.

3.1.2. Staurosporine binding to the ENT site. In this binding site, located at the entrance of the acetylcholine-binding site, residues G169, E170, R171 (via π–cation interaction), Q181, S388, K391, D393 and W400 bind the indolocarbazol fragment of the molecule. In contrast, only residues Q181 and L183 interact with the other non-aromatic fragments of the molecule.

3.1.3. Staurosporine binding to the EXC site. When this allosteric modulator binds to this site, located at the extracellular face of the receptor, its indolocarbazol moiety interacts with residues T95, D99, Y166 (through π–π interactions), L167 and V168. The non-indolocarbazol moiety interacts with