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Molecular modelling of subtypes (α_{2A}, α_{2B} and α_{2C}) of α_2-adrenoceptors: A comparative study

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ABSTRACT

The therapeutic usefulness of current agents that activate the three α_2-adrenoceptors, α_{2A}, α_{2B} and α_{2C} is limited by their lack of subtype selectivity. One approach to the development of subtype-selective agents is the *in silico* docking of potential ligands to the receptors in quantitative molecular modeling studies. Because the crystal structure of the α_2-adrenoceptors is not known, we used homology modeling based on the published structure of bovine rhodopsin.

We developed individual models for each of the three receptors, which were found to accurately represent published data from both radioligand binding mutagenesis experiments. Using 18 non-subtype-selective agents to validate the models, the calculated transformed and the experimental binding free energies were satisfactory correlated ($r^2_A = 0.888$, $r^2_B = 0.887$, $r^2_C = 0.790$). The binding pockets differed in size (482–619 Å^3) with the α_{2B} receptor subtype having the largest and the α_{2C} the smallest cavity. The binding sites for all three subtypes were found to be essentially identical with the exception of two subtype-specific residues, and thus we were unable to identify any significant differences in the interactions of ligands with the three receptor subtypes.

Although, the binding properties of all three receptors are very similar, the differences in pocket volume and two subtype-specific residues in the binding pocket might play an as yet undocumented role in subtype selectivity.

1. Introduction

α_2-Adrenoceptors belong to the superfamily of G protein-coupled receptors. Three highly homogenous subtypes, α_{2A}, α_{2B} and α_{2C} have been distinguished (Bylund et al., 1994). Non-selective α_2-adrenoceptor agonists have been used for the treatment of various diseases for a long time and the present state of their clinical and daily use has recently been summarized (Francesco et al., 2007; Crassous et al., 2007). Most experts agree, however, that a major limitation for their use is the lack of subtype-selectivity of currently available agents. Experiments with knock-out mice support the general clinical importance of the α_{2A} subtype, whereas the α_{2B} subtype may be responsible for the transient hypertensive phase following systematic administration of a non-selective α_2-agonist and the α_{2C} may be responsible for the control of adrenaline release from the adrenal medulla and it may also cooperate with α_{2A} in the feed-back inhibition of noradrenaline release in the presynaptic membrane of adrenergic neurons. However, the lack of truly subtype-selective agonists has seriously limited the understanding of the characteristic structural features of α_{2A}, α_{2B} and α_{2C} receptor agonists, the design and development of subtype-selective compounds, and, thereby, the more precise evaluation of the *in vivo* pharmacological roles of α_2-adrenoceptor subtypes. Therefore, molecular modelling studies, which may provide some insights into the structural requirements of receptor binding, may be of great value to discover new and more selective compounds.

Homology models of α_{2A}-adrenoceptor and docking of ligands to it have been described by several research groups. For the first GPCR models, a low resolution cryo-electron microscope (Henderson et al., 1990) and X-ray (Schertler et al., 1993) structure of bacteriorhodopsin was used as template. Next, to overcome the limitations of the models based on bacteriorhodopsin, which is not a GPCR and, in fact, shows a relatively low homology with human adrenoceptors, bovine rhodopsin-based homology models of α_{2A}-adrenoceptor were developed. An extensive analysis of ligand binding to bovine rhodopsin-based and engineered mutant receptors was carried out using AutoDock 2.4 and GRID Version 16 (Salminen et al., 1999). The combined use of molecular modelling methods and experimental data provided some understanding of

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ligand binding and revealed the superiority of a bovine rhodopsin-based model to study human adrenoceptors.

The same model was also used in another study in which two sets of ligand binding experiments were carried out (Nyronen et al., 2001). First, based on competition experiments with both an agonist and an antagonist radioligand, an atomic resolution model for the binding mode of adrenaline, noradrenaline, and their close structural analogues was provided, as well as a structure-based explanation for the binding affinity differences of the *R*- and *S*-enantiomers. Second, a model of how these ligands could promote the activation of α_{2A}-AR was presented based on ability of the ligands to promote receptor activation by measuring agonist-induced stimulation of [^{35}S]GTPγS binding.

Since the appearance of the first bovine rhodopsin structure in the literature (Palczewiski et al., 2000; Protein data bank code: 1F88), Nyronen's group has published a series of normal and mutated α_{2A}-AR models based on the structure of bovine rhodopsin (Peltonen et al., 2003). Twelve α_2-adrenergic agonists were used for docking to the models with automated docking methods. Radioligand binding assays and functional [^{35}S]GTPγS binding assays were performed to experimentally corroborate the predicted binding modes.

In the most current report of the Nyronen's research group (Xhaard et al., 2005), structural models for the three human α_2-AR subtypes were constructed by using another bovine rhodopsin X-ray structure (Teller et al., 2001; Protein database code: 1HZX); 20-20 alternative spatial arrangements of an all atom model were generated by MODELLER for each α_2-subtype. Surprisingly, no electrostatic interaction between the protonated moiety of the ligands and the carboxylate group of D3.32 could be detected, which had always been considered to be a key interaction for the natural agonists. A more detailed review of modelling α_1- and α_2-adrenoceptors was published recently (Carrieri and Fano, 2007).

Although all the above models provided some *qualitative* description for the ligand binding, neither quantitative predictions for the free energies of binding (ΔG_b) nor detailed analysis for subtype selectivity have been made. In rational drug discovery, estimation of ΔG_b of bioactive ligands to their macromolecular targets is an essential step (Hetenyi et al., 2006). Although, instrumental techniques for the measurement of ΔG_b are available, such as isothermal titration calorimetry (Leavitt and Freire, 2001) or amyloid aggregation (Kardos et al., 2004), they have not been widely applied to molecular modeling. On the other hand, many different *in silico* strategies for the structure-based calculation of ΔG_b have been developed. The use of a single protein–ligand complex structure (a crystallographic structure or a homology model energy minimum) represents one strategy for the calculation of ΔG_b. This approach requires a scoring function, along with a parameter set appropriate for the type of ligand molecules investigated. The scoring functions developed for rapid calculation of ΔG_b are primarily implemented to drive the docking simulations.

Our research group recently described an α_{2A}-adrenoceptor homology model and docking of 15 well known α_2-adrenoceptor agonists to obtain a quantitative model for making predictions of α_{2A}-adrenoceptor affinity of agonists (Balogh et al., 2007).

In another study, the homology models of all three adrenoceptor subtypes were made and a series of biphenyl derivatives – some of them with a slight functional selectivity for the α_{2C}-subtype – were docked to the models (Gentili et al., 2004). Binding free energy values were also calculated and an explanation of subtype selectivity based on structural features was provided.

In the present study, we describe the atomic resolution homology models of the α_{2A}-, α_{2B}- and α_{2C}-adrenoceptors and the computational docking of known α_2-adrenoceptor agonists to those models with a quantitative characterization.

2. Experimental procedures

The computational procedure we followed is briefly described below, for details of modelling, docking and scoring, Berendsen et al., 1995; Gasteiger and Marsili, 1980; Hooft et al., 1996; Lindahl et al., 2001; Mehler and Solmayer, 1991; Pedretti et al., 2004; Rasmussen et al., 2007; SYBYL, 2009; Guha et al., 2006; see the Supplementary Material (tables and figures appearing there are distinguished with an *S*).

2.1. Modelling of the receptors and ligands

For the receptor models, the crystal structure of bovine rhodopsin with a resolution of 2.2 Å (Okada et al., 2004; Protein database code: 1U19) was used as a template. The sequence alignment was made with BioEdit Biological Sequence Alignment editor Program (Hall, 1999). The three subtypes together with the bovine rhodopsin were aligned (see Figure S1) with Clustal W multiple alignment algorithm (Chenna et al., 2003). Modeller 9v4 was used for model building (Sali et al., 1993), and 100 homology models were created for all 3 adrenoceptor subtypes. Models were ranked by the 'modeller objective function' calculated by Modeller, the top five models for each subtype were selected for further investigation, and models with the best docking results were selected as final models. Models were validated with the web version of iMolTalk Structural Bioinformatics Toolkit (Diemand and Scheib, 2004).

Eighteen known agonists of α_2-adrenoceptors were selected from the literature with their binding affinities (Table S2) (Jasper et al., 1998). These compounds represent a fairly structurally diverse set of α_2-AR agonists, without any significant subtype selectivity. All possible torsion angles of each molecule were released and set rotatable. In most cases, protonation on the imidazoline ring of the ligands was made to get a symmetrically protonated structure (Remko et al., 2001), in a few cases, alternative protonations were also investigated and the thermodynamically more reliable structures were used.

2.2. Docking and scoring

All docking calculations were performed with AutoDock 4.0 and AutoDock Tools program package (Morris et al., 1998), with a slightly modified parameter set. At the end of the docking, ligand structures with the most favorable free energy of binding were selected as docked conformations. Molecular visualization was made with Pymol (DeLano, 2002) molecular visualization program. The free energies of binding of the ligands ($\Delta G_b^{(calc)}$) to the proteins were calculated by using the scoring function implemented in AutoDock. The binding affinities of the ligands were measured on cloned human α_{2A}-, α_{2B}- and α_{2C}-ARs using [^{3}H]MK912 as the radioligand (Jasper et al., 1998). The experimental $\Delta G_b^{(exp)}$ values of the agonists were computed from their K_i values at $T = 300$ K (Table S3) using the equation $\Delta G_b^{(exp)} = RT(\ln K_i)$.

3. Results

3.1. Characterization of the binding site

The location of the binding site for subtype A was deduced from mutagenesis data (Wang et al., 1991). In all three subtypes, all three key binding residues were also recognized by the Pocket Finder program (Hendlich et al., 1997). Cavities containing the binding sites are funnel-shaped, with the upper and wider part containing the binding residues and a narrow neck in the direction of the intracellular space. The α_{2C} receptor subtype has the smallest cavity by volume (482 Å^3), the subtype A is of medium sized (584 Å^3), and the cavity of subtype B has the largest volume (619 Å^3) (Figure S2).

For higher accuracy and to get more reliable structures for the protein–ligand complex, torsion angles of the side chains of key residues D3.32 (α_{2A}: Asp113, α_{2B}: Asp92, α_{2C}: Asp131), S5.42 (α_{2A}: Ser200, α_{2B}: Ser176, α_{2C}: Ser214) S5.46 (α_{2A}: Ser204, α_{2B}: Ser180; α_{2C}: Ser218) were allowed to freely move (for numbering of residues, the system of Ballesteros and Weinstein, 1995 was used). It should be noted that the docked conformations of the ligands were found in contact with those key residues, providing additional validation of the model (Figure S3).

Residues close to the binding site were also carefully compared. There were two different features: residues in position 5.31 were different in all three subtypes, whereas at location 5.43, the B subtype had a residue different from those of subtypes A and C. In other features, the binding sites seem to be conservative: the remaining eight residues were identical and they were overlapping in all three subtypes (Fig. 1).