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A critical pocket close to the glutamate binding site of mGlu receptors opens new possibilities for agonist design

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ABSTRACT

A recent publication from Ogawa et al. suggested a possible allosteric chloride binding site in the extracellular domain of metabotropic glutamate receptors (mGluRs) by comparison with a similar site found in atrial natriuretic peptide receptor. We simultaneously reported about (*S*)-PCEP an agonist of subtype 4 mGluR that would bind to a similar pocket, adjacent to the glutamate binding site. Here we disclose LSP1-2093, a new derivative of (*S*)-PCEP that holds a nitrophenyl substituent. Docking experiments predict that the nitro group binds to the receptor at the putative chloride ion site. It is thus possible to take advantage of this putative chloride binding site to develop new types of mGluR agonists. This pocket is present in the structural family of Leucine Isoleucine Valine Binding Protein that includes class C GPCRs, suggesting that extended agonists may be identified at receptors bearing such a structural domain.

1. Introduction

In 1993 P. O'Hara and colleagues were the first to demonstrate that both metabotropic glutamate receptors (mGluRs) and ionotropic glutamate receptors hold an amino terminal domain (ATD) that adopts the fold of LIVBP a bacterial periplasmic binding protein and that was thus named the LIVBP domain (O'Hara et al., 1993). They showed that this domain constitutes the glutamate binding site of mGluRs. Later it was found that in NMDA receptors, this domain is the binding domain for zinc and that it modulates the glutamate activation of these receptors. Since that early prediction, much progress has been made and crystal structures of both mGluR and NMDA ATDs have been disclosed (Karakas et al., 2009; Kunishima et al., 2000; Muto et al., 2007).

Periplasmic binding proteins are involved in high affinity active transport of various nutrients such as sugars, inorganic anions and amino acids (Leucine Isoleucine and Valine for LIVBP). A large number have been crystallized and display the same 3D structure with two globular domains linked by a hinge region allowing open and closed conformations (Quiocho and Ledvina, 1996). In the open form, the ligand binds to a first lobe and then is trapped upon closing of the domain in a similar manner as the venus flytrap, a carnivorous plant, catches its prey. By analogy, the LIVBP domain of mGluRs and NMDA receptors has been often named the venus flytrap (VFT) domain.

The metabotropic glutamate receptors belong to the class C of the G-protein coupled receptor family (Pin et al., 2003). All members of that family possess an LIVBP-like ATD connected to a hepta helix transmembrane domain. These receptors function as dimers with a contact surface between the lobes 1 of the ATDs (Kunishima et al., 2000). Agonists stabilize the closed LIVBP domains that result in a relative rotation of two domains then triggering G-protein activation. Eight subtypes of mGluRs have been identified and classified in three groups according to their sequence identity, transduction pathways and pharmacological

Abbreviations: ACPD, 1-aminocyclopentane 1,3,4-dicarboxylic acid; ACPT, 1-aminocyclopentane 1,3,4-tricarboxylic acid; ANP, atrial natriuretic peptide; AP4, 2-amino-4-phosphonobutyric acid; ATD, amino terminal domain; GPCR, G-protein coupled receptor; DCG-IV, (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine; HTS, high throughput screening; LIVBP, Leucine Isoleucine Valine Binding Protein; LSP1-2093, 3-amino-3-carboxypropyl(hydroxy(3-nitrophenyl)methyl)phosphinic acid; mGluR, metabotropic glutamate receptor; NMDA, *N*-methyl D-aspartate; OlfC, olfactory class C GPCRs; PCEP, 3-amino-3-carboxypropyl-2′-carboxyethylphosphinic acid; PDB, Protein Data Bank; VFT, Venus FlyTrap.

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profile. Group-I receptors (mGlu1 and mGlu5) are linked to PLC activation while group-II (mGlu2 and mGlu3) and group-III (mGlu4, mGlu6, mGlu7 and mGlu8) inhibit adenylate cyclase (Niswender and Conn, 2010).

Other proteins adopt the LIVBP-like fold. In the SCOP classification (L-arabinose binding protein-like family) 16 different types are listed (http://scop.mrc-lmb.cam.ac.uk/scop/data/scop.b.d.bea.b.b.html). Many of these proteins function as monomers but several others need to form a dimer in order to be active as seen with class C GPCRs. In this latter group are found the hormone binding domain of the atrial natriuretic peptide receptor (ANPR) and DNA-binding protein purine-, lac- and trehalose repressors. It was known that ANPRs require chloride for their activity. Recently Ogawa et al. showed that it may play an allosteric role in the binding of ANP (Ogawa et al., 2010). Moreover when comparing the crystal structures of ANPR and mGlu1R ATDs, these authors noted that the ANPR chloride binding site is conserved in mGlu1R. Thus they suggested that chloride may also allosterically regulate mGluR activation. In this article, we show that indeed a binding site similar to that of chloride in ANPR is present in several LIVBP-like proteins and that it may be a site for developing a new type of ligands. In fact, we have recently described an adjacent binding pocket to glutamate in mGlu4R that coincides with the ANPR chloride site and where new orthosteric agonists may be binding (Selvam et al., 2010). Here we disclose LSP1-2093, a new molecule of this type.

2. Materials and methods

2.1. Pharmacology

HEK293 cells (Human Embryonic Kidney cells) were transiently transfected by electroporation with rat clones of mGlu4, mGlu6, mGlu7 and mGlu8 receptors as described elsewhere (Brabet et al., 1998). A high affinity glutamate transporter EAAC1 was co-transfected with the receptor to avoid the influence of extracellular glutamate. Receptors were co-transfected with a chimeric Gq/Gi-protein. This modified Gq-protein allows the monitoring of naturally Gi-coupled receptor activity by measurements of inositol phospholipid hydrolysis (Gomeza et al., 1996).

[^{3}H]Inositol phosphate ([^{3}H]-InsP) accumulation experiments were performed in 96-well microplates, as described previously (Goudet et al., 2004). Briefly, transfected cells were incubated overnight with [^{3}H]-myoinositol (16 Ci/mmole, Amersham, Buckinghamshire, UK). The day after, ambient glutamate was degraded by incubation with glutamate pyruvate transferase in the presence of pyruvic acid then cells were stimulated with agonists for 30 minutes in the presence of 10 mM LiCl. [^{3}H]-InsP accumulated during receptor stimulation was recovered by anion exchange chromatography and radioactivity was counted using a Wallac 1450 Microbeta stintillation and luminescence counter (Perkin Elmer, Courtaboeuf, France). Results are expressed as the ratio between [^{3}H]-InsP and total radioactivity in each sample. All points are from triplicates. LSP1-2093 was synthesized in the laboratory of F. Acher (Acher et al., 2007).

2.2. Structure analysis and docking of LSP1-2093 in mGlu4R aminoterminal domain

All calculations were performed in Discovery Studio 2.5.5 (Accelrys Software Inc., San Diego, 92121 CA). Due to the lack of crystal structure for mGlu4 subtype amino terminal domain, LSP1-2093 was docked in a homology model that had been previously generated (Bertrand et al., 2002; Selvam et al., 2010). Our homology model was built upon 2 templates 1ewk (mGlu1R ATD) and 2e4u (mGlu3R ATD) (Selvam et al., 2010). The ligand was initially positioned in the binding site using CDOCKER. CDOCKER uses a CHARMm-based molecular dynamics scheme to dock ligands into a receptor binding site (Wu et al., 2003). Random ligand conformations are generated using high-temperature molecular dynamics. The conformations are then translated into the binding site. Candidate poses are then created using random rigid-body rotations followed by simulated annealing. A final energy minimization is then used to refine the ligand poses. As CDOCKER takes into account only ligand flexibility, protein-ligand interactions were further optimized by one nano second molecular dynamics using CHARMm where only side chains were flexible while the backbone was harmonically restrained. Once the trajectory was equilibrated, snapshots of the trajectory were analyzed in terms of protein-ligand contacts and the selected ones were submitted to energy minimization leading to the figures presented in this article (Fig. 5).

3. Results

3.1. LIVBP-like domains and homologous ANPR chloride binding site

The LIVBP domain is composed of two similar sub-domains (lobe1 and lobe2) each consisting of a central cluster of beta-sheets sandwiched between alpha-helices and lining a cleft in between. The chloride site of ANPR is found in lobe 1 between β1, β2, β3 sheets and α1, α2, α3 helices (Fig. 1) (He et al., 2006; Ogawa et al., 2010). In the orientation of Fig. 1, the ion is situated above the cleft, below two hydrophobic clusters and holds together the lower part of β2 and β3 sheets by making three hydrogen bonds with main chain NH of G85 and C86 and with hydroxyl of S53 (Fig. 1 and SI-1). Ogawa et al. demonstrated that chloride may be replaced by bromide as found in ANPR PDB structure 3ak3 and displayed in resulting Fig. 1 and SI-1 (Ogawa et al., 2010). When checking the analogous site in structures of the LIVBP-like family members that have been deposited at the PDB, we found several structures with the adequate distance between β2 and β3 sheets. However in some of them, the link between those sheets is provided by direct

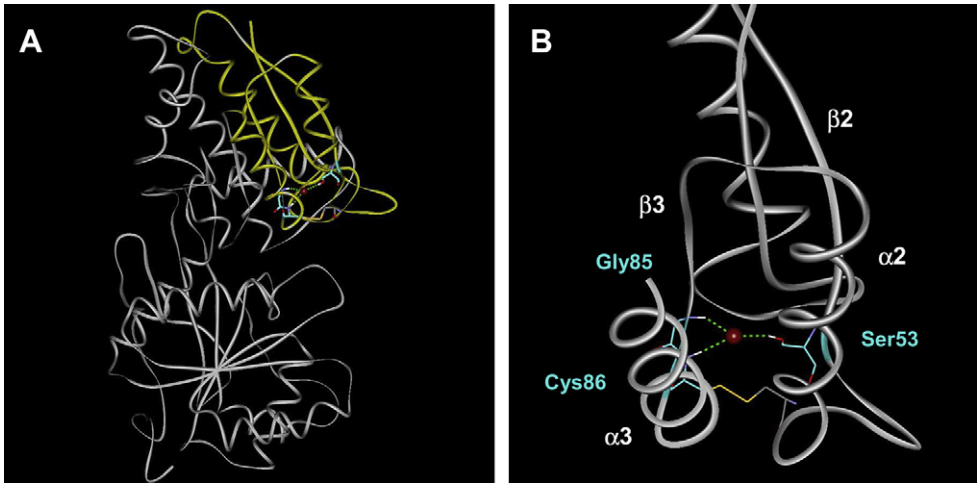

Fig. 1. Crystal structure of ANP receptor extracellular domain (monomer) bound with bromide (PDB identifier 3a3k). A) Ribbon diagram with β1, β2, β3 sheets and α1, α2, α3 helices highlighted in yellow; residues binding the bromide ion are displayed. B) Expanded view of the bromide binding site. Atom colors: carbon cyan, hydrogen white, oxygen red, nitrogen blue, sulphur yellow, bromine brown. Hydrogens have been removed for clarity except for those involved in hydrogen bonds that are displayed as green dotted lines.