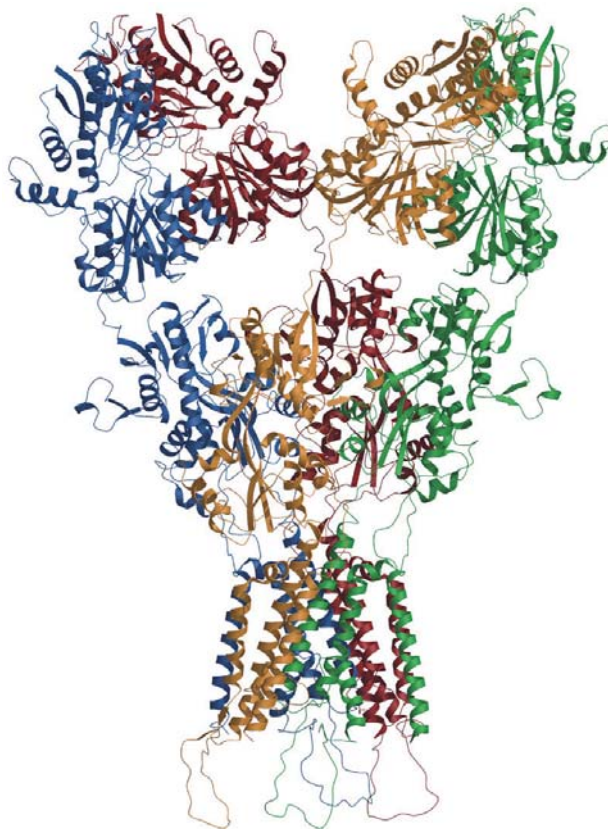


Pekka Postila

Dynamics of the Ligand-Binding Domains of Ionotropic Glutamate Receptors



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Domains of Ionotropic Glutamate Receptors

Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston Ylistönrinteellä, salissa YAA303
marraskuun 27. päivänä 2010 kello 12.

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UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2010

Dynamics of the Ligand-Binding
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JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE 214

Pekka Postila

Dynamics of the Ligand-Binding
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UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2010

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Cover picture by Pekka Postila

URN:ISBN:978-951-39-4182-6

ISBN 978-951-39-4182-6 (PDF)

ISBN 978-951-39-4081-2 (nid.)

ISSN 1456-9701

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Jyväskylä University Printing House, Jyväskylä 2010

Just remember what ol'
Jack Burton does when
the earth quakes, and the
poison arrows fall from
the sky, and the pillars of
Heaven shake. Yeah, Jack
Burton just looks that big
ol' storm right square in
the eye and he says,
"Give me your best shot,
pal. I can take it."

The Pork Chop Express

ABSTRACT

Postila, Pekka

Dynamics of the ligand-binding domains of ionotropic glutamate receptors.

Jyväskylä: University of Jyväskylä, 2010, 54 p.

(Jyväskylä Studies in Biological and Environmental Science,

ISSN 1456-9701; 214)

ISBN 978-951-39-4182-6 (PDF), 978-951-39-4081-2 (nid.)

Yhteenveto: Ionotrooppisten glutamaattireseptoreiden
ligandinsitomisdomeenien dynamiikka.

Diss.

Ionotropic glutamate receptors (iGluRs) transmit fast neuronal impulses in the synapses of mammalian brain. The binding of neurotransmitter L-glutamate closes the bilobed iGluR cleft of the extracellular ligand-binding domain (LBD). The screw-axis bending of iGluR-LBD in turn opens the tetrameric transmembrane ion channel and influx of cations depolarizes the neuron. The isolated iGluR-LBD referred here as the ligand-binding core (LBC) has been crystallized in complex with various ligands. Full agonists close the iGluR-LBC and, conversely, antagonists block both the receptor cleft closure and ion channel opening. Some partial agonists produce intermediate iGluR-LBC closure in addition to partial activation. In this thesis is demonstrated that receptor cleft closure can be recreated by inserting ligands inside the closed iGluR-LBCs and simulating the complexes with all-atom molecular dynamics (MD) with explicit solvent. During the MD simulations the iGluR cleft either opens (antagonist, partial agonist) or stays closed (full agonist). Accordingly, the closure stages and binding interactions were predicted for novel compounds (*e.g.* dysiherbaine analogs) without prior crystallographic data. The MD simulations were also used to interpret experimental ligand-binding affinity and receptor subtype specificity data. Although bulky partial agonists and antagonists usually produce full-scale receptor cleft opening in MD simulations, some partial agonists only disrupt the receptor cleft hydrogen bonding. The full agonist-iGluR complex simulations suggest that the stability of cleft closure is receptor subtype-specific. Moreover, the ligand-receptor complex simulations provided a more dynamic view of the iGluR-LBD dynamics than what the crystallographic studies have implied. Because iGluRs contribute to various neuropathologies such as epilepsy and migraine, the receptor family is important target for rational drug discovery.

Keywords: ionotropic glutamate receptor, ligand-binding core, ligand-binding domain, molecular dynamics, full agonist, partial agonist, antagonist

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ABSTRACT

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which are referred to in the text by their Roman numerals.

- I Lash LL, Sanders JM, Akiyama N, Shoji M, Postila P, Pentikäinen OT, Sasaki M, Sakai R, Swanson GT. 2008. Novel analogs and stereoisomers of the marine toxin neodysiherbaine with specificity for kainate receptors. *Journal of Pharmacology and Experimental Therapeutics*. 324:484-496.
- II Frydenvang K, Lash LL, Naur P, Postila PA, Pickering DS, Smith CM, Gajhede M, Sasaki M, Sakai R, Pentikäinen OT, Swanson GT, Kastrup JS. 2009. Full domain closure of the ligand-binding core of the ionotropic glutamate receptor iGluR5 induced by the high affinity agonist dysiherbaine and the functional antagonist 8,9-dideoxyneodysiherbaine. *Journal of Biological Chemistry*. 284:14219-14229.
- III Postila PA, Swanson GT, Pentikäinen OT. Exploring kainate receptor pharmacology using molecular dynamics simulations. *Neuropharmacology*. 58:515-527.
- IV Lash-Van Wyhe LL, Postila PA, Tsubone K, Sasaki M, Pentikäinen OT, Sakai R, Swanson GT. 2010. Pharmacological activity of C10-substituted analogs of the high-affinity kainate receptor agonist dysiherbaine. *Neuropharmacology*. 58:640-649.
- V Postila PA, Ylilauri M, Pentikäinen OT. 2010. Partial Agonism of Ionotropic Glutamate Receptors Indicated by an Interdomain Hydrogen Bond. Submitted manuscript.

RESPONSIBILITIES OF PEKKA POSTILA IN THE THESIS ARTICLES

- Article I: I planned the modeling experiments together with Olli Pentikäinen. I did the modeling experiments described in the article. I wrote the paragraphs involving modeling together with Olli Pentikäinen.
- Article II: I planned the modeling experiments together with Olli Pentikäinen. I did the modeling experiments described in the article. I wrote the paragraphs involving modeling together with Olli Pentikäinen.
- Article III: I planned the article together with Olli Pentikäinen. I did the modeling experiments described in the article. I wrote the article with the help of Olli Pentikäinen and Geoffrey Swanson.
- Article IV: I planned the modeling experiments together with Olli Pentikäinen. I did the modeling experiments described in the article. I wrote the paragraphs involving modeling together with Olli Pentikäinen.
- Article V: I planned the article together with Olli Pentikäinen. I did the modeling experiments described in the article except the GluN1 receptor simulations that were done by Mikko Ylilauri. I wrote the article.

ABBREVIATIONS

2D	two-dimensional
3D	three-dimensional
AMPA	(2S,3S,4S)-3-(carboxymethyl)-4-prop-1-en-2-ylpyrrolidine-2-carboxylic acid
<i>apo</i>	receptor structure without a bound ligand
ATD	amino-terminal domain, N-terminal domain, X domain
CNS	central nervous system
CTD	carboxy-terminal domain
DH	dysis herbaine
GluA1-4	AMPA receptor subunits
GluK1-5	kainate receptor subunits
GluN1-3	NMDA receptor subunits
GluR	glutamate receptor
H-bond	hydrogen bond
iGluR	ionotropic glutamate receptor
kainate	(2S,3S,4S)-3-(carboxymethyl)-4-prop-1-en-2-ylpyrrolidine-2-carboxylic acid
LBC	ligand-binding core
LBD	ligand-binding domain
MD	molecular dynamics
mGluR	metabotropic glutamate receptor
MSVIII-19	8,9-deoxy-neodysis herbaine; neodysis herbaine analog
neoDH	neodysis herbaine
NMDA	N-methyl-D-aspartate
PDB	protein database
TARP	transmembrane AMPA receptor regulatory protein
TM1-4	transmembrane helices or domains
TMD	transmembrane domain

1 INTRODUCTION

Fast neuronal signaling is a prerequisite for the efficient functioning of mammalian central nervous system (CNS). Individual neurons are connected *via* specialized junctions called chemical synapses into neuronal networks. The synaptic vesicles loaded with chemical transmitters fuse with the presynaptic cell membrane and release their contents into the synaptic cleft. Then, the neurotransmitters bind into the receptor proteins embedded on the cell membrane of the adjacent postsynaptic neuron. The neurotransmitter binding causes changes that either directly or by means of intermediary mechanisms cause ion channel opening in the postsynaptic neuron. The influx of cations depolarizes the neuron *i.e.* the excitatory postsynaptic potential changes and the nerve impulse travels along the axon or the dendrite.

Because synapses have a central position in the signal transduction pathways, their receptors contribute to neurobiology and various neuropathologies. There exist several synthetic and natural compounds that either block or promote function of these synaptic receptors. Therefore, the signal transduction of the CNS can be adjusted by ligands that bind into these receptors. However, before drug molecules can be successfully designed, the exact effects of ligand binding for the target proteins need to be understood. The drug molecules also have to be selective only for a certain receptor subtype to produce the desired effects.

Most excitatory neurotransmission in the CNS is mediated by ionotropic glutamate receptors (iGluRs). In this thesis was determined in detail the effects of ligand binding to the closure of the bilobed ligand-binding domain (LBD) of iGluRs. Because the level of iGluR-LBD closure is directly coupled to the ion channel opening, it is important to distinguish which ligand-receptor interactions are required for different functions. A multi-step computational approach involving molecular dynamics (MD) simulations was utilized to produce a dynamic view of the ligand-receptor binding interactions. The MD simulations were also used widely to interpret experimental data and to predict the binding effects of novel ligands on iGluR-LBD closure.

2 REVIEW OF LITERATURE

2.1 Glutamate receptors

L-glutamate binding into glutamate receptors (GluRs) promotes the entry of ions into the postsynaptic neuron, which increases the likelihood of excitation of the neuron. The mammalian GluRs are divided into iGluRs and metabotropic glutamate receptors (mGluRs; Fig. 1) but also bacteria have structurally related bilobed proteins (O'Hara et al. 1993). The bacterial amino acid binding proteins (BBPs) such as lysine-, arginine-, ornithine-binding protein (LAOBP; Kang et al. 1991) and leucine/isoleucine/valine-binding protein (LIVPB; Sack et al. 1989) are involved in periplasmic nutrient transport (O'Hara et al. 1993).

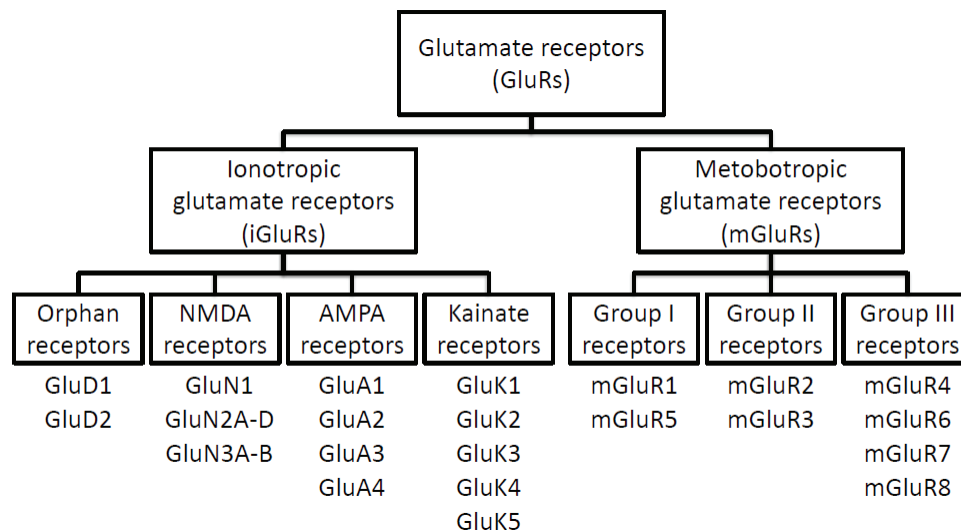


FIGURE 1 The glutamate receptor (GluR) family is divided to ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs).

The binding of L-glutamate into iGluRs induces prompt excitation because it directly opens cationic ion channel formed by the receptor complex (O'Hara et

al. 1993). The mGluRs do not form ion channels like iGluRs but they produce a G-protein or guanine nucleotide-binding protein coupled modulatory effects that affect for example the neuronal excitability of voltage-dependent Ca^{2+} channels (Endoh 2004).

The iGluRs have been linked to a variety of neurological diseases such as migraine (Filla et al. 2002, Sang et al. 2004), epilepsy (Barton et al. 2003), pain (Dominguez et al. 2005, Gilron et al. 2000, Sang et al. 1998), cerebral ischemia (O'Neill et al. 1998), anxiety disorders (Alt et al. 2004), and Parkinson's disease (Greenamyre et al. 1994, Klockgether et al. 1991). Although iGluRs are crucial for the normal functioning of the CNS, they also have more peripheral functions modulating the pain responses in skin (Ault & Hildebrand 1993), insulin secretion in pancreatic islet cells (Inagaki et al. 1995), as well as inducing bone resorption (Chenu et al. 1998) and histamine secretion from mast cells (Purcell et al. 1996).

2.2 Ionotropic glutamate receptors

The iGluRs are divided into AMPA (2*S*,3*S*,4*S*)-3-(carboxymethyl)-4-prop-1-en-2-ylpyrrolidine-2-carboxylic acid), NMDA (*N*-methyl-D-aspartate), and kainate (2*S*,3*S*,4*S*)-3-(carboxymethyl)-4-prop-1-en-2-ylpyrrolidine-2-carboxylic acid) receptors (Fig. 1) based on their sequence differences and ligand binding differences (For review see, Hollmann & Heinemann 1994). In addition, there exist orphan receptors GluD1 (GluR δ 1) and GluD2 (GluR δ 2; Fig. 1) that are categorized as iGluRs based on the sequence similarity (Lomeli et al. 1993, Yamazaki et al. 1992), however, their function is mainly unknown.

2.2.1 AMPA receptors

The AMPA subunits (GluA1-4; Fig. 1) GluA1 (GluR1; GLU_{A1}), GluA2 (GluR2; GLU_{A2}), GluA3 (GluR3; GLU_{A3}), and GluA4 (GluR4; GLU_{A4}) form heteromeric receptors that mediate majority of the fast excitatory neurotransmission in mammalian brain (Hollmann & Heinemann 1994). AMPA receptors promote Na^+ influx but are impermeable to Ca^{2+} if the tetramer complex contains GluA2 subunit (Burnashev et al. 1992). The AMPA subunits exist as alternatively spliced flip or flop variants (Sommer et al. 1990).

2.2.2 Kainate receptors

Kainate receptors (GluK1-5; Fig. 1) form homomeric assemblies of GluK1 (GluR5; GLU_{K5}), GluK2 (GluR6, GLU_{K6}), and GluK3 (GluR7; GLU_{K7}) or heteromeric assemblies with GluK4 (KA1; GLU_{K1}) and GluK5 (KA2; GLU_{K2}). Kainate receptors promote Na^+ and Ca^{2+} influx (For review see, Kew & Kemp 2005). Presynaptic kainate receptors control L-glutamate release in hippocampus (Chittajallu et al. 1996); however, the postsynaptic kainate receptors can also produce slow excitation in addition to AMPA-type fast excitation (Castillo et al. 1997).

2.2.3 NMDA receptors

NMDA receptor family (GluN1-3; Fig. 1) is composed of GluN1 (NMDA-R1; GLU_{N1}), GluN2A-D (NMDA-R2A-D; GLU_{N2A-D}), and GluN3A-B (NMDA-R3A-B; GLU_{N3A-B}) subunits (Hollmann & Heinemann 1994). The functional NMDA receptors are formed as heteromeric assemblies of GluN1 and one or two GluN2 subunits or GluN1 together with both GluN2 and GluN3 subunits. NMDA receptors produce much slower activation and deactivation than kainate and AMPA receptors (Kew & Kemp 2005). However, the fast activation by AMPA and kainate receptors removes the voltage-dependent Mg^{2+} block from NMDA receptor ion channels (Johnson & Ascher 1990, Nowak et al. 1984). NMDA receptors promote the influx of Ca^{2+} , Na^+ , K^+ ions (MacDermott et al. 1986). The amino-terminal domains (ATDs) are allosterically modulated by Zn^{2+} ions that in turn cause NMDA receptor inactivation (Paoletti et al. 2000).

2.3 Ionotropic glutamate receptor structure

Each iGluR monomer (Fig. 2) is composed of extracellular ATD and ligand-binding domain (LBD), four transmembrane helices (TM1-TM4) that form transmembrane domain (TMD), and cytoplasmic carboxy-terminal domain (CTD). The amino-terminus of iGluR is extracellular and the carboxy-terminus intracellular (Hollmann & Heinemann 1994). The iGluRs are composed of four monomer subunits (Fig. 2) that are arranged as dimers of dimers or as tetramer shaped like the capital letter 'Y' (Fig. 3; Béhé et al. 1995, Sobolevsky et al. 2009). Both ATDs and LBDs pair as dimers that form together the tetramer; however, the dimer pairs come from different subunits. Thus the extracellular domains are tightly intertwined on top of the ion channel (Fig. 3).

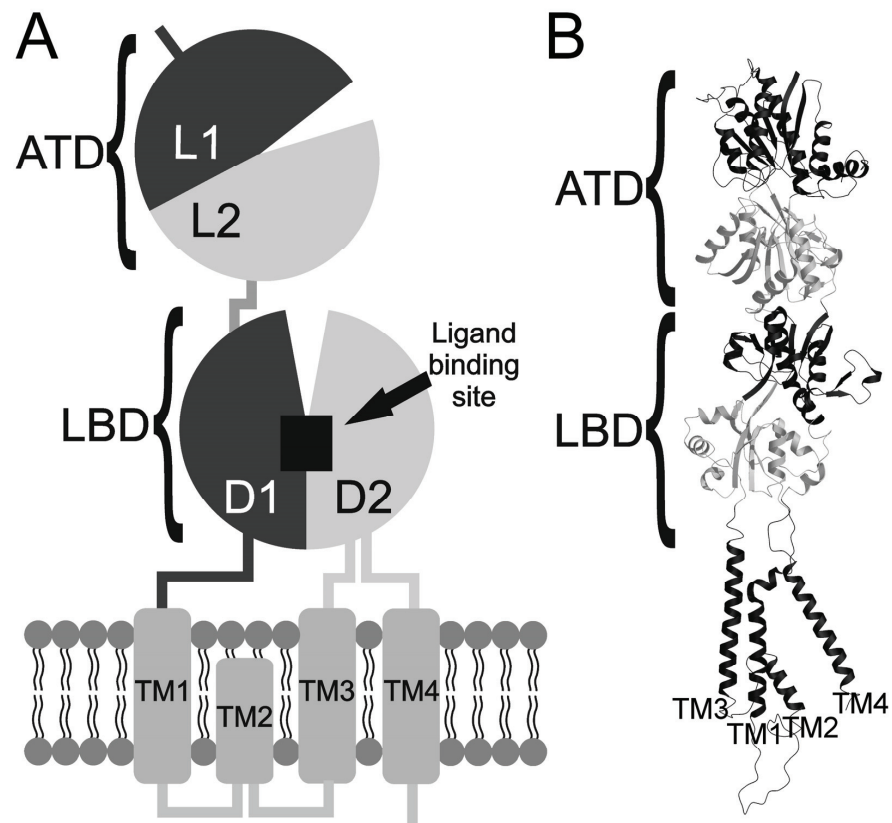


FIGURE 2 The ionotropic glutamate receptor (iGluR) monomer structure shown as (A) a cartoon representation and (B) as a 3D homology model. To fill the missing loops in the cytoplasmic area of ion channel a homology model was built based on the GluA2 crystal structure (PDB: 3KG2; Sobolevsky et al. 2009) and rat GRIA2 sequence (Keinänen et al. 1990). The monomer includes extracellular amino-terminal domain (ATD) and ligand-binding domain (LBD), transmembrane helices (TM1-4) embedded in cell membrane, and cytoplasmic carboxy-terminal.

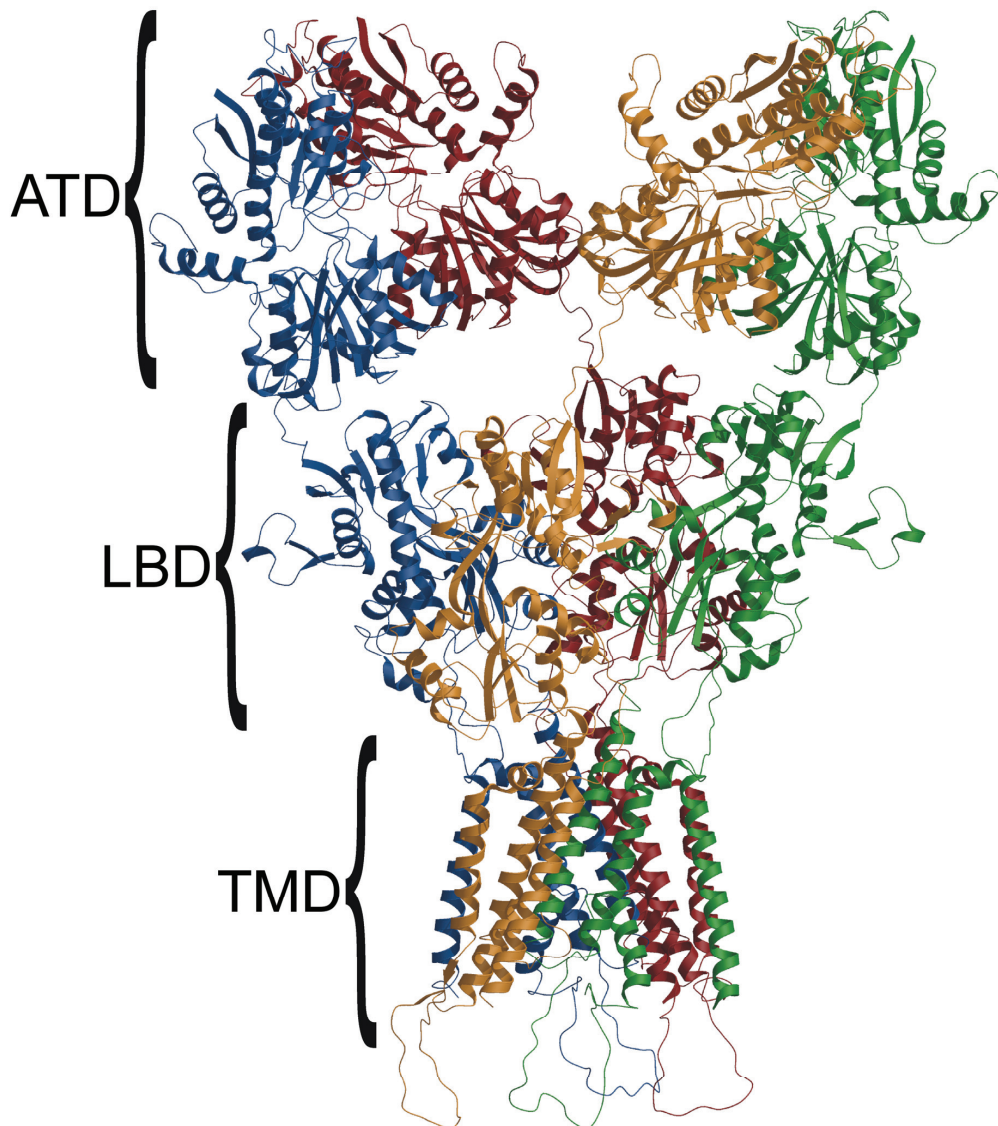


FIGURE 3 GluA2 receptor tetramer structure. The tetramer is composed of four amino-terminal domains (ATDs), ligand-binding domains (LBDs), and transmembrane domains (TMDs) that together form the ion channel. The carboxy-terminal domain (CTD) is missing from the homology model. The monomers forming the tetramer are shown with different colors. For further information see Fig. 2.

2.3.1 Amino-terminal domain

The ~400 residue iGluR-ATD has a clamshell-like shape with amino-terminal L1 and carboxyl-terminal L2 lobes (Figs. 2 and 3; Jin et al. 2009). The ATD is not needed for iGluR function or assembly (Pasternack et al. 2002). However, the subclass-specific assembly of iGluRs is controlled by extracellular ATDs (Ayalon & Stern-Bach 2001, Ayalon et al. 2005, Leuschner & Hoch. 1999). According to Xia et al. a tripeptide IGI motif in the ATD of AMPA receptors is responsible for anterograde trafficking of the receptor from the endoplasmic reticulum to the cell membrane (Xia et al. 2002). The GluA2-ATD has been reported to control the size and quantity of dendritic spines and induce their formation (Passafaro et al. 2003). The isolated iGluR-ATD has been crystallized from several sources and by a number of research groups (Clayton et al. 2009, Jin et al. 2009, Kumar et al. 2009) and it is structurally related to LIVBP and mGluR-LBDs (O'Hara et al. 1993). The binding of negative allosteric modulators such as zinc ions closes the ATD cleft of NMDA receptors, which in turn causes receptor inactivation by destabilizing the LBD dimer interface (Gielen et al. 2008).

2.3.2 Ligand-binding domain

The ~250 residue iGluR-LBD (Figs. 2 and 3) is composed of S1 and S2 segments that are intertwined into two functional lobes (or domains), D1 and D2, linked by a flexible hinge formed primarily by antiparallel β -strands (Armstrong et al. 1998). When full agonists such as L-glutamate bind inside the hinged clamshell-like gorge formed by the two globular domains, the receptor cleft closes (Fig. 4A; Armstrong & Gouaux 2000). Before the successful crystallization of isolated iGluR-LBDs, homology modeling based on BBPs was used to understand the structure (Lampinen et al. 1998, Paas et al. 1996). The isolated iGluR-LBDs, which are referred as the ligand-binding cores (LBCs) have been crystallized without and in complex with ligands to various closure stages (Fig. 4). In the crystallization studies the transmembrane domains TM1 and TM3 are usually jointed together by introducing an artificial Gly545-Thr546 linker (GluK1 numbering). Mounting evidence suggests that the iGluR-LBD closure stage controls directly the transmembrane ion channel opening (Armstrong & Gouaux 2000).

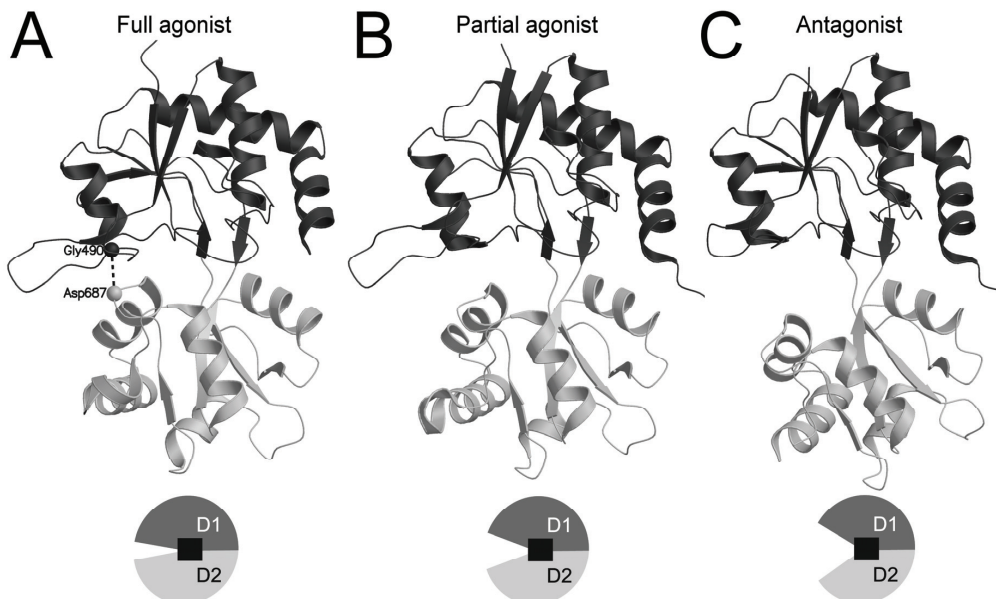


FIGURE 4 The main closure stages of GluK1-LBC shown with GluK1-LBC crystal structures. (A) The GluK1-LBC with full agonist L-glutamate complex (PDB: 1YCJ) is fully closed as shown by the Gly490^N-Asp687^O H-bond. (B) The receptor cleft of partial agonist domoate-GluK1 complex (PDB: 2PBW) shows an intermediate level of closure. (C) The antagonist (S)-ATPO bound GluK1-LBC (PDB: 1VSO) is fully open.

2.3.3 Transmembrane domain and carboxy-terminal domain

The four transmembrane helices (TM1-4) of each receptor monomer form together the tetrameric TMD or ion channel (Fig. 3). The ion channel structure follows an axis of ~ 4 -fold rotational symmetry. To be exact, TM2 is not a transmembrane helice but a cytoplasm-facing re-entrant membrane loop. In the antagonist bound GluA2 tetramer crystal structure TM3 helices are arranged inside the ion channel and the TM4 helices reside in the exterior coordinating extensive subunit-subunit interactions (Sobolevsky et al. 2009). The 3D structure of carboxy-terminal domain (CTD), whose length varies considerably between receptor subunits, has not been solved as of yet. The CTD is required for the assembly of functional iGluRs (Ayalon & Stern-Bach 2001). The receptor localization, biosynthesis, subcellular distribution, and protein-protein interactions are controlled *via* the phosphorylation sites in the carboxy-termini (For review see, Wang et al. 2005). For example the carboxy-terminus of GluA1 receptor has phosphorylation sites for both protein kinases A and C (Roche et al. 1996).

2.4 Coupling ligand-binding domain closure to activation

The closure stage of iGluR-LBD in response to ligand binding can be determined experimentally by fluorescence resonance energy transfer-based assay (Mankiewicz et al. 2008, Ramanoudjame et al. 2006). Also nuclear magnetic resonance measurements (Ahmed et al. 2007), the Fourier transform infrared spectroscopy (Jayaraman et al. 2000), and MD simulations (Lau & Roux 2007) have been useful in determining the receptor dynamics. However, the detailed view of the iGluR-LBD closure and receptor activation has been mostly provided by the crystallographic studies.

2.4.1 Full agonism

According to a crystallographic study without a bound ligand (*apo*) the GluA2 cleft stays open. The binding of full agonists such as the endogenous neurotransmitter L-glutamate is needed to induce screw-axis bending or closure of the open iGluR cleft (Fig. 4A). This receptor cleft closure is coupled to ion channel opening (Armstrong & Gouaux 2000) and agonist efficacy (Jin et al. 2003, Mankiewicz et al. 2008). In another word, high affinity binding does not ensure strong iGluR activation; instead, the ligand binding has to induce full receptor cleft closure to produce maximal response. Accordingly, binding of a full agonist leads into the entry of ion channel specific cations into the cytoplasm and the depolarization of the neuron. In addition to natural neurotransmitter L-glutamate there exist several receptor-specific full agonists that close the iGluR-LBD and induce receptor activation. For example the crystal structures of AMPA-GluA2 (Armstrong & Gouaux 2000), quisqualate-GluK2 (Mayer. 2005), and D-serine-GluN1 (Furukawa & Gouaux 2003) complexes show full receptor cleft closure. The activation of NMDA receptors is a notable exception as it requires simultaneous binding of L-glutamate to GluN2 and co-agonist glycine to GluN1 (Furukawa et al. 2005). Full iGluR-LBC closure has been achieved with full agonist using umbrella sampling technique (Lau & Roux 2007).

2.4.2 Partial agonism

By definition partial agonists for iGluRs are ligands whose binding produces weaker maximum response than the endogenous neurotransmitter L-glutamate. The extent of iGluR cleft closure is flexible and ligand-specific, accordingly, the partial agonist-binding seems to produce intermediate or partial closure of the receptor cleft (Fig. 4B; Armstrong & Gouaux 2000, Furukawa & Gouaux 2003, Jin et al. 2003, Mayer. 2005, Nanao et al. 2005) as well as partial activation. The partially closed receptor cleft has been determined for domoate-GluK1 (Hald et al. 2007), kainate-GluK2 (Mayer. 2005), and kainate-GluA2 (Armstrong & Gouaux 2000) complexes using crystallography. However, a docking study

suggests that small partial agonists for GluK2 would prefer the fully closed conformation instead of intermediate closure (Fay et al. 2009). Also, prior MD simulation studies suggest that partial agonist binding within the ligand-binding pocket is more flexible than with full agonists (Arinaminpathy et al. 2002, Arinaminpathy et al. 2006). With AMPA receptors the open iGluR-LBC is not linked directly to antagonism but to partial agonism, if transmembrane AMPA receptor regulatory proteins (TARPs) are also expressed with the GluA2 receptor (Menuz et al. 2007). The TARPs are AMPA receptor auxiliary proteins that affect both the functional properties of AMPA receptor channel gating, pharmacology, and cell membrane trafficking (For review see, Milstein & Nicoll 2008). In addition, the crystallization implies perplexingly that the NMDA-LBCs are fully closed with both full agonists and partial agonists (Furukawa & Gouaux 2003, Inanobe et al. 2005).

2.4.3 Antagonism

When the iGluR-LBD is in open conformation, the transmembrane helices keep close to each other (Fig. 3; Sobolevsky et al. 2009), and no ions pass through the ion channel. Inverse agonists or antagonists prevent the closure of the iGluR-LBD by restraining the receptor cleft open (Fig. 4C) and thus keeping the ion channel closed. The GluA2 receptor cleft of both *apo* and antagonist bound crystal structures are wide open according to crystallization (Armstrong & Gouaux 2000), however, nuclear magnetic resonance study indicate some conformational flexibility (Ahmed et al. 2007). According to a MD simulation study the iGluR-LBC could close fully even without a bound agonist (Bjerrum & Biggin 2008). The function blocking antagonists are sought after because they are useful for pharmacological studies and drug discovery projects. The discovery of iGluR subtype-specific and competitive antagonists would be especially useful for discriminating different receptor functions in the CNS. The ligand-binding pocket of GluK1 receptor is relatively large, therefore it can house several bulky antagonists such as LY466195 (Weiss et al. 2006). Accordingly, GluK1-LBC has been crystallized into open conformation in complex with antagonists (S)-ATPO (Hald et al. 2007), UBP310, and UBP302 (Mayer et al. 2006). In addition, crystallography indicates that GluA2 cleft stays open with bound antagonists CNQX (Menuz et al. 2007), DNQX (Armstrong & Gouaux 2000), and (S)-ATPO (Hogner et al. 2003).

2.5 Desensitization

Both AMPA and kainate receptors bind L-glutamate with low-affinity and deactivate quickly as the neurotransmitter detaches. Moreover, the iGluRs cannot induce postsynaptic excitation indefinitely even if the iGluR-LBD remains continuously occupied by an agonist. A process called desensitization assures that the bound agonist cannot keep the ion channel endlessly open and; thus, the neuron is able to recover from the agonist-induced excitation. In continued presence of L-glutamate AMPA and kainate receptors desensitize rapidly; however, AMPA receptors recover from desensitization ~10-fold faster than kainate receptors (For review, Dingledine et al. 1999). The flop splice variants of GluA3 and GluA4 receptors induce shorter desensitization time constants (~1 ms) than the same flip variants (~3 ms; Mosbacher et al. 1994).

The pace or recovery from the desensitization does not depend solely on the receptor subunit composition but also the agonist ligands have unique properties. For example L-glutamate desensitizes GluK1 receptor within ~4.1 ms and with kainate this can take from 1.5 ms to 3 s (Swanson et al. 1997). The desensitization of kainate receptors can be prevented by introducing plant lectin concanavalin A (Schiffer et al. 1997). Also cyclothiazide blocks effectively the desensitization of AMPA receptors (Patneau et al. 1993). Similarly as TARPs modulate AMPA receptor activation and desensitization (Milstein & Nicoll 2008) a brain-specific protein NETO2 reportedly modulates kainate receptor function by inducing slower entry into the desensitization and faster recovery (Zhang et al. 2009). At structural level the desensitization has been linked to the D1-D1 or dimer interface rearrangements after ion channel opening (Armstrong et al. 2006, Sobolevsky et al. 2009, Sun et al. 2002). Without Cl⁻ ions both the dimer destabilization and the desensitization reportedly increases (Plested & Mayer 2007).

3 AIMS OF THE STUDY

The structure-activity relationship of ligand-iGluR complexes is crucial for future drug design projects. Accordingly, the main purpose of this thesis was to determine how ligand binding affects the closure stages of iGluR-LBCs. All-atom MD simulations with explicit solvent were performed using existing iGluR-LBC crystal structures to find out, how the ligand-receptor complexes interact in solvent. The aim was also to see if the MD simulations could be used to recreate the receptor cleft closure stages and ligand-binding modes seen in the crystal structures. For novel compounds that lacked prior structural data the aim was to predict the ligand binding interactions and receptor cleft closure stages. The modeling results were also used to explain the experimental binding affinity and receptor subtype specificity data. In addition, the simulations were expected to shed light on the mechanisms that underlie partial agonism and desensitization.

4 MATERIALS AND METHODS

4.1 Databases

The crystal structures used in the thesis were acquired from the Protein Data Bank (PDB; Berman et al. 2000; <http://www.pdb.org/>). The amino acid sequences of proteins were extracted from Swiss-Prot (Bairoch & Apweiler 2000). The 2D structures of ligands were acquired from various sources in the literature.

4.2 Homology modeling

When there was no crystal structure of the ligand-receptor complex available or the structure was incomplete (*e.g.* missing loops), homology modeling was used. The crystal structure was aligned with the amino acid sequence using MALIGN in BODIL (Lehtonen et al. 2004) and based on the alignment homology models were built using NEST in JACKAL (Petrey et al. 2003) or MODELLER9v7 (Sali & Blundell 1993).

4.3 Ligand molecules

The ligand molecules were drawn in 3D and energy minimized using SYBYL7.3 (Tripos Inc., St Louis, MO). The ligand conformations were then geometry-optimized and electrostatic potentials were calculated quantum mechanically using GAUSSIAN03 (Gaussian Inc., Wallingford, CT) at the HF/6-31+G* level using a continuum (PCM) water model. Because HF/6-31+G* basis set does not include iodine, the electronic potentials for 5-I-willardiine were calculated using HF/3-21G and Merz-Kollman radius of 2.3 for iodine (Singh & Kollman 1984).

In cases, where the studied ligand had already been crystallized with the protein, the 3D conformation of the ligand was acquired directly from the crystal structure without geometry-optimization (V). The restrained electrostatic potential methodology (Bayly et al. 1993, Cieplak et al. 1995, Cornell et al. 1993) was used to calculate the atom-centered point charges from the electrostatic potentials.

4.4 Structure comparison and ligand positioning

Structure comparisons and in some cases ligand positioning (V) were acquired by superimpositioning the C α -atoms of the target structure with a ligand-receptor complex using VERTAA in BODIL. Alternatively, the ligands were flexibly docked using GOLD3.1 (Jones et al. 1995, Jones et al. 1997). In each case, ligand positioning inside the ligand-binding pocket was chosen based on the docking scoring and by comparing the positioning to similar crystallized ligand-receptor complexes. A central position in the ligand-binding pocket was used as a search area of a 15 Å radius sphere for docking. For example with GluK2 receptor the O^{OH/O.3}-atom of Tyr488 was chosen as the central position.

4.5 Molecular dynamics simulations

4.5.1 Simulation settings

The MD simulations of the ligand-LBC complexes were run either with monomer (IV, V) or dimer structures (I-IV). The charges of chemically comparable atoms were set equal for each ligand molecule. The extra chains, ions, ligands *etc.* of crystal structures were removed using BODIL. Also extra water molecules in too close proximity (~ 1.4 Å) of the docked ligands were removed if original crystal structure was not used directly. The Gly545-Thr546 linker (GluK1 numbering) was removed to allow more flexible movement of the D1-hinge-D2 region. Disulphide bridges were built between adjacent cysteines (GluA2: Cys739-Cys794; GluK1: Cys750-Cys804; GluN1: Cys420-Cys454 and Cys436-Cys455) and the protonation state of histidines was chosen according to nearby water molecules and residues. TLEAP in ANTECHAMBER-1.27 (Wang et al. 2006) was used to (a) solvate the ligand-receptor complexes with water boxes of transferable intermolecular potential three-point (TIP3P) water molecules 13 Å in every direction, (b) appropriate counter ions (Na⁺ or Cl⁻) were added to neutralize the charged ligand-receptor complexes, (c) AMBER force field parameters for the protein (parm99) and ligands (gaff) were generated, and (d) hydrogen atoms were added.

4.5.2 Simulation runs

The two energy minimization and three MD simulation steps were sequentially performed using NAMD2.6 (Phillips et al. 2005) in constant volume. (a) Water molecules, counter-ions, and side chains of residues were energy minimized with the conjugate gradient algorithm for 15,000 steps while the C^α-atoms were restrained with the harmonic force of 5 kcal mol⁻¹ Å⁻². (b) Next, the entire system was energy minimized without constraints for 15000 steps. (c) Finally, the system was MD simulated with restrained C^α-atoms for 30,000 steps and then (d) also in constant pressure for 30,000 steps. (e) The actual production MD simulation ranging from 14-46 ns were repeated usually two to three times using same set-up without constraints.

During the minimization and simulation steps the system was kept at 300 K with a Langevin damping coefficient of 5 ps⁻¹ for all non-hydrogen atoms; however, 400 K was also tested for closed GluA2-LBC simulations with kainate and L-glutamate (V, Fig. S3). Nosé-Hoover Langevin piston (Feller. 1995) was used to keep the pressure at 1 atm with an oscillation timescale of 200 fs and a damping timescale of 100 fs. An integration time step of 2 fs was used under a multiple time stepping scheme (Schlick et al. 1999). The bonded and short-range interactions were acquired every time step and long range electrostatic interactions every third step. A cutoff of 12 Å was used for the short-range electrostatic interactions and van der Waals forces. A switching function was enforced for the van der Waals forces to make the cutoff smoother. The periodic boundary conditions were used in the minimization and simulation steps. The long-range electrostatics were counted with the particle-mesh Ewald (PME) method (Darden et al. 1993, Essmann et al. 1995, Sagui & Darden 1999, Toukmaji et al. 2000). The H-bonds were restrained using the SHAKE algorithm (Ryckaert et al. 1977).

4.5.3 Simulation analysis

PTRAJ 6.5 (I-IV, standalone version: www.chpc.utah.edu/~cheatham/software.html, accessed 1.5.2007) or PTRAJ in ANTECHAMBER1.27 (V, Wang et al. 2006) was used to extract snap shot structures at 360 ps interval and distances between selected atom pairs from the simulation trajectories. The atom pairs used in the distance measurements were selected from opposite sides of the bilobed iGluR-LBC (III, Fig. 3; V, Fig. 1, Fig. S2). The distance of 3.4 Å was considered as the upper limit for an H-bond, when considering H-bonding in the MD simulations. The closure angles of MD snap shot structures were compared to the open antagonist UBP302 bound GluK1-LBC crystal structure (Mayer et al. 2006) using HINGEFIND algorithm (III, Fig. 5; Wriggers & Schulten 1997).

4.6 Visualization

Figures showing ligand binding interactions or structure comparisons were rendered using BODIL, MOLSCRIPT (Kraulis 1991), and RASTER3D (Merritt. 1997). The 2D images of ligands were created using ISISDRAW or SYMYX DRAW3.1 (Symyx Technologies, Inc., Santa Clara, CA, USA).

4.7 Experimental data

All the experimental data presented in the thesis were produced by our collaborators. The site directed mutagenesis, electrophysiology, and radioligand binding assays were done in Professor Geoffrey Swanson's laboratory at Northwestern University Feinberg School of Medicine, Chicago, USA (I, II, IV). The crystallography was done in Professor Jette Kastrup's laboratory at University of Copenhagen, Denmark (II). The dysiherbaine (DH) analogs were acquired from the laboratories of Professor Ryuichi Sakai in Hokkaido University, Japan and Professor Sasaki in Tohoku University, Japan (I, II, IV). Rest of the experimental data utilized in the thesis was acquired from literature.

5 REVIEW OF THE RESULTS

5.1 Recreating receptor cleft closure (III, V)

The iGluR-LBCs have been crystallized in complex with full agonists, partial agonists, and antagonists into closed, partially open, and open conformations, respectively (Fig. 4; V, Tables S2 and S3). Thus, there exist a set of compounds whose effects on both iGluR ion channel function and cleft closure are well known. The crystal structures are nevertheless only static snapshots of the ligand-receptor interactions that exist in solution. The MD simulations provide a more dynamic view of ligand-binding interactions and receptor cleft closure. More importantly, there exist kainate receptor-specific dysiherbaine (DH) analogs (III, Fig. 1, IV, Fig. 1) and a large set of small partial agonists for GluK2 receptor that lack crystallographic data (V, Fig. S1; Fay et al. 2009). To predict the effects of novel ligands on iGluR cleft closure; they were docked or inserted inside the closed iGluR-LBCs and simulated with MD. For this new simulation data to be comparable to the established findings, also the effects of the well-known ligands on the closed receptor cleft were simulated.

5.1.1 Full agonists keep receptor cleft closed (III, V)

The MD simulations suggest that the iGluR cleft stays closed with bound full agonists. Full agonist L-glutamate keeps the receptor clefts of GluK1 (PDB: 1YCJ; III, Fig. 5B) and GluA2 (PDB: 1FTJ, C chain; V, Fig 2 C, Table S2) effectively shut in MD simulations. The receptor cleft of full agonist AMPA-GluA2 complex also remained tightly shut (V, Fig. 2A, Table S2), however; the cleft closure was firmest in full agonist glycine-GluN1 (PDB: 1PB7, A chain; V, Fig. 6B, Table S6) and D-serine-GluN1 (PDB: 1PB8, A chain; V, Table S6) simulations. This cleft closure stability in full agonist binding is best indicated by the Gly490^N-Asp687^O H-bonding for GluK1 (III, Fig. 5A and B), the Gly472^N-Ser673^O H-bonding for GluA2 (IV, Fig. 2A and C), and the Gly485^N-Gln686^O H-bonding for GluN1 (V, Fig. 6B). The formed H-bond (V, Fig. S2) indicates both full iGluR

cleft closure and full agonism. The angle measurements performed for ligand-GluK1 simulations with Hingefind algorithm also suggested that this particular H-bond is a good sign of receptor cleft closure (III, Fig. 5).

In L-glutamate-GluK2 complex simulations (V, Fig. 2E) the receptor cleft closure was not as firm as in equivalent GluK1, GluA2, or GluN1 simulations. Similar periodic disruptions in the Gly489^N-Asp687^O H-bonding were also present in SYM 2081-GluK2 simulations using both the original crystal structure (PDB: 1SD3, V, blue lines in Fig. 2F) and the previously L-glutamate bound structure (PDB: 1S50, V, red lines in Fig. 2F). The results indicate that GluK2 cleft cannot retain the same level of tightness or firmness in closure as the simulations progress. The wobbly cleft closure suggests that less force or a smaller partial agonist is required to open the GluK2 cleft, accordingly there exist numerous small partial agonists for GluK2 (V, Fig. S1). These small partial agonists could not wedge the GluK2 cleft open similarly as for example bulky kainate does. Despite the unstable cleft closure observed in full agonist-GluK2 simulations, as a whole the fully closed iGluR cleft and lasting interdomain H-bonding in MD simulations point toward full agonism.

5.1.2 Initial H-bonding needed for tight receptor cleft closure (V)

Full agonists can be distinguished from partial agonists and antagonists by concentrating on the interdomain H-bonding. Therefore, the importance of initial H-bonding for full agonist simulations was studied next using an alternative L-glutamate-GluA2 structure (PDB: 1FTJ, A chain). In this conformation the peptide bond between Asp672 and Ser673 is flipped and the Gly472^N-Ser673^O H-bond (V, Fig. S2) is not formed (Armstrong & Gouaux 2000). If this alternative L-glutamate-GluA2 complex was MD simulated, the H-bond did not form regularly (V, Fig. 2B, Table S1). In fact the Gly472^N-Ser673^O H-bond formed only in one of three L-glutamate-GluA2 simulations if it was not there from the beginning (V, magenta line in Fig. 2D). The closure stages of flipped and unflipped GluA2-LBC structures are very similar if their C^α-atoms are superimposed (V, Fig. 2B). As even the natural neurotransmitter was not able to force full GluA2 cleft closure, the subsequent closed conformation simulations were done with fully closed and unflipped iGluR-LBCs that had the equivalent H-bond from the beginning.

5.1.3 Bulky partial agonists open receptor cleft partially (III, V)

Bulky partial agonists such as kainate and domoate (V, Fig. S1) wedge the iGluR cleft partially open in the crystal structures (Armstrong & Gouaux 2000, Mayer. 2005, Nanao et al. 2005). These original crystal structures were MD simulated to acquire a less static view of partial agonist-iGluR complexes. The intermediate receptor cleft closure of kainate-GluA2 (PDB: 1FTK, A chain; V, Fig. 3B) and domoate-GluK2 (PDB: 1YAE, B chain; V, Fig. 3F) complexes were preserved in the MD simulations. However, the Glu489^N-Asp687^O H-bond formed regularly in kainate-GluK2 (PDB: 1TT1, A chain, V, Fig. 3D) simulations.

The MD simulations suggest that GluK2 cleft could fluctuate between fully closed and partially open conformations with bound partial agonists. Moreover, the partial receptor activation is not necessarily linked to intermediate iGluR cleft closure but to the inability of partial agonists to upkeep equally firm receptor cleft closure as full agonists.

Partial agonist domoate produced intermediate cleft closure in MD simulations in complex with the closed LBCs of kainate receptors GluK1 (PDB: 1YCJ, III, Fig. 5D) or GluK2 (PDB: 1S50 A chain, V, Fig. 3G). Similarly, partial agonist kainate opened the closed GluK2 cleft (PDB: 1S50, A chain, V, Fig. 3E). The opening induced by these bulky partial agonists was usually immediate and it was close to the level of cleft closure seen in crystal structures (Armstrong & Gouaux 2000, Mayer. 2005, Nanao et al. 2005). However, in kainate-GluA2 (PDB: 1FTM, A chain, V, Fig. 3C) simulations the closed cleft stayed shut and minimal opening took place only in one simulation (V, magenta line in Fig. 3C). This inability of kainate to open the closed GluA2-LBC underlines the importance of initial receptor cleft closure stage for bulky partial agonists and antagonists in MD simulations. Because kainate only barely fit inside the closed GluA2-LBC, the initial positioning of the ligand in relation to the residues in the ligand-binding pocket was far from optimal. Therefore, the MD force field could not adjust kainate-receptor complex correctly and the receptor cleft did not open. By raising the simulation temperature from 300 K to 400 K kainate was able to align correctly and the GluA2 cleft opened partially (V, Fig. S3). The increased flexibility produced by the elevated temperature allowed kainate to position correctly and its ring system stayed close to the conformation seen in the crystal structure (Armstrong & Gouaux 2000).

5.1.4 Partial agonists that destabilize GluA2 and GluN1 cleft closure (V)

5-substituted willardiines or 5-R-willardiines (V, Fig. S1) are partial agonists that also produce intermediate closure stages in complex with GluA2-LBC in crystallographic and fluorescence resonance energy transfer studies (V, Fig. 4A, Table S2; Jin et al. 2003, Mankiewicz et al. 2008). In MD simulations the receptor cleft closure of original 5-R-willardiine-GluA2 crystal structures did not change substantially as indicated by the Gly472^N-Ser673^O H-bonding (V, Fig. 4B and D). Especially the 5-Br-willardiine-GluA2 crystal structure simulations suggested that the partially open GluA2 cleft is able to close temporally with these partial agonists (V, Fig. 4F). The periodic cleft closure was similar to the fluctuation seen in the kainate-GluK2 crystal structure simulations (V, Fig. 3D).

When the 5-R-willardiines were simulated in complex with the closed GluA2-LBC (PDB: 1S50, A chain), the receptor cleft did not usually open. Unlike in 300 K kainate-GluA2 simulations (V, Fig. 3C), the Gly472^N-Ser673^O H-bonding was disrupted routinely in 5-R-willardiine-GluA2 simulations (V, Fig. 4C, E, and G). Moreover, the Gly472^N-Ser673^O H-bonding with 5-R-willardiines was substantially more erratic than with full agonists AMPA and L-glutamate (V, Fig. 2A and C). The results indicate that 5-R-willardiines either destabilize GluA2 cleft closure or simply separate the lobes. Because the H-bonding

diverged consistently between full agonist and partial agonist simulations, large-scale GluA2 cleft opening with partial agonists was not needed for *in silico* ligand categorization.

The Gly485^N-Gln686^O H-bond was disrupted when the closed GluN1-LBCs were MD simulated with partial agonists ACPC (PDB: 1Y20; V, Fig. 6C) and D-cycloserine (PDB: 1PB9; V, Fig. 6C). The H-bonding disruptions were immediate and extensive with D-cycloserine but they took more time and were lesser in scale with ACPC. However, there were no H-bonding disruptions in partial agonist ACBC-GluN1 (PDB: 1Y1Z; V, Fig. 6E) simulations. In contrast, with full agonists glycine (V, Fig. 6B) and D-serine there were no disruptions in the Gly485^N-Gln686^O H-bonding or substantial GluN1 cleft opening. Therefore, the modeling suggests for the first time that partial agonist binding affects the GluN1 cleft closure (V, Fig. 6A).

5.1.5 Antagonists open receptor cleft (III, V)

The crystallography suggests that without a bound ligand and with bound antagonists the iGluR-LBCs are fully open (Armstrong & Gouaux 2000, Hogner et al. 2003). When a fully closed *apo*-GluK1-LBC (PDB: 1YCJ) was simulated, the receptor cleft did not open (III, Fig. 5I). Thus, the GluK1 cleft opening or disruptions in the interdomain H-bonding should result solely from the effects of ligand binding. The *apo* receptor cleft closure stability likely varies between iGluR subtypes, for example the closed GluK2-LBC did not remain stably closed even with bound full agonists (V, Fig. 2E and F). The receptor cleft of *apo*-GluA2-LBC (PDB: 1FTO) crystal structure remained open in MD simulations without reaching the Gly472^N-Ser673^O H-bonding range (V, Table S2). As a whole, the *apo* simulations indicate that the iGluR cleft cannot open or close without a bound ligand in MD simulations. On the other hand, antagonists LY466195 (IV, Table 2, Fig. 5E, Table 2) and DNQX (IV, Table 2) opened the closed GluK1 cleft notably in MD simulations. Accordingly, the receptor cleft was open also in the LY466195-GluK1 (PDB: 2QS4) and DNQX-GluA2 (PDB: 1FTL) crystal structures. The results suggest that antagonist bound (open cleft) conformation can be recreated or predicted by simulating the antagonists in complex with closed iGluR-LBCs. The initial iGluR cleft closure or H-bonding is expected to be equally important for recreating or predicting successfully closure stage with antagonists as with bulky partial agonists (V, Fig. 3C).

5.2 Predicting receptor cleft closure (I-V)

Thus far the results have indicated that MD simulations can be used to reproduce the appropriate iGluR-LBC closure stages, when the studied ligands are inserted inside the closed receptor cleft. Next logical step was to utilize the

MD protocol to predict the iGluR cleft closure for molecules lacking prior crystallographic data. These predictions were performed for small GluK2 partial agonists (V, Fig. S1) and a variety of dysiherbaine (DH) based analogs (III, Fig. 1; IV, Fig. 1), which lacked prior crystallographic data. With DH and its analogs the modeling protocol was also used to explain experimental ligand-binding data and receptor subtype specificities (I-V).

5.2.1 Small partial agonists destabilize GluK2 cleft closure (V)

The Gly489^N-Asp687^O H-bonding of initially closed GluK2-LBC (PDB: 1S50, A chain) was in general more destabilized with the small partial agonists for GluK2 (V, Fig. 5) than with the full agonists (V, Fig. 2E and F). However, the closed GluK2 cleft opened completely and consistently only in L-cysteate-GluK2 simulations (V, Fig. 5D). The lack of GluK2 cleft opening in MD simulations and a previous docking study (Fay et al. 2009) suggest that these small partial agonists could in fact prefer the closed cleft conformation. As a whole, the modeling results indicated that full agonists and partial agonists for GluK2 could not be categorized solely using MD simulations (V, Table S3). The similarity in the tightness of receptor cleft closure for both full and partial agonists (V, Fig. 2E and F *vs.* Fig. 5) suggests that GluK2 cleft closure is less stable than with GluK1, GluA2, and GluN1 receptors. Although this is problematic for *in silico* ligand categorization, it also provides an explanation, why these relatively small partial agonists for GluK2 can open the receptor cleft or destabilize its closure.

5.2.2 Dysiherbaine analogs (I-IV)

DH and neodysiherbaine (neoDH; III, Fig. 1) are potent convulsants and full agonists for kainate receptors (III, Table 1). DH was originally extracted from Micronesian marine sponge *Lendenfeldia chodrodes*, which was originally misidentified as *Dysidea herbacea* (Sakai et al. 1997, Sakai et al. 2001a, Sakai et al. 2001b). DH and neoDH are composed of a hydrophobic hydrofuroopyran ring fused with the L-glutamate backbone (III, Fig. 1). DH has a methylamine substituent and neoDH a hydroxyl group at the C8 position in the ring system (III, Fig. 1). There also exist a large set of synthetic DH and neoDH analogs with altered stereochemistries and/or functional groups at C2, C4, C8, C9, and C10 positions (III, Fig. 1; IV, Fig. 1). The experimental results indicated that even relatively minor changes to DH structure could result in major changes in the pharmacological profile (I, II, IV).

5.2.2.1 C8 positioned hydroxyl *vs.* aminomethyl group (II-IV)

The crystallography suggested that DH (PDB: 3GBA) retains both the canonical interactions shared with L-glutamate backbone and keeps the GluK1 receptor cleft closed (II, Fig. 2). The neoDH-GluK1 simulations demonstrated that the binding interactions of neoDH (PDB: 1YCJ; III, Fig. 4A) are close to those of DH

(III, Fig. 2B). Most notably the γ -carboxylate group and the 9-hydroxyl of neoDH form an intramolecular H-bond (III; Fig. 4A, Table I). There also exist direct H-bond between Glu738^N and the 9-hydroxyl with both of the full agonists (III; Figs. 2 *vs.* 4A, Table I). The GluK1 cleft remained closed with neoDH similarly as was seen with other full agonist-GluK1 simulations (III, Table 2). Accordingly, the almost identical ligand-binding properties of DH and neoDH assured that both the activating and desensitizing currents were also similar (III, Table 1).

Although DH and neoDH bind GluK1 with high affinity, the C8 substitution (II, Fig. 1) weakens the binding affinity of neoDH for GluK1 >15-fold (III, Table 1). With GluK2 the difference in binding affinity was in the same scale (III, Table 1); however, neoDH binding for GluK3 was drastically weaker if compared to DH (IV, DH: 5.4 nM *vs.* neoDH: 3005 nM, Table 1). The neoDH binding is substantially weaker because Tyr444 of GluK1 (Tyr443 with GluK2) is replaced with Phe446 in GluK3 (IV, Fig. 6). With GluK1 and GluK2 the side chain hydroxyl of Ser741/Thr741 aligns against Tyr444/443 and H-bonds with the 8-aminomethyl group of DH. The 8-aminomethyl also H-bonds favorably with the γ -carboxylate group of Glu738/739 and the side chain hydroxyl of Ser741 in GluK2 or Thr742 in GluK3. In contrast, the 8-hydroxyl of neoDH interacts unfavorably with negatively charged acceptor groups at the same sites. Although the side chain hydroxyls of Ser741 in GluK1 or Thr741 in GluK2 could donate an H-bond to 8-hydroxyl of neoDH, the H-bonding network would still be disturbed inside the ligand-binding pocket.

The MD simulation derived conclusions on neoDH and DH subtype specificity were confirmed by producing reciprocal mutants for the kainate receptors (IV, Fig. 7). The binding affinity of neoDH for GluK2 decreased to almost same level as with GluK3 when Tyr443 was mutated into phenylalanine. The neoDH binding for GluK2 was only moderately affected by A518T mutation. The single-site mutations GluK3 (F446Y) and GluK3 (T520A) produced only minor effect on neoDH binding affinity.

5.2.2.2 Modest effects of C8 position for dysiherbaine analog binding (I, III)

As the site-directed mutagenesis and MD simulations with DH-GluK1 and neoDH-GluK1 complexes indicated, the 8-aminomethyl group of DH is preferred to 8-hydroxyl of neoDH. However, further alterations made on the C8 position did not prevent high affinity binding with kainate receptors.

For instance, 8-deoxy-neoDH (I, Fig. 1), which lacks the C8-hydroxyl of neoDH, is a full agonist for GluK1 (I). The activating and desensitizing currents produced by 8-deoxy-neoDH with kainate receptors are similar to those of neoDH and DH (III, Table 1). Both neoDH and 8-deoxy-neoDH retain similar canonical interactions as the backbone of L-glutamate with the GluK1-LBC in MD simulations (PDB: 1YCJ; III, Figs. 4A and B). The ring systems of 8-deoxy-neoDH and neoDH align similarly against the hydrophobic residues (*e.g.* Tyr489) in the ligand-binding pocket. Moreover, the binding affinity of 8-deoxy-neoDH for GluK1 is slightly higher than that of the parent compound neoDH (I,

Table 1). The MD simulations suggest that the binding conformation of 8-deoxy-neoDH is either similar to that of neoDH (III, Fig. 4A) or alternatively the ligand stays extended without a direct H-bond to Glu738^N (III; Fig. 4B; Table 1). The GluK1 cleft stayed tightly shut with bound 8-deoxy-neoDH in MD simulations, which was expected behavior as the ligand was experimentally categorized as full agonist (I).

MD simulations were also analyzed to determine why the binding affinity of full agonist 8-deoxy-neoDH was >1000 fold weaker for GluK2 than for GluK1 (I, Table 1). The 9-hydroxyl of 8-deoxy-neoDH forms weaker H-bonds with Thr741/742 in GluK2 and GluK3 than with Ser741 in GluK1. The methyl group of threonine pushes unfavorably against Asn721 (Ser721 in GluK1), thus, the interdomain contact between Glu441 and Asn721 likely destabilizes. Alternatively, the binding of 8-deoxy-neoDH causes more compact hydrophobic packing of GluK2 ligand-binding pocket than with GluK1. The bulky side chain of Phe735 in GluK2 (Leu735 in GluK1) could displace a key water molecule that likely stabilizes the binding of 8-deoxy-neoDH with GluK1.

The reversal of 8-hydroxyl stereochemistry to produce 8-epi-neoDH (I, Fig. 1) did not affect the activation or desensitization of GluK1 receptor notably (III, Table 1). Also the effects on ligand-binding affinity were moderate, if it was compared to neoDH (I, Table 1). In 8-epi-neoDH-GluK1 (PDB: 1YCJ) simulations the reversed 8-hydroxyl forced the ring system to a flat conformation without intramolecular H-bonding (III, Fig. 4C). Even without the neoDH-like ring system bending, the 9-hydroxyl of 8-epi-neoDH was able to H-bond with Glu738^N *via* two water molecules (III; Fig. 4C: 11 and w5). The closed GluK1-LBC stayed very tightly closed in MD simulations with 8-epi-neoDH as was to be expected for a full agonist (I). The GluK1 cleft closure did not change if in addition to reversing the 8-hydroxyl stereochemistry also the 9-hydroxyl was substituted with fluorine to produce 9-F-8-epi-neoDH (I, Fig. 1). Full agonist 9-F-8-epi-neoDH assumed flat and extended conformation against the hydrophobic D1 residues of GluK1-LBC (PDB: 1YCJ) in MD simulations. The linkage to Glu738^N was formed *via* a water molecule (III; Fig. 4D: w3) to the oxygen at the six-membered ring of the 9-F-8-epi-neoDH. The planar conformation of 9-F-8-epi-neoDH assured that its binding affinity was only marginally weaker than that of 8-epi-neoDH (I, Fig. 1).

5.2.2.3 The C9 position is crucial for dysiherbaine analog binding (I, III)

The DH-like stereochemistry at C9 position is requirement for high affinity binding with DH analogs (III, Table 1). The high affinity DH analogs need a polar C9 positioned group and the stereochemistry at C2, C4, and C9 positions should mimic that of DH. Accordingly, if the 9-hydroxyl is removed from the neoDH ring system to produce 9-deoxy-neoDH (I, Fig. 1), the full agonist is transformed into a partial agonist (I). The binding mode of 9-deoxy-neoDH with closed GluK1-LBC (PDB: 1YCJ) is close to the neoDH-GluK1 complex (III, Fig. 4A) in MD simulations; however, without 9-hydroxyl 9-deoxy-neoDH cannot interact with Glu738^N (III, Fig. 6A). The 8-hydroxyl of 9-deoxy-neoDH is

forced into the same position as the 9-hydroxyl of 8-deoxy-neoDH in order to form an H-bond with Ser741. However, the unfavorable hydrophobic alignment of the ring system weakens the binding affinity of 9-deoxy-neoDH for GluK1 ~100-fold, if compared to 8-deoxy-neoDH. Similarly as with bulky partial agonists domoate (III, Fig. 5D), also 9-deoxy-neoDH opens the closed GluK1 cleft partially as indicated by the Gly490-Asp687 H-bonding (III, Fig. 5C).

The epimerization of 9-hydroxyl to produce 9-epi-neoDH (I, Fig. 1) decreased the ligand-binding affinity substantially in relation to the parent molecule neoDH (I, Table 1). Although the 8-hydroxyl of 9-epi-neoDH H-bonds with the side chain of Ser741 with closed GluK1-LBC (PDB: 1YCJ) in MD simulations, the inability of the reversed 9-hydroxyl to H-bond with Glu721^N affects unfavorably the ligand-binding affinity (III, Fig. 6C). Simultaneous inversion of stereochemistry at C8 and C9 positioned hydroxyls to produce 8,9-epi-neoDH (I, Fig. 1) reduced kainate receptor binding affinity drastically (I, Table 1). In the MD simulations, the 9-hydroxyl of 8,9-epi-neoDH could not H-bond with the surrounding residues or form intramolecular H-bond (III, Fig. 6D). The conventional alignment of the ring system against the hydrophobic D1 face and the canonical L-glutamate-like interactions kept the GluK1 cleft closed and assured binding for 8,9-epi-neoDH.

5.2.2.4 Without C8 and C9 positioned hydroxyls dysiherbaine-induced desensitization increased (I-III)

Both of the C8 and C9 positioned hydroxyls were removed from the neoDH structure to produce 8,9-deoxy-neoDH (or MSVIII-19; I, Fig. 1). The binding affinity of MSVIII-19 is only moderately lower than that of neoDH (I, Table 1). The MSVIII-19 binding causes rapid GluK1 desensitization and weak activation (III, Table 1); therefore, MSVIII-19 was originally categorized as an antagonist (Sanders et al. 2005). Further experiments indicated that MSVIII-19 is a very weak partial agonist or a functional antagonist for kainate receptors (II). The closed GluK1-LBC (PDB: 1YCJ) stayed shut with prebound MSVIII-19 in MD simulations as indicated by the Gly490^O-Asp687^N H-bonding (III, Fig. 5H). The same closure stage was also produced in a crystallographic study (II). The moderately high binding affinity of MSVIII-19 (I, Table 1) is based on the canonical interactions shared with L-glutamate and the DH-like alignment of the hydrophobic ring system with the D1 face residues. The MSVIII-19-GluK1 simulations suggest that without the C8 and C9 positioned hydroxyls the mobility of nearby residues such as Ser741 increases (III, Fig. 7C). In addition, the Glu441-Ser721 distance fluctuates considerably. This instability inside the receptor cleft could be connected to the rapid desensitization reported for MSVIII-19 with kainate receptors (II). Although the GluK1 cleft stays closed with MSVIII-19, this does not translate effectively as ion channel opening because rapid desensitization masks the activation.

5.2.2.5 The epimerization of C2 and C4 positions radically changes dysiherbaine analog binding (I, III)

The epimerization of C4 position in neoDH to produce 4-epi-neoDH (I, Fig. 1) weakened the ligand-binding properties significantly (III, Table 1). 4-epi-neoDH aligns its ring system against hydrophobic residues on the D2 face in GluK1-LBC (PDB: 1YCJ) MD simulations. This unfavorable arrangement is contrary to what was seen in the neoDH-GluK1 complex, in which the ligand's ring system aligns against the D1 face (III, Fig. 4A). The 9-hydroxyl of 4-epi-neoDH is connected to the side chain of Ser741 *via* a water bridge (III, w2 in Fig. 6E) and the C8 and C9 positioned hydroxyls disrupt the Gly490^O-Asp687^N H-bonding. The weak receptor activation and weak binding affinity for GluK1 as well as the inability of 4-epi-neoDH to induce strong desensitization (III, Table 1) result from the less than optimal binding interactions seen in the MD simulations (III, Fig. 6E). Additional epimerization of the C2 position in neoDH to produced a kainate receptor antagonist 2,4-epi-neoDH (I, Fig. 1) that opened the closed GluK1-LBC (PDB: 1YCJ) considerably in MD simulations (III, Fig. 5G). In 2,4-epi-neoDH-GluK1 simulations the ligand's ring system aligns against the D2 face in a same manner as that of 4-epi-neoDH (III, Fig. 7A). In addition, the ligand-binding affinity of 2,4-epi-neoDH for GluK1 was decreased even more than with 4-epi-neoDH if compared to the parent molecule neoDH (III, Table 1).

5.2.2.6 The C10 substituent confer GluK1 receptor specificity (V)

The insertion of C10 positioned groups into the neoDH and DH structures reduced ligand-binding affinities for kainate receptors and increased GluK1 subtype specificity (IV, Table 1). The 10-methoxymethyl group of 10-methoxymethyl-neoDH (or 10-MOM-neoDH; IV, Fig. 1) weakened the binding affinity for GluK1 only slightly if compared to parent compound neoDH (IV, Table 1). In MD simulations the 10-methoxymethyl group protrudes favorably into a hydrophobic niche formed by Ser721, Leu541, Ile725, Leu735, Met737, and Val745 in the ligand-binding site of GluK1-LBC (PDB: 1YCJ; IV, Fig. 8A). With GluK2 and GluK3 there are bulkier amino acids at positions 721/722 (GluK2/GluK3: asparagine) and 735 (GluK2: phenylalanine), which together reduce the space available for the hydrophobic 10-hydroxymethyl group (IV, Fig. 8B). Therefore, 10-MOM-neoDH binds to GluK2 and GluK3 receptors with considerably weaker affinity than to GluK1 receptor (IV, Table 1).

10-hydroxymethyl-neoDH (or 10-HM-neoDH; IV, Fig. 1) and 8-desmethyl-10-hydroxymethyl-DH (8-desmethyl-10-HM-DH; IV, Fig. 1) likely push their C10 positioned groups into the same hydrophobic niche as 10-MOM-neoDH. Although the 10-hydroxy-methyl group is smaller and more hydrophobic than the preferred 10-methoxymethyl group, it still has room to position inside the GluK1 niche. Therefore, the 10-hydroxymethyl group weakened the binding affinities of 10-HM-neoDH and 8-desmethyl-10-HM-DH for GluK1 receptor only moderately if compared to GluK2 and GluK3 receptors (IV, Table 1). This subtype specificity for GluK1 is most pronounced with 10-MOM-neoDH as its

10-hydroxymethyl group has optimal hydrophobicity for the niche (IV, Fig. 8). Although the C10 group of 10-MOM-neoDH is optimal for GluK1 binding, the C8 positioned amine group of 8-desmethyl-10-HM-DH assures slightly higher binding affinity for GluK1 (IV, Table 1). Both the 8-aminomethyl of DH and the 8-desmethyl of 8-desmethyl-10-HM-DH promote higher affinity for kainate receptors than the 8-hydroxyl of neoDH or 10-HM-neoDH.

6 DISCUSSION

6.1 Full tetramer structure *vs.* ligand-binding cores

The structure-function relationship of iGluRs is a complex phenomenon that is influenced by several components. The modeling approach used in the thesis is unable to take into consideration all these relevant factors, and, thus, it only complements the experimental methods. For example the effects of auxiliary proteins such as TARPs and NETO2, which reportedly alter the iGluR function by modulating ion channel function (Milstein & Nicoll 2008, Zhang et al. 2009), are out of the scope of this study. The recently published full tetramer structure of GluA2 receptor (Sobolevsky et al. 2009) is both a possibility and a challenge for future iGluR modeling studies. The full receptor model (Fig. 3) could be used in MD simulations to determine for example how the ligand-binding is exactly relayed to the transmembrane ion channel or how desensitization functions.

Because the full receptor simulations are computationally demanding, the ligand-receptor interactions are likely studied using the iGluR-LBC structures in future modeling studies as well. Furthermore, the thesis articles (I-IV) and previous studies (Pentikäinen et al. 2003, Pentikäinen et al. 2006, Sanders et al. 2005, Sanders et al. 2006) demonstrate that the ligand-iGluR-LBC complex simulations can help to explain both the binding affinities and receptor subtype specificities of ligands. Also the receptor cleft closure can be recreated or predicted for compounds by simulating them in complex with the closed iGluR-LBC (III, V). As the receptor cleft closure stages are linked to the ion channel opening (Armstrong & Gouaux 2000, Jin et al. 2003, Mankiewicz et al. 2008), the MD simulations can also be used to categorize ligands into full agonists, partial agonists, and antagonists (III, V).

6.2 More than one way to produce partial activation?

As a whole the MD simulations imply that full agonists induce tight interdomain H-bonding (III, Fig. 5, Table 2; V, Fig. 2) and keep the iGluR cleft closed. The past crystallization studies have implied that the iGluR cleft shows intermediate level of closure with bound partial agonists (Armstrong & Gouaux 2000, Furukawa & Gouaux 2003, Jin et al. 2003, Mayer. 2005, Nanao et al. 2005). Although bulky partial agonists generally wedge the fully shut iGluR cleft open with ease (III, Fig. 5D; V, Fig. 3), the opening can be of lesser extent in MD simulations (V, Figs. 4-6). Moreover, the closed iGluR cleft simulations suggest that the mechanism of full agonism is closely related to partial agonism. For example, in the MD simulations with original kainate-GluK2 and 5-Br-willardiine-GluA2 crystal structures (V, Figs. 3D and 4F, respectively) the partially open iGluR cleft closes fully repeatedly. Moreover, in the 9-deoxy-neoDH-GluK1 simulations (III, Fig. 5C) the fully closed GluK1 cleft opens gradually as the partial agonist adjusts inside the ligand-binding pocket. In addition, some partial agonists for GluA2, GluK2, and GluN1 were not able to open the closed iGluR cleft in MD simulations; instead they destabilized the interdomain H-bonding (V, Figs. 4-6). These H-bonding disruptions are a consistent sign of partial agonism for ligand-LBC simulations at least with GluA2 and GluN1 receptors (V, Figs. 4-6). Because weak partial agonist MSVIII-19 produced a fully closed GluK1-LBC in crystallographic study (II) and MD simulations (III, Fig. 5H), it also seems that the intermediate iGluR cleft closure might not be a necessity for partial agonism.

6.3 The stability of cleft closure differs between receptor subtypes

With GluK1, GluA2, and GluN1 receptors the interdomain H-bonding disruptions or receptor cleft opening were restricted to partial agonist or antagonist bound simulations. The simulation results for GluK2 receptor were less conclusive as the GluK2 cleft closure was somewhat unstable with full agonists as well. The wobbly Gly489^N-Asp687^O H-bonding prevented reliable *in silico* categorization of GluK2-specific ligands (V, Fig. 5). On the other hand, the loose GluK2 cleft closure provided a suitable explanation why there exist a multitude of relatively small partial agonists for GluK2 receptor (V, Fig. S1). If the opening of the GluK2 cleft does not require much force, small partial agonists could close the cleft and re-open it. MD simulations suggest that the re-opening could be caused by the unfavorable ligand-receptor repulsions. Indeed, a previous docking study also suggested that these small partial agonists prefer the closed GluK2 cleft (Fay et al. 2009). The cleft closure stability differences between receptor subtypes likely result from residue differences at the receptor

cleft area. These cleft closure stability differences could also affect desensitization and they should be studied more thoroughly in the future.

6.4 Partial agonism *vs.* desensitization

The receptor clefts of GluN1-LBC crystal structures with bound full agonists and partial agonists are both fully closed (V, Fig. 6A). The GluN1 cleft opening or H-bonding disruptions were consistently seen only in partial agonist simulations and not with full agonists (V, Fig. 4 and 6). Also, the MSVIII-19-GluK1 simulations (III, Fig. 5H) indicated that full iGluR cleft closure is not necessarily linked to full agonism. The experimental results have suggested that MSVIII-19 produces exceptionally rapid desensitization, which masks the partial activation almost altogether (I, II). It is possible that future experiments with different techniques could change the partial agonist status of MSVIII-19 yet again. The MSVIII-19-GluK1 simulations implied that disruptions in the H-bonding between the side chains of Glu441 and Ser721 (III, Figs. 5H and 7B) or erratic movements of their neighboring residues might be linked to the desensitization. In fact, the Glu441-Ser721 H-bond is known to affect the receptor cleft closure stability, desensitization (Lau & Roux 2007, Robert et al. 2005), and cell membrane trafficking (Gill et al. 2009, Penn et al. 2008). However, our MD simulations with GluA2, GluK2, and GluN1 receptors did not indicate that this particular H-bond could be used to categorize ligands as partial agonists (V, shown for GluA2 in Table S1). Thus, further experiments for instance with full tetramer iGluR structure are needed to understand the possible linkage of partial agonism and desensitization. As the exact GluA2 tetramer structure is now known (Sobolevsky et al. 2009) the significance of dimer interface interactions for iGluR desensitization (Armstrong et al. 2006, Sun et al. 2002) can be investigated in the future modeling studies.

7 CONCLUSIONS

The main findings of the thesis are explained below.

1. The iGluR cleft closure can be recreated using the modeling protocol introduced in the study. Full agonists usually keep the receptor cleft closed in MD simulations similarly as indicated by the crystallographic data. Bulky partial agonists and antagonists open the closed iGluR-LBC partially or completely, respectively. Although the reproduced closure stages were not necessarily identical to those acquired from x-ray crystallography, the modeling results followed similar trends.
2. The MD simulations can be used to predict ligand-binding modes and receptor cleft closure stages in complex with various compounds. The modeling predicted that full agonists such as 8-deoxy-neoDH keep the GluK1 cleft closed and that partial agonist 9-deoxy-neoDH opens the receptor cleft partially. Adversely, antagonist 2,4-epi-neoDH opened the GluK1 cleft completely in MD simulations. However, the simulations predicted also that the GluK1 cleft is fully closed in complex with a weak partial agonist or functional antagonist MSVIII-19. Although this result uncoupled the iGluR cleft closure from receptor activation, the modeling results were later confirmed by crystallography. The electrophysiology indicates that MSVIII-19 binding produces rapid kainate receptor desensitization, which masks the activation almost completely. In addition, the GluK2 cleft closure was also predicted for a large set of small partial agonists.
3. The favorability of ligand-receptor interactions can be deduced by analyzing the MD simulation trajectories in detail. In simulations the prebound ligands adjust themselves into conformations that best complement the neighboring residues. This information demonstrated effectively that relatively small alterations to the DH (or neoDH) structure could change the ligand-receptor interactions. The C9

position was found to have largest impact on DH analog binding affinities. Also any alterations to the stereochemistry at C2 and C4 positions were found to be detrimental for the binding affinities of the DH analogs.

4. The binding affinities of DH analogs vary notably between different kainate receptor subunits. Therefore, MD simulations and sequence comparisons were used to understand the subtype specificities of the DH analogs. For example, 10-MOM-neoDH prefers GluK1 over GluK2 because the ligand's hydrophobic 10-methoxymethyl group positions itself optimally inside the hydrophobic niche in the ligand-binding pocket of GluK1 receptor. The modeling results explaining receptor subtype specificities were in several cases confirmed by producing reciprocal mutants.
5. Partial agonists can produce intermediate iGluR cleft closure in MD simulations and crystallographic studies. However, even the partially closed iGluR-LBC could periodically close with some bulky partial agonists in MD simulations. Moreover, the small GluK2 partial agonists or GluA2 partial agonists, 5-R-willardiines, predominantly destabilized the closed receptor cleft instead of opening it completely. The H-bonding disruptions or cleft opening for GluK1, GluA2, and GluN1 receptors seemed to result exclusively from partial agonist (or antagonist) binding.
6. The MD simulations with full agonist-LBC complexes show that the tightness or firmness of receptor cleft closure varies between iGluR subunits. With GluA2 and GluN1 receptors the cleft does not open or show H-bonding disruptions without a bound partial agonist. The GluK2 cleft closure was least stable as indicated by the erratic Gly489^N-Asp687^O H-bonding. The loose GluK2 cleft closure makes *in silico* ligand categorization difficult but it also provides an explanation for the relatively small size of some GluK2-specific partial agonists.

Acknowledgements

This thesis was carried out at the University of Jyväskylä, at the Department of Biological and Environmental Science. The thesis work was funded by ISB – National Doctoral Programme in Informational and Structural Biology. Equally important for the thesis were the computing resources of CSC – IT Center for Science.

I especially want to thank my supervisor Dr. Olli Pentikäinen for his invaluable help and guidance during the thesis work. I almost escaped from Jyväskylä in 2006, but I am happy that he convinced me to stay for a few more years.

I acknowledge the reviewers Prof. Kari Keinänen and Dr. André H. Juffer for their significant input on finishing the thesis. I am also pleased that Dr. Mikael Peräkylä accepted the role of opponent for the dissertation. Thanks to all co-authors, collaborators, and my thesis committee members Prof. Jari Yläne and Dr. Tommi Nyrönen for their input to this thesis.

I want to thank group members Elisa Nurminen, Jarkko Koivunen, Salla Virtanen, Mikko Ylilauri, Jason Ahokas, and Sanna Niinivehmas for enduring my repetitive presentations and sometimes very peculiar humor. The same applies to the whole SMB staff.

Similarly, I thank Prof. Geoffrey Swanson, Helene Lyons-Swanson, Bryan Copits, Shanti Frausto, and Dr. Martin Gill for the time I spend as their guest at Northwestern in Chicago. I truly enjoyed the atmosphere of your lab and your company.

I also want to recognize my girlfriend Elisa Lylander for putting up with my occasionally grumpy mood during this endeavor. Finally, I give special credit to my mother since she has provided me unconditional support during my seemingly endless educational career.

YHTEENVETO (RÉSUMÉ IN FINNISH)

Ionotrooppisten glutamaattireseptoreiden ligandinsitomisdomeenien dynamiikka.

Nopea signaalinvälitys on perusedellytys tehokkaalle ihmisen aivojen toiminnalle. Hermoimpulsseja välittävät hermosolut kytkeytyvät verkoiksi kemiallisissa yhdyskohdissa eli synapseissa. Välittäjäaineet erittyvät signaalia tuovasta hermosolusta ja sitoutuvat seuraavan hermosolun solukalvolla oleviin reseptoriproteiineihin. Välittäjäaineiden sitoutuminen aiheuttaa joko suoraan tai välillisesti ionikanavien aukeamista hermosolujen solukalvoilla. Hermosolun sisään virranneet ionit puolestaan tuottavat hermoimpulssin, joka siirtyy viejä- tai tuojaaraketta pitkin eteenpäin.

Koska synapseilla on keskeinen asema signaalinvälityksessä, niiden toimintaa säätelevät reseptorit vaikuttavat osaltaan moniin neurologisiin tauteihin. Useat keinotekoisesti valmistetut ja luonnon yhdisteet joko estävät tai edistävät reseptorien toimintaa. Hermovasteita voidaan siis säädellä reseptoreihin sitoutuvien kemiallisten pienmolekyyliden eli ligandien avulla. Ennen lääkeaineiden rationaalista suunnittelua täytyy kuitenkin tietää kuinka erilaiset ligandit tarkalleen ottaen vaikuttavat kohdeproteiininsa toimintaan. Tämän lisäksi tulee varmistaa, että hoitokäyttöön tarkoitetut yhdisteet ovat mahdollisimman valikoivia kohdeproteiininsa suhteen, jotta voidaan välttää haitallisia sivuvaikutuksia.

Ionotrooppiset glutamaattireseptorit (iGluR:t) muodostavat ionikanavia, joiden avautumista säätelee hermovälittäjäaine glutamaatin sitoutuminen reseptorin ligandinsitomisdomeeniin (LBD). Solunulkopuolinen iGluR-LBD koostuu saranan yhdistämisestä D1- ja D2-domeeneista, jotka ovat yhteydessä solukalvon neljästä reseptorialayksiköstä muodostuvaan ionikanavaan. Kun täysi agonisti kuten esimerkiksi glutamaatti sitoutuu domeenien väliin, iGluR-LBD vääntyy kiinni ja ionikanava avautuu hetkeksi. Röntgenkristallografialla ratkaistut kolmiulotteiset proteiinirakenteet ovat osoittaneet, että iGluR-LBD pysyy auki ilman ligandia tai reseptoriin sitoutuneen antagonistin estäessä sulkeutumista. Kun iGluR-LBD on auki tai glutamaatti ei voi sitoutua, ionikanava pysyy kiinni. Jotkin osittaiset agonistit tuottavat välikokoista iGluR-LBD:n sulkeutumista sekä vain osittaista reseptorin aktivaatiota. Laaja tutkimusaineisto tukeekin hypoteesia, jonka mukaan iGluR-LBD:n sulkeutumisaste on suoraan verrannollinen ionikanavan avautumisen määrään.

Tässä väitöskirjassa osoitetaan, että röntgenkristallografialla määritetyt iGluR-LBD:iden sulkeutumisasteet voidaan ennustaa tai tuottaa uudelleen laskennallisesti. Menetelmässä ligandit laitetaan sulkeutuneen iGluR-LBD:n sisälle esimerkiksi telakoimalla ja syntyneitä ligandi-reseptoriyhdistelmiä simuloidaan virtuaalisissa vesilaatikoissa molekyyldynamiikalla (MD). MD-simulaatioissa iGluR-LBD:t joko avautuivat (antagonisti, osittainen agonisti) tai pysyivät kiinni (täysi agonisti) sitoutuneen ligandin ominaisuuksien mukaan.

Erityisesti kookkaat partiaaliset agonistit ja antagonistit pystyivät avaamaan kiinni olevat iGluR-LBD:t simulaatioissa. Vaikka menetelmää rajoittaa muun muassa suljetun ligandinsitomistaskun koko, MD-simulaatioiden tulokset ovat hyvin lähellä kiteytystuloksia. Menetelmää käytettiin väitöksessä ennustamaan ja selittämään uusien dysiherbaiini-nimiseen (DH) myrkkyyden pohjautuvien yhdisteiden farmakologiaa kainaattireseptoreiden kanssa. MD-simulaatiot ennustivat sekä LBD:n avautumisasteet että tarkat sitoutumisvuorovaikutukset DH-yhdisteille GluK1-kainaattireseptorin kanssa. MD-simulaatiot ennustivat, että täydet agonistit kuten 8-deoksi-neoDH pitävät GluK1-LBD:n kiinni ja partiaallinen agonisti 9-deoksi-neoDH avaa sen osittain. Vastaavasti antagonistit 2,4-epi-neoDH avasi GluK1-LBD:n täysin. Heikko partiaallinen agonisti MSVIII-19 sitä vastoin tuotti sekä MD-simulaatioissa että röntgenkristallografialla kiinni olevan GluK1-LBD:n. Tämä poikkeava tulos johtuu ilmeisesti MSVIII-19 voimakkaasta kyvystä aiheuttaa desensitisaatiota, mikä estää kainaattireseptoreiden aktivaatiota LBD:n sulkeutumisesta huolimatta.

Ligandit ja sitoutumiseen osallistuvat reseptorin aminohapot asettuvat mahdollisimman edullisesti toisiinsa nähden MD-simulaatioissa. Ligandin ja reseptorin välisiä vuorovaikutuksia tarkastelemalla voi selittää ligandien sitoutumisen reseptorikohtaisia voimakkuuseroja. Vaikka menetelmä onkin tietokonepohjainen, sitä voidaan hyödyntää kokeellisia mittaustuloksia tulkittaessa. MD-simulaatiot osoittivat, että pienetkin muutokset esimerkiksi DH:n perusrakenteeseen voivat aiheuttaa voimakkaita eroja sitoutumisvoimakkuuksissa kainaattireseptorialayksiköihin. Erityisesti DH:n rengasrakenteen hiilen 9 funktionaalisella ryhmällä on suuri vaikutus DH-yhdisteiden sitoutumisvoimakkuuteen. Lisäksi stereokemian muutos hiilien 2 ja 4 kohdalla aiheuttaa merkittäviä muutoksia DH-yhdisteiden sitoutumisessa. MD-simulaatioita käytettiin myös selittämään DH-yhdisteiden sitoutumisvoimakkuuksissa olevia eroja kainaattireseptoreiden välillä. Esimerkiksi 10-MOM-neoDH sitoutuu valikoivasti juuri GluK1-kainaattireseptoriin, sillä kyseisellä ligandilla on hiilessä 10 funktionaalinen ryhmä, joka löytää optimaalisen taskun vain GluK1-kainaattireseptorin ligandinsitomistaskusta. Useissa tapauksissa MD-simulaatioiden pohjalta tehtyjä päätelmiä käytettiin pohjana mutaatioiden suunnittelulle, joiden avulla reseptorialayksiköiden välisiä ligandin sitomisvoimakkuuseroja voitiin selvittää kokeellisesti.

Partiaaliset agonistit tuottavat välikokoista avautumista iGluR-LBD:ille sekä MD-simulaatioiden että röntgenkristallografian mukaan. Jotkin MD-simulaatiot kuitenkin osoittivat, että välikokoisesti avautunut iGluR-LBD saattaa sulkeutua myös partiaalisten agonistien kanssa. Kun GluA2-, GluK2- tai GluN1-reseptoreita simuloitiin partiaalisten agonistien kanssa, jo kiinni olevat LBD:t eivät aina kyenneet avautumaan välikokoisesti. Sen sijaan iGluR-LBD:n sulkeutuminen muuttui epävakaaksi joidenkin sitoutuneiden partiaalisten agonistien kanssa, mikä näkyi erään D1- ja D2-domeenien välisen vetysidoksen jatkuvana katkeiluna. GluA2- ja GluN1-reseptoreilla vetysidoksen katkeilu oli havaittavissa vain simuloitaessa partiaalisia agonisteja. GluK2-kainaattireseptorin kohdalla sulkeutuminen oli kuitenkin epävakaata myös

täysien agonistien kanssa. Tämän pohjalta voidaan päätellä, että iGluR-LBD:n kiinnipysymisessä on selkeitä reseptorikohtaisia eroja. LBD:n epävakaata sulkeutumista MD-simulaatioissa voi osaltaan selittää, miksi GluK2-kainaattireseptorille on olemassa monia reseptorin ligandinsitomistaskun kokoon nähden pieniä partiaalisia agonisteja.

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