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*

Received for publication, November 10, 2008, and in revised form, February 19, 2009 Published, JBC Papers in Press, March 18, 2009, DOI 10.1074/jbc.M808547200

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The prevailing structural model for ligand activation of ionotropic glutamate receptors posits that agonist efficacy arises from the stability and magnitude of induced domain closure in the ligand-binding core structure. Here we describe an exception to the correlation between ligand efficacy and domain closure. A weakly efficacious partial agonist of very low potency for homomeric iGluR5 kainate receptors, 8,9-dideoxyneodysiherbaine (MSVIII-19), induced a fully closed iGluR5 ligand-binding core. The degree of relative domain closure, ~30°, was similar to that we resolved with the structurally related high affinity agonist dysiherbaine and to that of l-glutamate. The pharmacological activity of MSVIII-19 was confirmed in patch clamp recordings from transfected HEK293 cells, where MSVIII-19 predominantly inhibits iGluR5-2a, with little activation apparent at a high concentration (1 mM) of MSVIII-19 (<1% of mean glutamate-evoked currents). To determine the efficacy of the ligand quantitatively, we constructed concentration-response relationships for MSVIII-19 following potentiation of steady-state currents with concanavalin A (EC50 = 3.6 μM) and on the nondesensitizing receptor mutant iGluR5-2b(Y506C/L768C) (EC50 = 8.1 μM). MSVIII-19 exhibited a maximum of 16% of full agonist efficacy, as measured in parallel recordings with glutamate. Molecular dynamics simulations and electrophysiological recordings confirm that the specificity of MSVIII-19 for iGluR5 is partly attributable to interdomain hydrogen bond residues Glu241 and Ser221 in the iGluR5-S1S2 structure. The weaker interactions of MSVIII-19 with iGluR5 compared with dysiherbaine, together with altered stability of the interdomain interaction, may be responsible for the apparent uncoupling of domain closure and channel opening in this kainate receptor subunit.

Ionotropic glutamate receptors (iGluRs)3 are central to fast excitatory synaptic transmission in the central nervous system and are involved in numerous physiological and pathophysiological processes. The iGluRs consist of three different classes of receptors, N-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate receptors (1), which are assembled as tetramers in a dimer of dimers configuration (2, 3). These receptors can be considered as multidomain proteins, composed of an extracellular N-terminal domain, a ligand-binding core made of discontinuous S1 and S2 segments that form two lobes (domains D1 and D2), three transmembrane-spanning regions (M1–M3) with a re-entrant loop between M1 and M2, and finally a cytoplasmic region (1).

Ligand-binding cores of iGluRs assume tertiary structures in solution that reproduce the pharmacological profiles of full-length receptors. Crystallographic studies of ligand-binding core complexes from representative members of all three iGluR subtypes (4–6) as well as the ligand-binding core of the structurally related δ2 subunit in complex with d-serine (7) have yielded unprecedented insight into structural correlates of iGluR function. Binding of agonists to iGluR ligand-binding cores can be described as a “Venus flytrap” mechanism. In the

8 This work was supported, in whole or in part, by National Institutes of Health, NINDS, Grant R01 NS44322 (to G. T. S.). This work was also supported by grants from the Dansync Center for Synchrotron Radiation, the Danish Medical Research Council, the European Community (Access to Research Infrastructure Action of the Improving Human Potential Programme) (to K. F., P. N., M. G., and J. S. K.) and by the Sigrid Jusélius Foundation (to O. T. P.).

The atomic coordinates and structure factors (codes 3GBA and 3GBB) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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3 The abbreviations used are: iGluR, ionotropic glutamate receptor; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, 6-cyano-7-nitroquinoxaline-2,3-dione; ConA, concanavalin A; DH, dysiherbaine; iGluR5-S1S2, ligand-binding core of the kainate receptor subunit iGluR5; MD, molecular dynamics; MSVIII-19, 8,9-dideoxyneodysiherbaine; NMDA, N-methyl-D-aspartic acid; CI, confidence interval.
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resting state, the ligand-binding core is present in an open form that is stabilized by antagonists (4, 8, 9). When an agonist binds to the ligand-binding core, a rotational change in conformation occurs, resulting in domain closure of the D1 and D2 lobes around a central hinge region (4, 6). In full-length receptors, this domain closure is thought to result in the opening of the ion channel (receptor activation). The extents of domain closure of ligand-binding cores of AMPA and kainate receptor subunits are correlated with the activation and the desensitization of the receptor (9, 10).

However, previous studies have questioned the association between the degree of domain closure of the ligand-binding core and channel opening or agonist efficacy. For example, AMPA was shown to induce a more closed structure of the ligand-binding core of the mutated iGluR2(L650T) than was expected from its partial agonist efficacy (11, 12). No correlation between domain closure and agonist efficacy has been demonstrated for the NR1 subunit of NMDA receptors (13).

In this study, we present the first example of a nonmutated kainate receptor that lacks the correlation between domain closure and efficacy. We tested if two structurally related kainate receptor ligands, one an agonist and one described previously as an antagonist (14), conformed to the prevailing structural model of ligand-induced activity. The high affinity agonist dysiherbaine (DH) is a natural excitotoxin originally isolated from a marine sponge (15, 16), whereas 8,9-dideoxyneoherbaine (MSVIII-19) is a synthetic analog that inhibits activation of iGluR5 receptors (14). To investigate receptor interactions with the two closely related compounds as well as the degree of domain closure introduced by the compounds, we determined the crystal structures of DH and MSVIII-19 in complex with the ligand-binding core of the kainate receptor subunit iGluR5 (iGluR5-S1S2). These two structures, along with functional studies, provide novel insights into the mechanism of kainate receptor activation, inhibition, and desensitization.

EXPERIMENTAL PROCEDURES

X-ray Structure Determination—The iGluR5-S1S2 construct developed by Naur et al. was used, and the protein was expressed and purified essentially as reported (17). DH was isolated from its natural source, and MSVIII-19 was synthesized as described previously (15, 18).

iGluR5-S1S2 in complex with DH and MSVIII-19, respectively, was crystallized by the hanging drop vapor diffusion method at 7 °C. For crystallization of the DH complex, the protein complex solution contained 2.0 mg/ml iGluR5-S1S2 and 5 mM DH, and for crystallization of the MSVIII-19 complex, 2.5 mg/ml iGluR5-S1S2 and 5 mM MSVIII-19 were used. The protein buffer was 10 mM Hepes, pH 7.0, 20 mM sodium chloride, and 1 mM EDTA. Crystals were obtained in drops consisting of 1 μl of complex solution and 1 μl of reservoir solution of 15–20% polyethylene glycol 8000, 0.05 M ammonium sulfate, 0.1 M phosphate-citrate buffer, pH 4.5 (DH) and 16–17% polyethylene glycol 4000, 0.05–0.075 M lithium sulfate, 0.1 M phosphate-citrate buffer, pH 4.5 (MSVIII-19). The reservoir volume was 0.5 ml. The crystals were flash-cooled to 100 K using ~20% glycerol added to the reservoir solutions as a cryoprotectant.

Synchrotron data were collected at the I911-2 beamline (MAX-Lab, Lund, Sweden), equipped with a MARCCD detector and at a wavelength of 1.000 Å (DH) and 1.043 Å (MSVIII-19). Full data sets were collected to 1.35 Å (DH) and 2.1 Å resolution (MSVIII-19). Diffraction data of the DH complex were processed with the programs MOSFLM (19) and SCALA implemented in the program CCP4i (20), whereas the data of the MSVIII-19 complex were processed with the program XIA using XDS (21). For crystal data and data collection statistics, see Table 1.

The structures were solved by molecular replacement, using the program Phaser within CCP4i. The structure of iGluR5-S1S2 in complex with l-glutamate (Protein Data Bank code 1YCJ (17)) was used as a search model for phasing the data, including protein atoms of molecule A only. Clear solutions comprising four molecules in the DH complex (molA, molB, molC, and molD) and two molecules (molA and molB) in the MSVIII-19 complex were obtained.

Subsequently, the amino acid residues were traced using ARP/wARP within CCP4i, except for a few amino acids that were manually built using the program COOT (22). Afterwards, DH and MSVIII-19 were unambiguously fitted into the electron densities within the ligand-binding sites. The structures were further subjected to refinements in REFMAC5 within CCP4i. The final refinements of the DH complex included anisotropic displacement parameters and riding hydrogen atoms. The last rounds of refinements of the MSVIII-19 complex were done using CNS (23). Between each refinement step, the structure was inspected and corrected using the program COOT. Gradually, water molecules as well as glycerol and sulfate ions (which could potentially also be phosphate ions) in the DH complex were added to the structure.

The iGluR5-S1S2 construct comprises Gly, the amino acid residues 430–544 from segment S1 of the membrane-bound receptor, a two-amino acid linker Gly-Thr, and residues 667–805 from segment S2 (numbering with signal peptide). In the DH complex, all amino acid residues of molA and molB could be modeled, whereas in molC residues 430–433, 679, and 714 and in molD residues 430–433, 453, 679, and 714 could not be modeled. In the MSVIII-19 complex, all amino acid residues except for residue 430 in molA could be modeled. A summary of structure refinements is presented in Table 1.

Structure Analysis—DYNDOM (24) was employed for analysis of ligand-induced domain closure. The CCP4 program CONTACTS was used in the analysis of protein-ligand interactions. Figures were prepared with PyMOL (25). Library files for refinement of ligands were obtained using the PRODRG server (26).

iGluR5-S1S2 Radioligand Binding Assay—For competition binding, 10 ng of iGluR5-S1S2 protein was incubated with 0.8–1.2 nM 3H-labeled (2S,4R)-4-methylglutamic acid (47.9 Ci/mmol; ARC Inc., St. Louis, MO) for 2 h on ice in assay buffer (50 mM Tris-HCl, 10% (v/v) glycerol, pH 7.1, at 4 °C) in the presence of 1 pm to 0.10 mM competitor. Nonspecific binding was determined in the presence of 1 mM L-glutamate. Samples were filtered onto Schleicher & Schuell 0.2 μM ME 24 mixed cellulose ester filters and were washed twice with 2 ml of ice-cold assay buffer. Radioactivity was determined by liquid scin-
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Radioligand Binding Studies at Full-length iGluR5 and iGluR6—Site mutagenesis of receptor cDNAs was carried out using the QuikChange protocol (Stratagene, Inc., La Jolla, CA) according to the manufacturer's instructions. Radioligand binding to iGluR5-2a(Q) and iGluR6a(Q) receptors or mutants was performed as described previously (28). Briefly, membranes were prepared from HEK293 cells transfected with wild type and mutant kainate receptor cDNAs. DH and MSVIII-19 were used to displace \[^3H\]kainate (10–20 nM; PerkinElmer Life Sciences). Nonspecific binding was determined in the presence of 1 mM \(\text{L-glutamate}\). After a 1-h incubation at 4 °C, samples were harvested by rapid filtration onto Whatman GF/C membranes, scintillation fluid was added, samples were incubated for 1 h at room temperature, and dpm values were measured on a Beckman LS5000TD scintillation counter (Beckman Coulter Inc., Fullerton, CA). Two replicates at each ligand concentration were measured in a single displacement experiment, and the mean of the replicates was used to determine specific binding. Specific binding was calculated after subtraction of the mean from total binding, and data were plotted and fit with a one-site competition curve with fixed minima (0%) and maxima (100%) using Prism 4 (GraphPad Software, Inc.).

Values were calculated with the Cheng-Prusoff equation using the determined IC\(_{50}\) values and the radioligand \(K_d\) values (iGluR5-2a, 73 nM (29); iGluR6a, 13 nM (30); iGluR6a(N721S), 62 nM (30); iGluR5-2a(S721N), 55 nM (95% confidence interval (CI), 32–78 nM), this study). The \(K_d\) value for iGluR5-2a(S721N) was determined in saturation isotherm experiments with varying concentrations of \[^3H\]kainate under the same conditions as those in the displacement assays. These data were plotted and fit with a one-site binding curve (hyperbola) using Prism 4.

Electrophysiology—iGluR5-2b(Y506C/L768C) cDNA was generously provided by Dr. Mark Mayer (NICHD, National Institutes of Health). HEK293 cells were maintained and transfected with receptor and enhanced green fluorescent protein cDNAs, as described previously (28). All recordings were made from receptor subunits that were unedited at the Q/R site. Internal solution consisted of 110 mM CsF, 30 mM CsCl, 4 mM NaCl, 0.5 mM CaCl\(_2\), 10 mM HEPES, and 5 mM EGTA and was adjusted to pH 7.3 with CsOH. The external solution contained 150 mM NaCl, 2.8 mM KCl, 2 mM CaCl\(_2\), 1 mM MgCl\(_2\), and 10 mM HEPES, adjusted to pH 7.3 with NaOH. Compounds were applied with fast application through a three-barrel glass tube mounted on a piezo-bimorph (30), and whole cell recordings were made with Axopatch 200B amplifier (Axon Instruments). Data were analyzed with Origin 7.5 (OriginLab Corp.) and Prism4 (GraphPad Software Inc.): inhibition-response curves were plotted and fit with a one-site competition curve constrained to fixed minima (0) and maxima (100).

Molecular Dynamics Simulations—The ligand structures were sketched with Sybyl (Tripos, Inc.) and geometry optimized by quantum mechanics using Gaussian03 (31) at the HF/6-31+G* level with water in continuum solvent model (the PCM model). With the iGluR5-S1S2 structures, chain A was used for DH, and chain B was used for MSVIII-19. For iGluR6, the structure of iGluR6-S1S2 with \(\text{L-glutamate}\) (Protein Data Bank code 1S50 (6)) was used. The positions of DH and MSVIII-19 within the binding site of iGluR6-S1S2 were acquired by superpositioning the iGluR6-S1S2 structure with the iGluR5-S1S2 structures in complex with DH and MSVIII-19, respectively, using Veretaa in the program Bodil (32). In iGluR6, the side chain of Asn\(^{722}\) was adjusted to a position comparable with that observed in iGluR6-S1S2 complexes with larger ligands than glutamate. Water molecules from the crystal structures situated too close (1.4 Å radius) to the ligands were removed to accommodate the different sized ligands.

Tleap in Antechamber-1.27 (33) was used to (a) set the force field parameters for the protein (parm99) and ligands (gaff), (b) add hydrogen atoms, (c) neutralize the system with chloride ions for iGluR5 and sodium ions for iGluR6, and (d) solvate the system with a rectangular box (13 Å in every dimension) of transferable intermolecular potential three-point water molecules (TIP3P). The build complexes were used as starting structures for molecular dynamics (MD) simulations. The electrostatic potentials of the optimized ligand molecules were computed with Gaussian03 (HF/6–31+G*). The RESP methodology (34) was used to create the atom-centered point charges from electrostatic potentials. The charges for chemically comparable atoms were set to identical values. The two-step energy minimization and the three-step MD simulation were run with NAMD 2.6 (35). First, the water molecules, counterions, and amino acid side chains were minimized with the conjugate gradient algorithm (3000 steps) as the rest of the complex was constrained by restraining Cα atoms into their initial positions with harmonic force of 5 kcal mol\(^{-1}\) Å\(^{-2}\). Second, the whole complex was minimized without constraints (9000 steps) to ensure complete equilibration of the system. The initial MD simulations in which the Cα atoms were restrained as in the energy minimization were performed in constant volume (9000 steps) and then in constant pressure (9000 steps) as well. The production simulation was performed without constraints for 2.4 ns. The complex was held at a constant temperature of 300 K with Langevin dynamics for all non-hydrogen atoms, using a Langevin damping coefficient of 5 ps\(^{-1}\). A constant pressure of 1 atmosphere was upheld by a Nosé-Hoover Langevin piston (36) with an oscillation time scale of 200 fs and damping time scale of 100 fs. An integration time step of 2 fs was used under a multiple time-stepping scheme (37). The bonded and short range interactions were calculated at every time step, and long range electrostatic interactions were calculated at every third step. A 12 Å cut-off was used for van der Waals and short range electrostatic interactions. For the van der Waals interactions, a switching function was enforced to smooth the cut-off. The simulations were conducted under periodic boundary conditions with full system, long range electrostatics using the particle-mesh Ewald method (38). Bonds involving hydrogen atoms were restrained by the SHAKE algorithm (39).
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The MD trajectories were analyzed in detail with ptraj6.5 (standalone version available on the World Wide Web). Fig. 6 was constructed using Bodil, Molscript (40), and Raster3D (41).

RESULTS

To understand the structural basis for the divergent pharmacological activity of MSVIII-19, an apparent iGluR5 receptor antagonist, and its substituted structural analog, the high affinity agonist DH, we determined the crystal structures of the two compounds bound to the ligand-binding core construct of iGluR5. In addition to an l-glutamate backbone, DH contains a bicyclic hydrofuropyran ring system with two important functional groups at C8 and C9 (Fig. 1). MSVIII-19 is a synthetic analog of DH without the substituents at C8 and C9 (Fig. 1). The binding affinities of DH and MSVIII-19 were determined for the iGluR5-S1S2 construct by competition studies with \( ^3 \text{H}-\text{H}18528 \) and \( ^3 \text{H}-\text{H}11005 \) ligand. DH and MSVIII-19 mimic the L-glutamate binding to iGluR5-S1S2 in complex with DH and MSVIII-19, respectively (Table 1).

![FIGURE 1. Chemical structures of dysiberaine and MSVIII-19. The two compounds differ in positions C8 (NH-Me substituent in DH versus H in MSVIII-19) and C9 (OH versus H).](image)

| Table 1 Crystal data and statistics of data collection and refinements of iGluR5-S1S2 in complex with DH and MSVIII-19, respectively |
|---------------------------------------------------------------|
| **Crystal data**                                              | **DH** | **MSVIII-19** |
| Space group                                                  | \( p1 \) | \( p1 \) |
| Unit cell parameters (Å)                                      | \( a = 44.9 \) | \( a = 44.9 \) |
|                                                            | \( b = 68.9 \) | \( b = 68.9 \) |
|                                                            | \( c = 90.3 \) | \( c = 67.0 \) |
|                                                            | \( a = 92.7 \) | \( a = 100.9 \) |
| Non-hydrogen atoms                                          | 9,881 | 5,409 |
| Amino acid residues                                         | 2,015 | 2,122 |
| DH or MSVIII-19                                             | 4 | 2 |
| Water/sulfate ions/glyceral chloride ions                    | 1,412 | 346 |
| \( R_{	ext{free}} \) (%)                                     | 16.5 | 20.1 |
| \( R_{	ext{free}} \) (%)                                     | 19.3 | 25.1 |
| r.m.s. deviation bond lengths (Å)                           | 0.007 | 0.007 |
| r.m.s. deviation angles (degrees)                           | 1.2 | 1.2 |
| Residues in allowed regions of Ramachandran plot(%)         | 99.2 | 98.4 |
| Protein atoms (molA/B/C/D)                                   | 11/12/13 | 24/21 |
| Water/sulfate ions/glyceral chloride ions                    | 6/67/67 | 13/13 |
| No. of observations                                         | 762,939 | 64,434 |
| Average redundancy                                          | 3.6 | 10.2 |
| Average R values (Å)                                        | 7.9 | 10.4 |
| r.m.s.d., root mean square                                   | 27/2/16 | 

\( ^3 \text{H}-\text{H}18528 \) and \( ^3 \text{H}-\text{H}11005 \) binding sites within the iGluR5 ligand-binding core are comprised of residues Glu441, Tyr489, Pro516, Leu517, Thr518, Arg523, and Tyr761 located in the D1 domain and Val685, Gly686, Ser689, Thr690, Ser721, Met737, Glu738, and Ser741 within domain D2. In both structures, the \( \alpha \)-amino acid moiety and the distal carboxylic group at the furo[3,2-b]pyran ring system occupy positions identical to those of the \( \alpha \)-amino acid moiety and \( \gamma \)-carboxylate group of L-glutamate, respectively, when bound to iGluR5-S1S2 (17) and similarly reproduce hydrogen-bonding contacts to protein residues (Fig. 2 and Table 2).

The 9-hydroxyl group of DH forms contacts with the backbone nitrogen atom of Glu738 (Fig. 2A). This D2 residue also forms potential hydrogen bonds to the \( \alpha \)-amino acid moiety and 8-methylamino group of DH and is therefore an important residue for anchoring DH in iGluR5-S1S2. In addition, the 8-methylamino group of DH makes a hydrogen bond to the side chain of Ser741. The six–membered ring oxygen atom forms an indirect hydrogen bond to Val685 (N) and Leu736 (O) via the W2 water molecule (Table 3).
Four water molecules (W3, W5, W6, and W7) observed in the iGluR5-S1S2/glutamate complex (17) are not seen in the present two complexes (Fig. 2, C and D). In the iGluR5-S1S2-DH complex, the nitrogen atom of the 8-methylamino group of DH mimics one of these water molecules (W7), and the 9-hydroxyl group of DH mimics a second water molecule (W5). The third additional water molecule in the iGluR5-S1S2-glutamate complex (W3) is not present in the iGluR5-S1S2-DH complex and would be located only 1.9 Å from the six-membered ring oxygen atom of DH. Last, W6, which forms a hydrogen bond with the α-amino group in the iGluR5-S1S2-glutamate complex, is absent due to steric hindrance in the iGluR5-S1S2-DH complex. The closer positioning of the furo[3,2-b]pyran ring system of MSVIII-19 toward residues Glu441, Tyr489, Pro516, Ser721, and Ser741 in the iGluR5-S1S2 complex with MSVIII-19 also excludes water molecules in this region.

Glu441 in D1 and Ser721 in D2 are known to form an important interdomain interaction (6, 17). In the iGluR5-S1S2-DH complex, both side chain oxygen atoms of Glu441 form a hydrogen bond with the Ser721 side chain. In contrast, only one hydrogen bond is present in the iGluR5-S1S2-MSVIII-19 complex (Fig. 2); this occurs because the side chain of Ser721 points away from ligand in this structure. This altered interdomain lock in the MSVIII-19 complex arises from the different location of the MSVIII-19 ring system compared with DH.

**FIGURE 2.** The binding mode of DH and MSVIII-19 in the ligand-binding site of iGluR5-S1S2 (in cross-eyed stereo view). The omit $F_o - F$ electron density maps contoured at the 3σ level are shown. Potential hydrogen-bonding residues of iGluR5-S1S2 (white) are represented as sticks, and dashed lines indicate hydrogen bonds (within 3.2 Å). Water molecules forming hydrogen bonds to the ligands are shown as red spheres. A, the iGluR5-S1S2 structure with DH (molecule A; yellow). B, the iGluR5-S1S2 structure with MSVIII-19 (molecule A; cyan). C, the iGluR5-S1S2 structure with L-glutamate for comparison (Protein Data Bank code 1YCJ, molecule A; salmon). D, superimposition of the iGluR5-S1S2 with DH, MSVIII-19, and L-glutamate on D1 residues. For clarity, only the ligands and water molecules are shown. Color coding of ligands is as in A–C. Water molecules have been displayed in colors according to the ligands.

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The agonist DH induces full domain closure of iGluR5-S1S2, the closure being 30° relative to the most open iGluR5-S1S2 structure in complex with the antagonist UBP302 (Protein Data Bank code 2F35, molecule A (8)). The domain closure was determined relative to this antagonist structure, since no apo structure is yet available for iGluR5.

Surprisingly, MSVIII-19, characterized previously as an antagonist on iGluR5, induces full domain closure in the MSVIII-19 complex with iGluR5-S1S2 (molecule A, 28°; molecule B, 30°) (Fig. 3), which is difficult to reconcile with the hypothesis that antagonists stabilize more open forms of the iGluR ligand-binding core. This prompted us to explore in more detail the nature of MSVIII-19 activity on iGluR5 kainate receptors. Two general explanations could account for the divergent pharmacological and structural data: (a) MSVIII-19 might be a full agonist rather than an antagonist, or (b) the structure of iGluR5-S1S2 in complex with...
TABLE 2
Potential hydrogen bonds within 3.2 Å between iGluR5-S1S2 and the agonist DH (molecule A; chemical structures of DH and MSVIII-19 are shown in Fig. 1) and the functional antagonist MSVIII-19, respectively. Contacts observed for L-glutamate bound to iGluR5-S1S2 (Protein Data Bank code 1YC), molecule A (17) are included for comparison.

| Glutamate | DH | MSVIII-19 |
|-----------|----|-----------|
| α-Carboxylate oxygen<sup>a</sup> | | |
| Thr<sub>218</sub> N | 2.8 | 2.8 | 2.8 |
| Arg<sub>223</sub> N<sup>y</sup> | 2.8 | 2.9 | 3.0 |
| α-Carboxylate oxygen<sup>b</sup> | | |
| Arg<sub>223</sub> N<sup>y</sup> | 2.8 | 2.7 | 2.8 |
| Ser<sub>269</sub> N | 2.8 | 2.8 | 2.9 |
| α-Ammonium nitrogen<sup>c</sup> | | |
| Pro<sub>216</sub> O | 2.8 | 2.8 | 3.1 |
| Thr<sub>218</sub> O<sup>y</sup> | 2.9 | 2.9 | 2.7 |
| Glu<sub>278</sub> O<sup>e</sup> | 2.9 | 2.9 | 2.7 |
| Glu<sub>278</sub> O<sub>e2</sub> | (3.3) | 3.1 | (3.3) |
| W6 | 3.0 | – | – |
| Distal carboxylate oxygen<sup>d</sup> | | |
| Ser<sub>267</sub> N | 3.2 | 3.2 | (3.3) |
| Thr<sub>260</sub> N | 3.1 | 3.0 | 3.2 |
| W1 | 2.6 | 2.7 | 2.6 |
| Distal carboxylate oxygen<sup>e</sup> | | |
| Thr<sub>260</sub> O<sup>y</sup> | 2.7 | 2.7 | 2.7 |
| W2 | 3.0 | (3.6)<sup>a</sup> | 2.6 |
| W5 | 2.7 | – | – |
| Oxygen in 6-ring O2 | | |
| W2 | – | 3.0 | 2.4 |
| Oxygen in 5-ring O1 | | |
| Glu<sub>278</sub> O<sup>e</sup> | – | 3.1 | 3.0 |
| C8 substituent, NH–Me | | |
| Glu<sub>278</sub> O<sup>e</sup> | – | 3.0 | – |
| Ser<sub>267</sub> O<sup>y</sup> | 2.7 | – | – |
| C9 substituent, OH | | |
| Glu<sub>278</sub> N | – | 2.8 | – |

<sup>a</sup> Oxy atom in glutamate, O<sub>y</sub> atom in DH/MSVIII-19.
<sup>b</sup> O atom in glutamate, O<sub>y</sub> atom in DH/MSVIII-19.
<sup>c</sup> N atom in glutamate, N<sub>y</sub> atom in DH/MSVIII-19.
<sup>d</sup> O<sup>e</sup> atom in glutamate, O<sub>y</sub> atom in DH/MSVIII-19.
<sup>e</sup> O<sup>e2</sup> atom in glutamate, O<sub>y</sub> atom in DH/MSVIII-19.

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MSVIII-19 may not correlate with efficacy. In the next set of experiments, we sought to differentiate between these possibilities.

MSVIII-19 Is a Partial Agonist with Low Efficacy for iGluR5 Receptors—Next, the pharmacological activity of MSVIII-19 was characterized in more detail to determine if the iGluR5-S1S2-MSVIII-19 structure conforms to the prevailing hypothesis that agonist efficacy arises from the degree of domain closure. MSVIII-19 competitively displaces radiolabeled <sup>3</sup>H]kainate from iGluR5 receptors and potentially reduces activation when preapplied before glutamate (14). Therefore, it acts either through competitive antagonism, similar to a classic antagonist, such as 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), or through functional antagonism by favoring the desensitized state or inhibiting receptor activation at concentrations of ligand below that required to evoke currents. We previously were unable to detect iGluR5 receptor currents evoked by MSVIII-19 at a concentration (100 μM) over 4 orders of magnitude higher than the IC<sub>50</sub> value for inhibition of glutamate-evoked currents (23 nM) (14), which led us to characterize the molecule as an antagonist.

As a further attempt to detect agonist activity, we applied a higher concentration of MSVIII-19 (1 mM) to iGluR5-2a receptors in patch clamp recordings from transfected HEK293 cells. Control applications of saturating L-glutamate (10 mM) to whole cells in laminar solution streams elicited currents that activated rapidly (10–90% rise time of <2 ms) and subsequently desensitized with a time course similar to that described previously (Fig. 4A) (30). MSVIII-19 (1 mM) evoked nondesensitizing currents of very small amplitude in a majority of these recordings (10 of 12 cells, mean amplitude of 7.3 ± 1.3 pA) (Fig. 4A). The amplitudes of the MSVIII-19 currents were less than 1% of the mean glutamate-evoked currents in the same cells (mean of 888 ± 92 pA). Thus, MSVIII-19 appears to be an agonist of remarkably low potency and efficacy relative to its capacity to induce the desensitized state or inhibit receptor activation. We also examined an alternate possibility, that MSVIII-19 gates current with extremely fast entry into the desensitization state; currents with this kinetic characteristic could go undetected in whole cell recordings because of inherent limitations in the rapidity of solution exchange over intact cells (~0.8 ms with our application system). In outside-out patch recordings, which have higher temporal resolution, MSVIII-19 (1 mM) did not evoke detectable currents from iGluR5 receptors, despite the presence of control glutamate-evoked currents (n = 3; data not shown). Therefore, if MSVIII-19 does elicit desensitizing currents from iGluR5 receptors, they decay at a rate faster than we can detect in our patch recordings, which have a solution exchange time of <200 μs.

It was impractical to determine directly if MSVIII-19 acted as a full or partial agonist on iGluR5 receptors, because the concentrations involved were too high relative to our supply of the compound, and the currents were too small in amplitude to measure reliably at subsaturating concentrations; consequently, we took two alternate approaches to address the critical question of ligand efficacy. First, we examined the agonist activity of MSVIII-19 following potentiation of steady-state currents with the plant lectin concanavalin A (ConA). Before treatment with ConA, 10 mM L-glutamate elicits currents with the plant lectin concanavalin A (ConA). After treatment with ConA, 10 mM L-glutamate elicited rapidly desensitizing currents during 100-ms applications (Fig. 4B). Application of ConA (0.3 mg/ml, 2 min) potentiated the peak and steady-state currents elicited by glutamate from iGluR5-2a receptors. Subsequently, saturating concentrations of MSVIII-19 (10 μM) evoked nondesensitizing currents of low amplitude relative to the steady-state glutamate currents. A concentration–response relationship for the agonist activ-
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FIGURE 3. MSVIII-19 induces full domain closure in the ligand-binding core of iGluR5. A, superimposition of the MSVIII-19 (cyan) DH (yellow), and l-glutamate (Protein Data Bank code 1YJC, molecule A; salmon) complexes with iGluR5-S1S2 on D1 residues. B, superimposition of the MSVIII-19 (cyan) and UBP302 (Protein Data Bank code 2F35, molecule A; blue) complexes with iGluR5-S1S2 on D1 residues. The high degree of domain closure introduced by MSVIII-19 compared with UBP302 is evident from the change of the D2 domain position.

FIGURE 4. MSVIII-19 is a weak partial agonist for iGluR5 receptors. A, representative current responses from iGluR5 receptor-expressing HEK293 cells to rapid application of (S)-glutamate (10 mM) or MSVIII-19 (1 mM). Currents evoked by MSVIII-19 were detected in a subset of recordings. B, MSVIII-19 acts as a weak partial agonist after treatment with concanavalin A. The top trace shows a current evoked by glutamate (10 mM) from iGluR5 receptors in HEK293 cells before concanavalin A treatment. The middle current is from the same cell after incubation with concanavalin A (0.3 mg/ml, 2 min), demonstrating that the steady-state current is potentiated. As shown in the bottom trace, MSVIII-19 (10 μM) now acts as an agonist to evoke nondesensitizing currents from iGluR5 receptors. C, a concentration-response relationship for the MSVIII-19 activity was constructed by measuring the ratio of the steady-state currents evoked by glutamate application within each cell (n = 3–5 cells for each concentration). The analysis yielded an EC50 of 3.6 μM and a Hill slope of 2.4 (n = 3–5 cells for each concentration). All cells were held at −70 mV, and drugs were applied for either 100 ms (glutamate; black bars) or 1 s (MSVIII-19; gray bars).

It was generated by measuring the steady-state amplitudes of the MSVIII-19 current relative to the steady-state glutamate current within single cells (Fig. 4C). The data were well fitted with a sigmoidal concentration-response curve that yielded an EC50 of 3.6 μM and a Hill slope of 2.4 (n = 3–5 cells for each concentration, 95% CI for the EC50 was 3.3–3.9 μM). Notably, the mean amplitude of MSVIII-19 currents reached a maximum of 13% of steady-state currents evoked by glutamate. These data support the interpretation that MSVIII-19 is a partial agonist with low efficacy at iGluR5 receptors.

These conclusions were further supported in a second set of experiments in which we examined the activity of MSVIII-19 on the non-desensitizing receptor mutant iGluR5-2b(Y506C/L768C) (43). An introduced intrasubunit disulfide bond in these receptor subunits prevents desensitization after activation by either l-glutamate or MSVIII-19 (10 μM and 100 μM, respectively; Fig. 5A). Similar to the ConA-treated iGluR5 receptors, the mean amplitude of currents evoked by MSVIII-19 saturated at 16% of glutamate currents within individual recordings. A sigmoidal concentration-response curve yielded an EC50 of 8.1 μM (95% CI, 5.7–11.4 μM) and a Hill slope of 2.0 (n = 3–7 recordings at each concentration) (Fig. 5B). These data therefore confirm that MSVIII-19 acts as a partial agonist, only weakly activating the iGluR5 receptor, a conclusion that remains divergent from the conventional interpretation of a closed iGluR ligand-binding core.

Differences in Selectivity Profile of DH and MSVIII-19—DH and MSVIII-19 differ in their selectivity for kainate receptor subunits; DH exhibits high affinity for both iGluR5-2a (0.5 nM) and iGluR6a (1.3 nM), whereas MSVIII-19 is highly selective for the iGluR5-2a subunit (128 nM) and shows no detectable radioligand binding or functional inhibition of homomeric iGluR6a receptors (14). This divergence in affinity was puzzling, given the high similarity of the crystal structures. To explore the possible determinants of these differential pharmacological profiles, we carried out MD simulations using the present structures to initialize models of the iGluR6a ligand-binding core with the two compounds.

The binding conformations of DH to iGluR5 and iGluR6 are predicted to be very similar (Fig. 6A), which is not surprising, given its similar binding affinities for the two receptor subunits (16). In both cases, DH forms an intramolecular hydrogen bond between the 9-hydroxyl group and the γ-carboxylate group. Importantly, Asn721 in iGluR6a hydrogen-bonds to Glu441 (shown as an orange dotted line in Fig. 6A), establishing the interdomain contact observed between Ser721 and Glu441 in iGluR5 (6, 17). This interaction between the D1 and D2
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Binding Affinity and Functional Activity of MSVIII-19 Arise in Part from the Stability of the Interdomain Interaction between Residues 441 and 721—The MD simulations suggested that the interdomain contact between Glu441 and Ser721 in iGluR5 and the unfavorable substitution of Asn721 in iGluR6 could contribute to the action and subunit selectivity of MSVIII-19. We tested this hypothesis experimentally in ligand-binding and electrophysiological assays with site-specific mutants of iGluR5-2a and iGluR6a in which Ser and Asn721 were reciprocally substituted (iGluR5-2a(S721N) and iGluR6a(N721S)). In radioligand binding assays with membranes prepared from iGluR5-2a receptor-expressing HEK293 cells, MSVIII-19 displaced [3H]kainate with a \( K_i \) of 161 nM (Fig. 7A; \( n = 3 \) experiments at each concentration), which is similar to our previous analysis that yielded a \( K_i \) of 128 nM (14). MSVIII-19 did not displace the radioligand from iGluR6a receptors even at the highest concentrations assayed (30 and 100 \( \mu M \)). Substitution of asparagine for serine in iGluR5-2a(S721N) significantly reduced the displacement of radioligand by \( \sim 30 \)-fold to an estimated \( K_i \) of 4.1 \( \mu M \) (95% CI, 2.3–7.2 \( \mu M \)) when the data were fit as a single component. However, a two-site competition curve fit to the same data points was preferred in a comparison of models (\( F \) test, \( p < 0.001 \)) and yielded \( K_i \) values of 618 \( \mu M \) and 112 \( \mu M \) (Fig. 7A; \( n = 3–5 \) assays for each concentration), suggestive of substantial cooperativity in the binding of MSVIII-19 to this mutant receptor. The iGluR6a(N721S) receptor mutants exhibited detectable binding to MSVIII-19, in contrast to wild type iGluR6a receptors, with the estimated \( K_i \) of \( \sim 86 \mu M \) from the partial displacement curve fit to the data.

The alterations in binding affinity were paralleled by shifts in the potency of the inhibition of glutamate-evoked currents from the receptors and their reciprocal mutants. Preaplication of MSVIII-19 inhibits subsequent glutamate-evoked currents.
Residue 721 is an important determinant of MSVIII-19 affinity and functional activity in kainate receptor subunits. A, displacement of specific binding by [3H]kainate by a range of concentrations of MSVIII-19 from iGluR5-2a, iGluR6a, iGluR5-2a(S721N), or iGluR6a(N721S) receptors expressed in HEK293 cells. Curves were fit with a one-site competition curve with fixed minima (0%) and maxima (100%), with the exception of iGluR5(S721N), which was best fit with a two-site competition curve; n = 3–5 experiments for each concentration of analog on each receptor subunit. K<sub>j</sub> values derived from these fits were as follows: iGluR5, 161 nM; iGluR5(S721N), 618 nM and 112 μM; iGluR6a(N721S), ~86 μM. No displacement was detected from homotypic iGluR6a receptors, as reported previously (14). B, reciprocal mutation of residue 721 in iGluR5-2a and iGluR6a receptors affects functional antagonist activity. Inhibition-response relationships were constructed for MSVIII-19 functional antagonism of glutamate-evoked currents from iGluR5-2a, iGluR6a, iGluR5-2a(S721N), or iGluR6a(N721S) receptors. Glutamate (10 mM) was applied every 20 s for 1–2 min to establish base-line current amplitudes. Following this, MSVIII-19 was added to the external solution for 1 min before currents were again evoked with glutamate at 20-s intervals (while in the continued presence of MSVIII-19). Data for iGluR5-2a and iGluR6a are from Sanders et al. (14) and are shown for comparative purposes. The potency for inhibition was reduced 50-fold for iGluR5-2a(S721N) relative to wild type iGluR5-2a receptors (1.2 μM versus 23 nM, respectively; n = 4–8 cells for each concentration) (14). iGluR6a(N721S) receptors were inhibited with an IC<sub>50</sub> of 24.1 μM (n = 3–5 recordings at each concentration).

FIGURE 7. Residue 721 is an important determinant of MSVIII-19 affinity and functional activity in kainate receptor subunits. A, displacement of specific binding by [3H]kainate by a range of concentrations of MSVIII-19 from iGluR5-2a, iGluR6a, iGluR5-2a(S721N), or iGluR6a(N721S) receptors expressed in HEK293 cells. Curves were fit with a one-site competition curve with fixed minima (0%) and maxima (100%), with the exception of iGluR5(S721N), which was best fit with a two-site competition curve; n = 3–5 experiments for each concentration of analog on each receptor subunit. K<sub>j</sub> values derived from these fits were as follows: iGluR5, 161 nM; iGluR5(S721N), 618 nM and 112 μM; iGluR6a(N721S), ~86 μM. No displacement was detected from homotypic iGluR6a receptors, as reported previously (14). B, reciprocal mutation of residue 721 in iGluR5-2a and iGluR6a receptors affects functional antagonist activity. Inhibition-response relationships were constructed for MSVIII-19 functional antagonism of glutamate-evoked currents from iGluR5-2a, iGluR6a, iGluR5-2a(S721N), or iGluR6a(N721S) receptors. Glutamate (10 mM) was applied every 20 s for 1–2 min to establish base-line current amplitudes. Following this, MSVIII-19 was added to the external solution for 1 min before currents were again evoked with glutamate at 20-s intervals (while in the continued presence of MSVIII-19). Data for iGluR5-2a and iGluR6a are from Sanders et al. (14) and are shown for comparative purposes. The potency for inhibition was reduced 50-fold for iGluR5-2a(S721N) relative to wild type iGluR5-2a receptors (1.2 μM versus 23 nM, respectively; n = 4–8 cells for each concentration) (14). iGluR6a(N721S) receptors were inhibited with an IC<sub>50</sub> of 24.1 μM (n = 3–5 recordings at each concentration).

with an IC<sub>50</sub> of 23 nM (95% CI, 16 –32 nM) at a concentration of 10 μM glutamate but does not reduce currents from iGluR6a receptors (14). The potency for inhibition was markedly reduced for iGluR5-2a(S721N) receptors, to 1.2 μM (95% CI, 1.1–1.4 μM, n = 4–8 recordings at each concentration) (Fig. 7B). The Hill slope also increased from 0.6 to 1.0 for iGluR5-2a versus iGluR5-2a(S721N) receptors, respectively (p < 0.001). iGluR6a(N721S) receptors were inhibited with an IC<sub>50</sub> of 24.1 μM (95% CI, 19.3–30.4 μM, n = 3–5 recordings at each concentration), which, although low, was clearly an increase in potency from the completely insensitive wild type iGluR6a receptors.

These data therefore support the conclusions derived from the MD simulations, confirming that Ser<sup>721</sup> is a critical determinant of activity for iGluR5 and that Asn<sup>721</sup> disrupts MSVIII-19 binding stability in the ligand-binding core. We note, however, that these single reciprocal mutations did not completely reverse the binding or activity profiles, signifying that interactions between other residues also reduce affinity of MSVIII-19 for the iGluR6 subunit.

**DISCUSSION**

Here we provide evidence that ligand efficacy for activation of kainate receptors is not strictly correlated with the degree of domain closure in ligand-binding structures. Domain closure following ligand binding is thought to lead to ion channel opening and receptor desensitization resulting from destabilization of the dimer interface between D1 domains (44). Agonists promote a closed form of the ligand-binding core by movement of the D2 domain toward D1 (4, 6, 9, 17), whereas antagonists stabilize relatively open forms of the ligand-binding cleft (4, 8, 9). The strong correlation between a series of willardiine derivatives and agonist efficacy for the iGluR2 AMPA receptor provided a structural model for partial agonism and constituted the most compelling example of the relationship between domain rotation and pharmacological activity (10). However, we found that two closely related ligands, the high affinity agonist diyiherbaine and the weak partial agonist MSVIII-19, induced full domain closure in the ligand-binding core of iGluR5 (Fig. 3). Our data therefore suggest that domain closure can be uncoupled from agonist efficacy or that the functional correlate of domain closure differs in kainate receptors relative to AMPA receptors.

This conclusion relies critically on the demonstration that MSVIII-19 is not a full agonist for iGluR5 receptors. We previously characterized MSVIII-19 as an antagonist, because it potently inhibited receptor activation without apparent agonist activity (14). Here we found that MSVIII-19 was instead an agonist, albeit with a low potency relative to its larger structural analog, DH. Why did channel gating of iGluR5 receptors by MSVIII-19 require such a high concentration when effective “inhibition” occurs in the nanomolar range? We speculate that lower concentrations of this partial agonist might also have gated currents (consistent with the micromolar potency of ConA-treated receptors) but that the current amplitudes were below our level of detection in the recordings. In fact, although currents were detected upon application of 1 μM MSVIII-19, these were evident in only a subset of cells and were of very small amplitude. The potent antagonist activity characterized previously for MSVIII-19 therefore probably occurs through highly efficient desensitization of iGluR5 receptors instead of true competitive antagonism by this ligand. This explanation is consistent with the observation that low concentrations of glutamate and other agonists induce desensitization without detectable channel gating (45).

These recordings also suggested that MSVIII-19 acted as a partial agonist with low efficacy, but the agonist efficacy was difficult to measure directly, because the current amplitudes were too low. We therefore took approaches similar to those used in the seminal study that related the efficacy of AMPA receptor willardiine analogs to ligand-binding domain closure (10). First, steady-state currents from iGluR5 receptors were potentiated using the plant lectin concanavalin A, which allowed us to determine the efficacy of MSVIII-19 relative to L-glutamate. ConA previously was shown to selectively modulate open states that contribute to the equilibrium kainate receptor response (46). Second, we determined the con-
centration-response relationship for MSVIII-19 activation of a nondesensitizing mutant of the iGluR5 receptor (iGluR5-2b(Y506C/L768C)) (43). Therefore, under conditions similar to those that were used to demonstrate the strong correlation between AMPA receptor domain closure and agonist efficacy, we found that MSVIII-19 clearly acted as a weak partial agonist, with an efficacy <16% of that of L-glutamate, despite stabilizing a highly closed domain structure.

MSVIII-19 potently inhibits iGluR5 receptors at concentrations 4 or more orders of magnitude below those required for detectable agonist activity. Similarly, DH stabilizes a remarkably long lasting desensitized state of the iGluR5 (and iGluR6a) kainate receptor (47); indeed, complete (or nearly so) desensitization of kainate agonist currents, rather than agonist efficacy, is the most obvious pharmacological activity shared by L-glutamate, DH, and MSVIII-19. Given the divergence in nearly all other aspects of their activity as receptor ligands, it seems reasonable that the desensitized configuration represents the most stable structural state of both DH and MSVIII-19 bound to iGluR5. The extreme difference in potency for inhibition versus activation exhibited by MSVIII-19 could be interpreted in the context of a model previously proposed by Sun et al. (3), in which full domain closure and desensitization could occur without detectable gating via the disruption of the D1-D1 intersubunit interface that is thought to underlie desensitization in current models (48). It is possible that ConA strengthens this interface in the unliganded receptor, increasing the likelihood of channel gating, which is then manifested as a detectable MSVIII-19-evoked steady-state current.

Similar to the ConA-treated iGluR5 receptors, the receptor mutant iGluR5-2b(Y506C/L768C), where the dimer interface is locked in an active conformation by a covalent bond between cysteine residues, elicits nondesensitizing MSVIII-19-evoked steady-state currents.

We note that previous studies also have questioned the association between the degree of domain closure of the ligand-binding core and channel opening or agonist efficacy. Specifically, in both x-ray crystallographic (11) and fluorescence resonance energy transfer studies (12), the agonist AMPA induced a more closed structure of the mutated iGluR2(L650T) ligand-binding core than was expected from its partial agonist efficacy on this mutant AMPA receptor. Also, no correlation between domain closure and agonist efficacy has been demonstrated thus far for the NR1 glycine-binding subunit of NMDA receptors (13).

Although the canonical AMPA-receptor antagonist CNQX stabilizes an open conformation of the iGluR2 subunit (49), under some circumstances it can act as a partial agonist, demonstrating that the open structure does not necessarily preclude channel opening. In the presence of transmembrane AMPA receptor regulatory proteins, CNQX is a weak partial agonist, perhaps as a result of transmembrane AMPA receptor regulatory protein-dependent increases in agonist efficacy through a strengthening of the coupling between domain closure and channel opening (49). Also, CNQX acts as an agonist when bound to partially activated heteromeric iGluR5/KA2 kainate receptors, probably as a result of a similar increase in coupling efficiency in the receptor subunits (47).

Why does MSVIII-19 activate the receptor so poorly relative to its close analog DH? Within the ligand-binding site, the molecular basis for the apparent lack of agonist activity of MSVIII-19 can be accounted for by the absence of a single hydroxyl group on the C9 position of the molecule. Elimination of this moiety alone in a high affinity analog of dysiherbaine, 9-deoxynodosiherbaine, largely replicated the pharmacological profile of MSVIII-19 (28). In the structure of DH in complex with iGluR5-S1S2, the 9-hydroxyl group forms a direct hydrogen bond to the backbone of Glu738, which is absent in the analogous MSVIII-19 complex. With MSVIII-19, a water-mediated contact (W2) occurs between Glu738 and the ligand (Table 3). In addition, DH contains an 8-methylamino group that forms direct interactions with the Glu738 and Ser721 side chains (Table 2). In iGluR2, stabilization of binding by the equivalent glutamate, Glu705, has been suggested to be relevant to receptor desensitization rather than receptor activation (50). Furthermore, the extensive van der Waals interactions observed between DH and Val685 in iGluR5 are absent with bound MSVIII-19. The residue at this position in iGluR5 has previously been reported to display a key role in defining desensitization kinetics (51).

The interdomain contact between Glu441 and Ser721, defined as the interdomain lock (4, 17), also differs between the iGluR5-S1S2-MSVIII-19 and iGluR5-S1S2-DH complexes due to different conformations of the Ser721 side chain. This interdomain contact between Glu441 and Asn721 in iGluR6a is known to play a central role for receptor function and in the apparent affinity of agonists (30); our simulations suggest that in iGluR6, MSVIII-19 completely disrupts the 441–721 interaction, since Asn721 cannot adjust into an orientation that allows formation of a hydrogen bond from the Nδ2 atom to the Glu441 side chain (Fig. 6). Instead, the MD simulations suggest that the Nδ2 atom of Asn721 interacts with the W2 water molecule. In contrast, stable binding of MSVIII-19 to iGluR5 occurs, since Ser721 can adjust into a distinct orientation in which hydrogen bonding stabilizes the closure of D1-D2, which was supported by electrophysiological recordings. In ligand-binding and electrophysiological assays with site-specific mutants of iGluR5-2a and iGluR6a in which Ser and Asn721 were reciprocally substituted (iGluR5-2a(S721N) and iGluR6a(N721S) receptors), the alterations in binding affinity were paralleled by shifts in potency for inhibition of glutamate-evoked currents from the receptors and their reciprocal mutants. Hence, Ser721 is a primary determinant of iGluR5 versus iGluR6 affinity, and both apparent binding affinity and desensitizing activity of MSVIII-19 of iGluR5 receptors are strongly influenced by the stability of the interdomain interaction between residues 441 and 721. In summary, we postulate that the weaker interactions of MSVIII-19 with D2 residues of iGluR5, together with altered stability of the interdomain interaction between residues Glu441 and Ser721, may be responsible for the very weak coupling between domain closure and channel opening.

In conclusion, these studies provide new insight into the structural bases for complex physiological behavior of kainate receptors that diverges from the clear correlation between agonist efficacy and ligand-binding core closure that exists for AMPA receptors.
