Structural determinants of agonist-specific kinetics at the ionotropic glutamate receptor 2

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Glutamate receptors (GluRs) are the most abundant mediators of the fast excitatory neurotransmission in the human brain. Agonists will, after activation of the receptors, induce different degrees of desensitization. The efficacy of agonists strongly correlates with the agonist-induced closure of the ligand-binding domain. However, the differences in desensitization properties are less well understood. By using high-resolution x-ray structure of the GluR2 flop (GluR2o) ligand-binding core protein in complex with the partial glutamate receptor agonist (S)-2-amino-3-(3-hydroxy-5-tert-butyl-4-isoxazolyl)propionic acid [(S)-thio-ATPA], we show that (S)-thio-ATPA induces an 18° closure of the binding core similar to another partial agonist, (S)-2-amino-3-(4-bromo-3-hydroxy-5-isoxazolyl)propionic acid [(S)-Br-HIBO]. Despite the similar closure of the ligand-binding domain, we find in electrophysiological studies that (S)-thio-ATPA induced a 6.4-fold larger steady-state current than (RS)-Br-HIBO, and rapid agonist applications show that (S)-thio-ATPA induces a 3.6-fold higher steady-state/peak ratio and a 2.6-fold slower desensitization time constant than (RS)-Br-HIBO. Structural comparisons reveal that (S)-Br-HIBO, but not (S)-thio-ATPA, induces a twist of the ligand-binding core compared with the apostructure, and the agonist-specific conformation of Leu-650 correlates with the different kinetic profiles pointing at a key role in defining the desensitization kinetics. We conclude that, especially for intermediate efficacious agonists, the desensitization properties are influenced by additional ligand-induced factors beyond domain closure.

2-amino-3-(3-hydroxy-5-tert-butyl-4-isoxazolyl)propionic acid (thio-ATPA) | desensitization | domain closure | crystallization

The fast excitatory synaptic transmission between nerve cells is carried out primarily by ionotropic glutamate receptors (GluRs), mainly localized in the postsynaptic membrane. The GluRs are classified into three functionally and pharmacologically separate groups based on the potencies of their respective agonists, namely the 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA), kainate, and N-methyl-D-aspartic acid (NMDA) receptors (1, 2). The receptor is activated to an ion-conducting state upon agonist binding, and this process can be followed by desensitization.

The topological and structural characteristics of the GluRs have been intensively studied, and currently the favored structural models predict the AMPA receptors to form tetramers (3, 4) assembled of a pair of dimers (Fig. L4) (5–10). The mechanism by which binding of the agonist to the bilobed ligand-binding core is transmitted to the pore region is not understood in detail. However, recent studies of 5-substituted willardines nicely illustrate that a tight closure of the ligand-binding core of GluR2 more efficiently induces a gating conformation of the receptor complex as compared with partial agonists inducing more moderate closure of the binding core (11). A nondesensitizing receptor, harboring the L483Y mutation in the dimer interface (3, 10), has been used to characterize agonists of varying chemical structure on the AMPA receptor (11, 12). These studies indicate an apparent correlation between the degree of ligand-binding core closure and maximal evoked current in the standard Xenopus oocyte two-electrode voltage clamp setup.

A variety of AMPA analogues with distinct functional profiles have been synthesized since AMPA itself was originally described (13, 14). Thio-ATPA (Fig. 1B) was designed as a 3-isothiazolol analogue of 2-amino-3-(3-hydroxy-5-tert-butyl-4-isoxazolyl)propionic acid (ATPA) and is a potent agonist at the kainate receptor GluR5 (EC50 at 0.10 μM) only maintaining moderate activity on the AMPA receptors GluR1–4 (EC50 at 5.2–32 μM) (15). Interestingly, the current characteristics of (S)-thio-ATPA showed maximal current levels at 78–170% of those evoked by kainate and 120-1600% of those evoked by ATPA.

In this work, we explore the novel kinetic behaviors of (S)-thio-ATPA and a series of structurally distinct AMPA receptor agonists (Fig. 1B) by comparing the activities at the GluR2 flip (GluR2i) and flop (GluR2o) splice variants in a rapid agonist application setup and under two-electrode voltage clamp of Xenopus laevis oocytes. These results, combined with analysis of a high-resolution x-ray structure of the ligand-binding core construct of GluR2 (GluR2–S1S2J) complexed with (S)-thio-ATPA, form the basis of further understanding of the kinetic behavior of AMPA receptor agonists.

Materials and Methods

Glu Ligands and Reagents. (S)-AMPA, (RS)-2-amino-3-(4-bromo-3-hydroxy-5-isoxazolyl)propionic acid [(RS)-Br-HIBO], (S)-thio-ATPA, and (RS)-ATPA were kindly provided by P. Krosgsgaard-Larsen and U. Madsen (both of Danish University of Pharmaceutical Sciences, Copenhagen). Other reagents were purchased from Sigma.

Mutagenesis. The mutations were introduced by the overlap PCR method using Pfu polymerase, and all of the constructs were subsequently sequenced and inserted into the pGEMHE (16) oocyte expression vector. All recordings were performed on receptors unedited in the Q/R site (17).

Two-Electrode Voltage Clamp. Oocytes were isolated and prepared as described in ref. 18. cRNA was transcribed by using the mMACHINE T7 kit from Ambion (Austin, TX), according to the supplier’s instructions. To increase survival, some of the oocytes were kept in normal frog Ringer (NFR) (10 mM HepesNaOH, pH 7.4/115 mM NaCl/1.5 mM CaCl2/2.5 mM KCl/0.1 mM MgCl2) supplemented with 1% serum, 10 μg/ml penicillin, and 10 μg/ml streptomycin. Data under two-electrode voltage clamp were acquired and analyzed as described in ref. 18.

Abbreviations: AMPA, 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid; ATPA, 2-amino-3-(3-hydroxy-5-tert-butyl-4-isoxazolyl)propionic acid; Br-HIBO, 2-amino-3-(4-bromo-3-hydroxy-5-isoxazolyl)propionic acid; GluR, ionotropic glutamate receptor; GluR2i, GluR2 flip; GluR2o, GluR2 flop; GluR2-S1S2J, ligand-binding core construct of GluR2; NFR, normal frog Ringer.

Data deposition: The atomic coordinates and structure factors have been deposited in the Protein Data Bank, www.pdb.org (PDB ID code 2AIK).

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time constant. All traces displayed in the figures are individual responses.

**Expression and Purification of GluR2-S1S2J.** The GluR2-S1S2J construct was kindly provided by E. Gouaux (Columbia University, New York), and the protein was expressed, refolded, and purified essentially as described in refs. 6, 19, and 20.

**[3H]AMPA Binding.** Ligand binding was performed as described in ref. 19. Briefly, for saturation binding refolded GluR2-S1S2J protein (0.08 mg/ml) was incubated for 1 h on ice with 1–200 nM [3H]AMPA (11.1 Ci/mmol; 1 Ci = 37 GBq) (NEN) in 500 μl of binding buffer (100 mM thioecyanate/2.5 mM CaCl2/30 mM Tris-HCl pH 7.2). Competition experiments were performed under similar conditions, by using 20 nM [3H]AMPA and 0.2 nM to 1 mM (S)-thio-ATPA. Nonspecific binding was determined in the presence of 1 mM glutamate. The binding experiments were performed in triplicate. Kᵢ values were calculated by using the Cheng–Prusoff equation (21).

**Crystallization.** GluR2-S1S2J in complex with (S)-thio-ATPA was crystallized by the hanging drop vapor diffusion method at 6°C. The protein complex solution contained 5.6 mg/ml GluR2-S1S2J and 1.6 mg/ml (S)-thio-ATPA (molar ratio 1:32, in 10 mM Hapes, pH 7.4, 20 mM NaCl/1 mM EDTA). Crystals were obtained in drops consisting of 1 μl of complex solution and 1 μl of reservoir solution of 0.1 M NaCl, 0.1 M cacodylate buffer (pH 6.5), and 20% polyethylene glycol 8000. The reservoir volume was 0.49 ml. The crystals grew within 1 week to a maximum size of 0.1 mm.

**Data Collection.** The data of GluR2-S1S2J in complex with (S)-thio-ATPA were collected on beamline I11 at MAX Lab (Lund, Sweden) at 100 K by using a MAR345 image plate detector and wavelength of 1.0835 Å. The crystal was transferred to a cryosolution containing (S)-thio-ATPA and 0.1 M NaCl. 0.1 M cacodylate buffer (pH 6.5), 20% polyethylene glycol 8000, and 15% glycerol for a few seconds before flash-cooling.

Data were autoindexed and processed with the programs DENZO and SCALEPACK (HKL Research, Charlottesville, VA) (22). For crystal data and data collection statistics, see Table 4, which is published as supporting information on the PNAS web site. After data processing in SCALEPACK, data were transformed to CNS format by using the CCP4 suite of programs (23).

**Structure Determination and Refinements.** The structure was solved by molecular replacement using the program AMORE (24) from CCP4. The structure of GluR2-S1S2J in complex with the ligand (S)-2-amino-3-[3-hydroxy-5-(2-methyl-2H-tetrazol-5-yl)-4-isoaxazolyl]propionic acid [(S)-2-Me-Tet-AMPA] (12) was used as search model for phasing the data of the (S)-thio-ATPA complex, including protein atoms only.

Initially, the amino acid residues of GluR2-S1S2J were traced by using ARP/WARP (25), except for 17 amino acids, which were manually built by using program O ((26)). In addition, the ligand molecule (S)-thio-ATPA was fitted to the electron density, and water molecules were gradually added to the structure. Optimal geometry for the isothiazol ring of (S)-thio-ATPA was obtained by using ab initio methods. Parameter and topology files for programs O and CNS (Version 1.0) (27) were generated by the HIC-UP server (28). The structure was refined by using CNS, each step comprising positional and B-factor refinements. A summary of the structure refinements is presented in Table 4. Coordinates have been deposited in the Protein Data Bank (PDB ID code 2AIX).

The HINGE FIND script (29) implemented in VMD (30) was used for an analysis of ligand-induced D1/D2 domain closure. The interface accessible surface area was generated by the Protein–Protein Interaction Server (www.biochem.ucl.ac.uk/bsm/PP/server) (31),
**Results**

**X-Ray Structure of GluR2–S1S2J Complexed with (S)-Thio-ATPA.** To explore the molecular basis of the intriguing large maximal responses induced by thio-ATPA under two-electrode voltage clamp (15), we crystallized the soluble form of the ligand-binding core of GluR2o (GluR2–S1S2J) in complex with (S)-thio-ATPA. The structure of the complex of GluR2–S1S2J (S)-thio-ATPA was determined at 2.17 Å resolution (Rwork = 19.8%; Rfree = 22.9%), and it contained one molecule in the asymmetric unit of the crystal (Fig. 2B). The complex displays an intermediate degree of domain closure at 18° relative to the apoform (6). (S)-thio-ATPA binds with an extensive number of direct and indirect interactions to both D1 and D2 (Fig. 2C; see also Table 5, which is published as supporting information on the PNAS web site). The α-amino acid part of (S)-thio-ATPA binds to the receptor as observed for other agonists (6, 12). The hydroxyl group of the isothiazolol ring, which equals one of the oxygen atoms of the γ-carboxylate moiety of glutamate, makes direct contacts to D2 via three potential hydrogen bonds as well as indirect contacts through a water molecule (W1, Fig. 2C). (S)-thio-ATPA therefore resembles the glutamate-binding mode in GluR2–S1S2J rather than the (S)-AMPA binding mode (6). The interaction with D2 is further stabilized by two hydrogen bonds to the isothiazole nitrogen and two hydrogen bonds to the sulfur atom in (S)-thio-ATPA. The tert-butyl moiety at the 5-position of the isothiazolol ring points toward a partly hydrophobic pocket (12, 34), and is stabilized by van der Waals interactions to the side chains of Glu-402, Tyr-450, Pro-478, and Tyr-732 in D1 and to Leu-650, Thr-686, Glu-705, and Met-708 in D2. In addition to making contacts to the tert-butyl moiety, Glu-402 and Thr-686 also create the lock between D1 and D2 (6, 35).

The trans peptide bond between Asp-651 and Ser-652 has been reported to exist in two distinct ligand-dependent conformations (6). (S)-thio-ATPA stabilizes the peptide conformation similar to the apoform, kainate, and the (S)-Br-HIBO complexes but differs from the 180° flip observed for the (S)-AMPA and (S)-ATPA complexes (6, 12, 36) (Table 1). The peptide bond conformations exhibit different hydrogen-bonding patterns to W1. In the (S)-thio-ATPA complex, W1 is further hydrogen bonded to the backbone oxygen atom of Ser-652 (3.3 Å) and to the backbone nitrogen atoms of Thr-655 (3.2 Å) and Lys-656 (2.7 Å). In the (S)-ATPA and (S)-AMPA complexes, however, W1 makes hydrogen bonds to the oxygen atom of Leu-650, while still binding to the backbone nitrogen of Lys-656.

![Fig. 2](image-url)  
**Fig. 2.** Binding characteristics and structure of the ligand-binding core of GluR2 in complex with (S)-thio-ATPA. (A) Diagram of the [3H]AMPA displacement from GluR2–S1S2J by (S)-thio-ATPA. (B) Schematic drawing of the structure of GluR2–S1S2J in complex with (S)-thio-ATPA. Amino acid residues from segment S1 are red, and residues from S2 are orange. The Gly–Thr linker is displayed in yellow. (S)-thio-ATPA and the disulfide bridge are shown in ball-and-stick representation. (C) Binding mode of (S)-thio-ATPA, including potential hydrogen bonds within 3.3 Å (stippled lines). Nitrogen atoms are blue, oxygen atoms are red, and sulfur atoms are yellow. Water molecules are shown as red spheres.

MOLSCRIPT (32) and RASTER 3D (33) were used to prepare Fig. 2 and Fig. 4.

Table 1. Agonist efficacy, binding domain closure, and Asp-651–Ser-652 peptide bond conformation

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Efficacy, %</th>
<th>GluR2i</th>
<th>GluR2o</th>
<th>Domain closure, §</th>
<th>Peptide flip, ‡</th>
<th>Leu-650 conformation, ¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thio-ATPA</td>
<td>290 ± 6</td>
<td>78 ± 3</td>
<td>631 ± 14</td>
<td>95 ± 3</td>
<td>18</td>
<td>–</td>
</tr>
<tr>
<td>Glutamate</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>20</td>
<td>+/−</td>
</tr>
<tr>
<td>Br-HIBO</td>
<td>134 ± 6</td>
<td>90 ± 1</td>
<td>98 ± 2</td>
<td>92 ± 1</td>
<td>18</td>
<td>−</td>
</tr>
<tr>
<td>ATPA</td>
<td>102 ± 0.06</td>
<td>82 ± 1</td>
<td>180 ± 14</td>
<td>98 ± 1</td>
<td>21</td>
<td>++</td>
</tr>
<tr>
<td>Kainate</td>
<td>69 ± 1</td>
<td>4.4 ± 1.0</td>
<td>386 ± 28</td>
<td>31 ± 3</td>
<td>12</td>
<td>−</td>
</tr>
</tbody>
</table>

*Relative maximal response measured in oocytes and calculated as the ratio of the maximal glutamate response. Maximal response was obtained by 0.5 mM (S)-thio-ATPA, 0.9 mM glutamate, 0.5 mM (S)-Br-HIBO, 1.0 mM (R)-ATPA, or 1.0 mM kainate.

†Domain closure reflects the degree of ligand-induced closure of the ligand-binding core.

‡Peptide flip denotes unflipped (−) or flipped (+) conformations of the Asp-651–Ser-652 peptide bond (6).

§Values (°) given correspond to those of the torsional angles χ1/χ2 of the Leu-650 side chain. PDB ID codes: thio-ATPA (2AUX), glutamate (1FTJ, mol B), Br-HIBO (1M5C), ATPA (1NNP, mol A), kainate (1FW0).

¶Values from Armstrong and Gouaux (6).

Values from Hogner et al. (12).

**Values from Lunn et al. (36).**
### Dose-Response Relationships on GluR2 Splice Variants

Homomeric unedited forms of GluR2o were expressed in *Xenopus* oocytes and the potencies of selected agonists were determined under two-electrode voltage clamp (Fig. 1B and Table 2). Dose–response relationships are shown in Fig. 5, which is published as supporting information on the PNAS web site. The flop splice form responded to (S)-glutamate, (RS)-ATPA, and (S)-thio-ATPA with 3- to 5-fold lower EC_{50} values than the flip splice forms. The apparent differences in potency seem to reflect different desensitization kinetics of the splice forms, because recording from the nondesensitizing GluR2(L483Y) mutant almost entirely eliminates these differences in potency. The potency of (S)-Br-HIBO has previously been determined to 4.4 ± 0.6 μM at GluR2o(L483Y) (12).

### Agonist Efficacy on Wild-Type (WT) and Nondesensitizing Receptor Mutants

Saturating agonist concentrations were used to determine the maximal currents under a two-electrode voltage clamp. The values were normalized to the maximal current activated by (S)-glutamate (Table 1). A remarkable difference between the splice forms was observed for the kainate-induced current relative to the steady-state glutamate-induced current, whereas (S)-thio-ATPA was the most efficacious agonist on both of the splice forms. Despite the fact that (S)-Br-HIBO induced a domain closure similar to (S)-thio-ATPA, (RS)-Br-HIBO activated maximal currents that were 2.2- and 6.4-fold smaller than (S)-thio-ATPA on GluR2o and GluR2o, respectively (Table 1).

Because the amplitude of the maximal current recorded in oocytes is influenced by a number of parameters, including desensitization, we performed additional experiments on the nondesensitizing mutant GluR2o(L483Y) (10, 37). At this mutant, (S)-glutamate becomes the most efficacious agonist and, because kainate already induces nondesensitizing currents on the WT receptors (38), the ratio to the glutamate-activated currents becomes smaller on this receptor (Table 1). Analyzing (RS)-Br-HIBO on this mutant showed a maximal current similar to (S)-thio-ATPA on GluR2o(L483Y), while it was significantly higher (4.4 ± 1%; P = 0.001, t test) on GluR2o(L483Y), adding support to the model that the relative domain closure correlates with the steady-state conductance on GluR2o but surprisingly not for GluR2o (Table 1).

### Receptor Kinetics on GluR2o/Splice Variants

The desensitization time course was determined from outside-out patches isolated from *Xenopus* oocytes expressing high receptor levels (Table 3). By using rapid agonist application with saturating concentrations, (S)-thio-ATPA evoked a fast desensitizing current on GluR2o (τ = 6.6 ± 0.7 ms), whereas an almost nondesensitizing current was activated on GluR2o (Fig. 3d and Table 3), in strong contrast to the fast and almost complete desensitization induced by (S)-glutamate on both splice forms (Fig. 3b and Table 3).

### Comparison of (S)-Thio-ATPA and (S)-Br-HIBO in GluR2

(RS)-Br-HIBO induces currents on GluR1o and GluR3o, similar to those elicited by (S)-glutamate (39). Likewise, the kinetics of the (RS)-Br-HIBO-induced current on GluR2 resembles the (S)-glutamate-induced currents more than those elicited by (S)-thio-ATPA (Fig. 3 and Table 3). To explain the kinetic differences between (S)-thio-ATPA and (S)-Br-HIBO, we performed a more detailed comparison of the crystal structures. We found four noticeable differences between the two complexes. First, within the binding pocket, the same 17 amino acid residues are observed to be in contact with (S)-thio-ATPA and (S)-Br-HIBO. However, only 9 potential direct hydrogen bonds (distance < 3.3 Å) are formed to (S)-Br-HIBO as compared with 14 to (S)-thio-ATPA (Table 5).

Secondly, a twist of D1 relative to D2 (projection deviation angle of ~6°) occurs in the (S)-Br-HIBO structure as compared with the apo-GluR2-S1S2J. This twist is not observed in the structure of (S)-thio-ATPA (Fig. 4B). The twist might be induced to accommodate the ring system of (S)-Br-HIBO, which is positioned with an angle of ~30° to the ring system of (S)-thio-ATPA (Fig. 4B). (S)-Br-HIBO will not be able to bind in the nondesensitizing protein conformation seen in the (S)-thio-ATPA complex because of steric clashes with the D2 residues Leu-650, Thr-686, and Leu-704.

The last two differences between the (S)-Br-HIBO and (S)-thio-ATPA structures are observed in the conformation of the side chains of Leu-650 and Met-708. In the two structures, the Leu-650 side-chain χ1 angle adopts a gauche and an antf arrangement in the (S)-Br-HIBO and (S)-thio-ATPA structures, respectively (Table 1). The side chain of Met-708 is pushed toward Pro-403 due to an otherwise steric clash with the tert-butyl group of (S)-thio-ATPA, whereas the position of the S6-Cε part of the Met-708 side chain is optimized for favorable contacts to the bromide atom of (S)-Br-HIBO (Fig. 4B).

### Discussion

**Domain Closure Correlates with Efficacy at GluR2o but Not GluR2i**

The 3D structure of the GluR2o ligand-binding core has been determined for the apoform as well as a large number of complexes with antagonists and agonists that induce varied electrophysiological responses. Functional studies, primarily performed on GluR2i, have revealed a striking correlation between the degree of domain closure and the maximal response recorded as the peak current, or the relative maximal current in the presence of cyclothiazide or the nondesensitizing mutant GluR2o(L483Y) (11, 12, 40). We also observe a correlation between the degree of domain closure and the relative maximal current (Table 1) by using the nondesensitizing form of GluR2o, where glutamate and ATPA as full agonists induce the largest relative maximal current (98–100%) and a closure of 21° (6, 41). Br-HIBO and thio-ATPA induce an intermediate maximal current (92–95%) and a less tight domain closure of 18° (12), whereas the low domain closure (12°) induced by kainate (6) also induces the lowest maximal current (31%). Interestingly, when the relative maximal currents were determined on the nondesensitizing GluR2i mutant, there were concordance between the closure and the maximal current for many of the agonists except for the tert-butyl substituted compounds, (RS)-ATPA and (S)-thio-ATPA. They became less efficacious (the relative maximal current changed from 98% to 82% and 95% to...
Fig. 4. Superimposition of GluR2-S1S2J:(S)-thio-ATPA and GluR2-S1S2J:(S)-Br-HIBO dimers. Residues from D1 of protomer 1 (left) were used for the superimposition (rms deviation 0.45 Å on Cα atoms). (A) D1 residues of the GluR2-S1S2J:(S)-thio-ATPA dimer are colored red, and D2 residues are colored orange. The GluR2-S1S2J:(S)-Br-HIBO dimer is colored cyan. The agonists (S)-thio-ATPA and (S)-Br-HIBO are shown in ball-and-stick representation. Dotted lines indicate the distance between Ile-633 of the two protomers of the GluR2-S1S2J:(S)-thio-ATPA dimer (orange) and GluR2-S1S2J:(S)-Br-HIBO dimer (cyan). The D1/D2 domain closure is 18° in both structures, and the arrow indicates the different twist of D2 between the two structures. (B) The ligand-binding site. The (S)-thio-ATPA and (S)-Br-HIBO complexes are superimposed and colored as in A. Ligands are shown in ball-and-stick and water molecules as spheres. Nitrogen atoms are blue, oxygen atoms are red, and sulfur atoms are yellow.

Fig. 3. Rapid application on GluR2i and GluR2o. Representative traces from outside-out patches pulled from Xenopus oocytes expressing GluR2i (Upper) and GluR2o (Lower) showing responses to 100-ms application of 2.5 mM (S)-thio-ATPA (A), 10 mM (S)-glutamate (B), or 5 mM (RS)-Br-HIBO (C). All patches were clamped at −120 mV. Vertical and horizontal scale bars represent 500 pA and 20 ms, respectively, for all traces, except for 5 mM (RS)-Br-HIBO on GluR2o where the vertical bar is 200 pA.

78%, respectively), whereas no significant change was observed for (RS)-Br-HIBO.

Similar discrepancies between the GluR2o crystal structure and the GluR2i functional data were observed when comparing kainate, Br-willardiine, and I-willardiine. The latter two induced a 15° and 11° domain closure and relative maximal currents of 37% and 34% (11), respectively, whereas kainate induced a closure of 12° (6) together with a relative maximal current of 4.4% on GluR2i(L483Y) (Table 1). Thus, structurally different compounds might induce different degrees of closure in the GluR2i and GluR2o despite the fact that all residues involved in ligand interactions are conserved between the two splice forms. Alternatively, similar structures in the binding pocket might affect the subunit interface differently, thereby stabilizing other intersubunit configurations with different coupling efficiency to the conducting states of the channel (11).

Structural Differences Affecting the Desensitization. Despite the similar degree of domain closure induced by Br-HIBO and thio-ATPA, we observed a ≈3.5-fold difference in the steady-state/peak ratio and a >2.2-fold difference in decay time on GluR2o (corresponding to a 6.2- and >8-fold difference on GluR2i, respectively).

A comparison of the structures of the GluR2 ligand-binding core complexed with (S)-thio-ATPA and (S)-Br-HIBO revealed four marked differences between the two structures. Two involve the residues Leu-650 and Met-708. Leu-650 is located in a dynamic part of the binding pocket adjacent to Asp-651–Ser-652, reported to adopt multiple peptide-bond conformations (6). However, we find no correlation between the peptide flip conformation and the agonist-induced currents (Table 1). In addition, Leu-650 forms an interdomain bridge by means of nonpolar interactions and is able to adopt at least two markedly distinct conformations (Table 1 and Fig. 4B). (S)-Br-HIBO, (S)-glutamate, (S)-AMPA, and (S)-ATPA all induce similar conformations of Leu-650, whereas e.g., (S)-thio-ATPA and kainate induce the other conformation (6, 12, 36).

Mutating the Leu at the equivalent position to a Thr in GluR1 (42) or a Val in GluR4 (43) changes the desensitization properties of the receptors. NMR dynamics studies performed on the GluR2-binding domain (44) show that upon agonist binding, residues in D1 are relatively fixed compared with considerable structural changes in D2. In concordance, a recent study of GluR2(L650T) leads to a model suggesting that AMPA binding is initiated by interaction with preorganized residues in D1 followed by a Glu-705 mediated reorganization of the residues in D2 (45). The latter conformational change induced the domain closure and was proposed to couple to the ion channel gating (46). The conformational flexibility of Leu-650 appears critical for the transitions because the L650T change induced the domain closure and was proposed to couple to the ion channel gating (46). The conformational flexibility of Leu-650 appears critical for the transitions because the L650T change induced the domain closure and was proposed to couple to the ion channel gating (46). The conformational flexibility of Leu-650 appears critical for the transitions because the L650T change induced the domain closure and was proposed to couple to the ion channel gating (46).
between the (S)-thio-ATPA and (S)-Br-HIBO crystal structures (Fig. 4). Based on high-resolution structures of the S-substituted willardine derivatives, it has been proposed that conformational changes in Met-708 might be transmitted to the channel region via helix I (47). Analysis of complexes with the full agonist (S)-glutamate, (S)-AMPA, or (S)-ATPA shows that Met-708 adopts an induced fit involving rather large conformational changes without affecting the efficacy significantly (12, 34, 36) making conformational changes in Met-708 a less likely factor underlying the observed kinetic differences.

NMR and UV spectroscopic methods indicate that agonist binding to the binding domain may induce changes in the β-sheet content or orientation in D2, which has been proposed as a mechanism for coupling of the changes in the binding site to the channel activation (48). However, we are not able to detect significant differences in the β-sheet content in D2 for the agonists.

Twist of D2 Relative to D1. In the crystals of GluR2–S1S2J complexed with (S)-thio-ATPA, a twofold dimer is formed of two symmetry-related molecules with interface accessible surface area of 866 Å². This size is similar to those reported of other agonist complex structures (6, 12). A comprehensive mutagenesis study demonstrated that the stability of the interdomain interactions between helix D and helix I correlated strongly with the desensitization properties of glutamate at GluR2i (43). Similar interdomain interactions are found in the (S)-thio-ATPA, (S)-Br-HIBO, and (S)-glutamate complexes, suggesting that the intersubunit interface mainly formed between D1 in the two subunits (both helices D and J are located in D1) are of equal stability. However, the location of the two protomers comprising the (S)-thio-ATPA and (S)-Br-HIBO dimers is slightly different (see Fig. 4A). Alignment of the structures reveals a relative twist of 6° between D1 and D2 in the (S)-thio-ATPA complex as compared with the (S)-thio-ATPA complex (Fig. 4A). The twist also occurs to different degree in other complexes, e.g., in GluR2–S1S2J(S)-2-Me-Tet-AMPA and GluR2–S1S2J(S)-glutamate, where the three different molecules show changing twists ranging from 1–7° and 0.5–3°, respectively, even for the same ligand–receptor combination. Further structural studies will be needed to elucidate the effect of the twist on the kinetic properties. Interestingly, in accordance with the similar maximal current on GluR2o(L483Y) observed for the two agonists, (S)-Br-HIBO and (S)-thio-ATPA, the twist does not alter the distances between residues of the two protomers linked to the pore region in the receptor (Ile-633–Ile-633 distance of 34.8 and 34.5 Å in the (S)-thio-ATPA and (S)-Br-HIBO complexes, respectively; see Fig. 4A). In conclusion, we have used structurally different agonists to identify residues and mechanisms apart from domain closure that might influence the kinetic properties of GluR desensitization.

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