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Structure of an agonist-bound ionotropic glutamate receptor

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Abstract

Ionotropic glutamate receptors (iGluRs) mediate most excitatory neurotransmission in the central nervous system and function by opening their ion channel in response to binding of agonist glutamate. Here we report a structure of a homotetrameric rat GluA2 receptor in complex with partial agonist (*S*)-5-Nitrowillardiine. Comparison of this structure with the closed state structure in complex with competitive antagonist ZK 200775 suggests conformational changes that occur during iGluR gating. Guided by the structures, we engineered disulfide crosslinks to probe domain interactions that are important for iGluR gating events. The combination of structural information, kinetic modeling, biochemical and electrophysiological experiments provide s insight into the mechanism of iGluR gating.

Ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels that mediate most excitatory neurotransmission in the central nervous system (1). iGluRs are implicated in nearly all aspects of nervous system development and function, and their dysfunction is associated with devastating chronic neurodegenerative conditions such as Alzheimer's and Parkinson's diseases, psychiatric disorders such as schizophrenia and depression and acute disorders such as brain trauma and stroke (1–3). The iGluR family includes three major subtypes: AMPA, kainate (KA) and NMDA receptors, which have diverse kinetic and pharmacological properties and play disparate roles in cognition but share common structural features (1).

iGluRs function as assemblies of four subunits. Whereas the majority of iGluRs in the nervous system are heterotetramers composed of at least two types of subunits, AMPA and select KA subunits can form functional homotetramers. Each iGluR subunit has a modular design (4) and includes an extracellular amino-terminal domain (ATD) that controls subunit assembly, receptor trafficking, channel gating, agonist potency and allosteric modulation; a clamshell-shaped ligand-binding domain (LBD or S1S2) composed of two stretches of polypeptide, S1 and S2, and responsible for agonist/antagonist recognition; a transmembrane domain (TMD) that forms a cation-conducting channel and contributes to iGluR tetrameric stability and a cytoplasmic C-terminal domain involved in synaptic localization, trafficking, mobility and receptor regulation (1). Currently, structural information on iGluRs is limited to high resolution crystal structures of genetically excised, water soluble ATDs and LBDs and 3.6–4.0 Å resolution structures of an intact AMPA receptor in the antagonist bound

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closed state (5) and NMDA receptors in the allosterically inhibited agonist bound closed state (6, 7).

iGluRs function by opening their ion channel in response to binding of agonist glutamate (Glu) (1). This process of activation gating is accompanied by the typically slower process of desensitization, which leads to closure of the ion channel and causes a reduction of iGluR-mediated currents in the continuous presence of Glu. Desensitization represents a versatile mechanism for shaping synaptic transmission (8) and controlling it could be an effective way of regulating synaptic integration, modulating circuit function and altering iGluR activity in pathological conditions (1, 9). Here, we employed a combination of biochemical and biophysical approaches to study iGluR gating. We focused on AMPA subtype rat GluA2 receptors, for which a modified construct GluA2_{cryst} had previously yielded the structure in the closed antagonist bound state (5). We used a construct GluA2*, which is similar to GluA2_{cryst} but has several mutations reversed back towards the wild type GluA2 background (see Suppl. Materials).

For functional characterization, we expressed GluA2* in HEK 293 cells and used patch-clamp with fast solution exchange to record GluA2*-mediated currents. As is typical for AMPA subtype iGluRs at near-physiological membrane potentials, Glu application elicited an inward current that quickly decayed in the continuous presence of Glu as a result of desensitization (Fig. 1A). Desensitization in GluA2* receptors had a similar rate and extent as wild type receptors (Fig. S1A,D) and was blocked by the positive allosteric modulator cyclothiazide (CTZ) (10).

AMPA receptors can also be activated by partial agonists, which elicit smaller maximal whole-cell currents than the full agonist Glu but have similar single-channel conductance (11–13). In our studies, we used a partial agonist (*S*)-5-Nitrowillardiine (NOW). In crystal structures of isolated LBD, NOW demonstrated ability to keep the LBD clamshell completely closed (13), just like full agonists. NOW elicited currents with ~4 times smaller maximal amplitude than Glu and had similar potency for GluA2* and wild type GluA2 receptors (Fig. S1B–C). The rate of desensitization and to a greater extent recovery from it for NOW was apparently slower than for Glu (Fig. S1D–G). Kinetic modeling of the effects of full and partial agonists on AMPA receptors (14)(Fig. S2) closely reproduces our experimental data and predicts that in the presence of saturating concentrations of either, nearly all receptors accumulate in the agonist-bound desensitized states (D_A, Fig. 1B).

We purified GluA2* and crystallized it in the presence of NOW. The best crystals belonged to the C222₁ space group and diffracted to 4.8 Å resolution (Table S1). We solved the structure (GluA2_{NOW}) by molecular replacement, using the high resolution structure of the isolated ATD dimer (PDB ID: 3H5V) (15) and the ion channel domain from GluA2_{cryst} (PDB ID: 3KG2) (5) as search models. The NOW-bound structure of the isolated LBD (PDB ID: 3RTW) (13) did not work as a molecular replacement search model for LBD, suggesting that the LBD conformation is different in the context of GluA2_{NOW}. We solved the structure by separately using the D1 and D2 lobes of LBD as molecular replacement search models. The conformation of LBD in the GluA2_{NOW} structure most closely resembles the CNQX-bound conformation of the isolated ligand binding domain (PDB ID:

3B7D) (16) and their superposition yields a root mean square deviation (RMSD) of 0.31 Å. Phases were improved by multidomain non crystallographic symmetry (NCS) averaging, solvent flattening and histogram matching. The electron density maps were of sufficient clarity to build the majority of linkers connecting the LBD to the ion channel and the ATD to the LBD (Fig. S3). The resulting model that was refined to good crystallographic statistics and stereochemistry ($R_{\text{work}}/R_{\text{free}}$: 0.228/0.262, Table S1).

The GluA2_{NOW} structure (Fig. 1C) is shaped like the letter Y and has a domain arrangement similar to the antagonist ZK 200775 (ZK) bound closed state GluA2_{cryst} structure (5). For better comparison with GluA2_{NOW}, we co-crystallized GluA2* with ZK (Table S1). The resulting structure (GluA2_{ZK}) closely resembles the original GluA2_{cryst} structure (superposition of individual domains of GluA2_{ZK} and GluA2_{cryst} yields RMSD of 0.3 to 1 Å).

Grossly, GluA2_{NOW} and GluA2_{ZK} have similar architectures (Fig. 2A). The conformations of ATD are very similar (Fig. S4A). The ion channel of GluA2_{NOW} adopts a closed pore conformation that is also similar to GluA2_{ZK} (Fig. S4B–C, Fig. S5). Nevertheless, small differences in the cross-pore dimensions between two pairs of diagonal subunits A/C and B/D (Fig. S4D–G) suggest deeper expansion of the 2-fold rotational symmetry of extracellular domains into the extracellular half of the GluA2_{NOW} ion channel. The strongest difference in domain conformation was observed for the LBD: each clamshell is ~11° more closed in GluA2_{NOW} compared to GluA2_{ZK} (Fig. 2B). The closure of individual clamshells results in wider and shorter conformations of the back-to-back LBD dimers in GluA2_{NOW} (Fig. 2C–D).

The altered conformation of LBD dimers in GluA2_{NOW} results in increased tension in linkers connecting LBD to ATD and TMD. As ATD and the ion channel maintain their closed state-like conformations, the additional tension forces pull ATD dimers down, tilting them ~1.2° away from the overall two-fold axis of symmetry (Fig. 2A). The ~2.4° splaying of the ATD dimers away from each other occurs around a hinge point at the ATD dimer-dimer interface. This interface maintains nearly identical conformations in GluA2_{NOW} and GluA2_{ZK} strongly supporting a critical role of ATD in iGluR tetramerization (1). Simultaneously, the tension forces pull the ion channel up by making the whole structure ~2 Å shorter and push the two LBD dimers towards each other. As a result, the interface between the two LBD dimers in GluA2_{NOW} becomes tighter and covers a three times larger surface area than in GluA2_{ZK} (Fig. S4H–I). Accordingly, the GluA2_{NOW} structure becomes ~2 Å narrower at the level of the LBD.

We tested the functional significance of agonist-induced tightening of the interface between two LBD dimers predicted by the GluA2_{NOW} structure in experiments with GluA2-I664C receptors activated by Glu. Isoleucines I664 are located in the middle of the dimer-dimer interface – the Ca's are 13.5 Å apart in GluA2_{ZK} and 11.3 Å apart in GluA2_{NOW} (Fig. S6A). Crosslinking of I664C substituted cysteines has been shown in experiments with purified receptors (5). We compared the rates of GluA2-I664C receptor recovery from desensitization in reducing and non-reducing conditions. In non-reducing conditions, the recovery was incomplete (Fig. S6B–D), apparently due to slow peak current reduction. The

reduction was faster with longer exposures to Glu, and was accompanied by a decrease in the steady-state current amplitude without significant changes in the fast rate of desensitization and was completely reversed by DTT (Fig. S6E–F). The majority of receptors quickly become non-conducting in the presence of agonist, thus slow inactivation of GluA2-I664C receptors under non-reducing conditions is consistent with agonist-induced tightening of LBD dimer-dimer interface.

Compared to GluA2_{ZK}, the LBD clamshells in GluA2_{NOW} are ~11° more closed (Fig. 2B), presenting structural evidence of clamshell motion in the context of the intact, multi-domain iGluR, consistent with studies of isolated LBDs (17). The conformational change is smaller than in agonist- or partial agonist-bound structures of isolated LBD, where clamshells are up to ~14° more closed than in GluA2_{NOW} (Fig. S7). Despite different extents of clamshell closure between the isolated LBD and GluA2_{NOW}, the orientation of the NOW molecule in the binding pocket is similar (Fig. S8). However, increased distances between atoms of NOW and the binding pocket residues (Table S2) suggest weakened interactions. Perhaps the most unexpected feature of the GluA2_{NOW} structure is the conformation of the LBD dimer interface. Although the GluA2_{NOW} structure was crystallized at conditions apparently favoring desensitization (Fig. 1B), the D1-D1 interface appears nearly intact rather than ruptured (18). Indeed, the difference in distance between upper lobes D1 in GluA2_{NOW} and GluA2_{ZK} does not exceed ~1 Å, while the distance between lower lobes D2 is ~6 Å larger in GluA2_{NOW} (Fig. 2C–D).

To probe the LBD dimer interface conformation at conditions favoring different gating states in solution, we substituted individual residues at this interface with cysteines (Fig. 3A–B) and purified wild type and cysteine-substituted GluA2* receptors in their tetrameric form (Fig. S9). Cysteine crosslinking was tested in the presence or absence of reducing agent (2 mM DTT) and in the presence of different ligands. The GluA2_{NOW} and GluA2_{ZK} structures predicted position-specific differences in cross-interface distances between substituted cysteines (Fig. 3C) but nearly all of them were capable of forming crosslinks in non-reducing conditions favoring all gating states: closed, open and desensitized (Fig. 3D). We obtained similar results for the wild type and K493C full length GluA2 receptors (Fig. S10). Such high reactivity of cysteines at the dimer interface indicates that LBD is highly dynamic (13, 19–27) and its conformation fluctuates during gating. Because the cross-dimer disulfide bonds are unstable and reversible (20), they cannot abolish these fluctuations (Fig. 3D) but they can favor certain gating states.

To study the effect of LBD interface crosslinking on iGluR gating, we recorded Glu-activated currents from HEK 293 cells expressing wild type or cysteine-substituted receptors (Fig. 4A and Fig. S11). To separate effects on receptor activation and desensitization, we measured two parameters. The first parameter gives an estimate for the effect of crosslink on AMPA receptor activation (Fig. 4B). No difference in activation between reducing and non-reducing conditions was observed for wild type and K493C receptors. Crosslinking of cysteines in the lower D1-D1 interface (P494C and S497C) had a positive effect on activation. In a reducing environment, cysteine substitution of P494 almost entirely abolished iGluR function, while crosslinking recovered small currents, consistent with the very low probability of disulfide bond formation (Fig. 3D). The S497C receptors

demonstrated robust Glu-activated currents that had larger amplitudes under crosslinking conditions. Overall, strengthening of the D1-D1 interface seemed to promote channel ability to open, which was most obvious when cysteine substitutions themselves inhibited this ability. In contrast, crosslinking of cysteines substituted in the D2 lobe produced negative effects on iGluR activation that were stronger for positions located closer to the ion channel. Hence, the LBD D2 lobes interlocking appears to prevent their separation that is necessary for channel opening.

The second parameter reflects apparent changes in AMPA receptor desensitization, particularly its inhibition (Fig. 4C). No strong inhibition of desensitization was observed for wild type, K493C, or any receptors with cysteine substitutions in D2. Some of these mutant receptors had the rates and extents of desensitization somewhat different from wild type (Table S3) but those effects were due to mutations themselves and did not depend on the redox condition. Moreover, it is possible that the D2 cysteine crosslinks can promote desensitization by locking receptors in the non-conducting state unable to activate (Fig. 4B). Only P494C and S497C substitutions in the lower D1-D1 interface showed strongly reduced desensitization (Fig. 4C). While for P494C, inhibition of desensitization was apparently independent of redox condition, it was greatly enhanced by cysteine crosslinking in S497C receptors. The inhibition of desensitization observed in P494C and S497C receptors is reminiscent of the effect of the L483Y mutation (28) and of CTZ binding (10, 29), both of which take place at the D1-D1 interface.

Based on our mutational, structural and functional experiments in combination with previous work, we present two possible gating models (Fig. S12), both assume that LBDs are highly mobile (Fig. 3); agonist binding causes LBD clamshell closure (17); and channel opening occurs when LBD clamshells adopt their maximally closed conformation (22, 23, 30–32) represented by agonist-bound structures of isolated LBD (13, 17) with the D1-D1 interface intact (Fig. S7). The first model represents a traditional view (18) where the final desensitized state has the D1-D1 interface modified (Fig. S12A). In this model, the GluA2_{NOW} structure represents the agonist-bound closed state, which is predicted to be a transient state with negligible occupancy (Fig. 1B, Fig. S2F) insufficient to produce protein crystals. Nevertheless, such a scenario is plausible if only a limited range of conformations of the protein is accessible in the solubilized receptor or the crystal lattice contacts significantly affect protein conformation.

The second model (Fig. S12B) assumes two-step desensitization with GluA2_{NOW} representing a deep desensitized state. This model is consistent with the predictions of kinetic modeling that at high NOW concentrations, the majority of receptors accumulate in the deep desensitized state (D₂4 in Fig. S2). It also predicts that the same tension forces applied from ATD and ion channel through the connecting linkers that open LBD clamshells during deactivation help transition the receptor from the deep desensitized state back to the desensitized state. The second model therefore explains why mutations that change the rate of deactivation often produce similar effects on the rate of recovery from desensitization (14, 33, 34).

Independent of gating model, the entry into desensitization is associated with modification of the D1-D1 interface (Fig. S12) (18, 20, 35–37). One possible modification is represented by structures of the S729C and G725C crosslinked isolated LBDs (18) where the D1-D1 interface is ruptured. K493C crosslinking, however, does not affect desensitization (Fig. 4C) and argues against these structures representing the desensitized state of the intact receptor. Alternatively, the D1-D1 interface modification might be a rotation of the D1 lobes relative to each other that does not change the distance between K493 lysines but introduces relative displacement of pairs of other residues at the D1-D1 interface. Correspondingly, mutations like L483Y(28) or S497C (Fig. 4C, Fig. S11) or positive allosteric modulators like CTZ (10, 29) would block desensitization by imposing constraints on the D1-D1 interface rearrangement.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References and Notes

1. Traynelis SF, et al. *Pharmacol Rev.* Sep.2010 62:405. [PubMed: 20716669]
2. Bowie D. *CNS Neurol Disord Drug Targets.* Apr.2008 7:129. [PubMed: 18537642]
3. Paoletti P, Bellone C, Zhou Q. *Nat Rev Neurosci.* Jun.2013 14:383. [PubMed: 23686171]
4. Wo ZG, Oswald RE. *Trends Neurosci.* Apr.1995 18:161. [PubMed: 7539962]
5. Sobolevsky AI, Rosconi MP, Gouaux E. *Nature.* Dec 10.2009 462:745. [PubMed: 19946266]
6. Lee CH, et al. *Nature.* Jul 10.2014 511:191. [PubMed: 25008524]
7. Karakas E, Furukawa H. *Science.* May 30.2014 344:992. [PubMed: 24876489]
8. Jones MV, Westbrook GL. *Trends Neurosci.* Mar.1996 19:96. [PubMed: 9054063]
9. Black MD. *Psychopharmacology (Berl).* Apr.2005 179:154. [PubMed: 15672275]
10. Patneau DK, Vyklicky L, Mayer ML. *J. Neurosci.* 1993; 13:3496. [PubMed: 7688040]
11. Jin R, Banke T, Mayer ML, Traynelis SF, Gouaux E. *Nat Neurosci.* 2003; 6:803. [PubMed: 12872125]
12. Poon K, Nowak LM, Oswald RE. *Biophys J.* Sep 8.2010 99:1437. [PubMed: 20816055]
13. Poon K, Ahmed AH, Nowak LM, Oswald RE. *Mol Pharmacol.* Jul.2011 80:49. [PubMed: 21464198]
14. Robert A, Armstrong N, Gouaux JE, Howe JR. *J Neurosci.* Apr 13.2005 25:3752. [PubMed: 15829627]
15. Jin R, et al. *Embo J.* Jun 17.2009 28:1812. [PubMed: 19461580]
16. Menuz K, Stroud RM, Nicoll RA, Hays FA. *Science.* 2007; 318:815. [PubMed: 17975069]
17. Armstrong N, Gouaux E. *Neuron.* Oct.2000 28:165. [PubMed: 11086992]
18. Armstrong N, Jasti J, Beich-Frandsen M, Gouaux E. *Cell.* Oct 6.2006 127:85. [PubMed: 17018279]
19. Maltsev AS, Ahmed AH, Fenwick MK, Jane DE, Oswald RE. *Biochemistry.* Oct 7.2008 47:10600. [PubMed: 18795801]

20. Plested AJ, Mayer ML. J Neurosci. Sep 23.2009 29:11912. [PubMed: 19776277]
21. Fenwick MK, Oswald RE. J Biol Chem. Apr 16.2010 285:12334. [PubMed: 20110361]
22. Landes CF, Rambhadran A, Taylor JN, Salatan F, Jayaraman V. Nat Chem Biol. Mar.2011 7:168. [PubMed: 21297640]
23. Lau AY, Roux B. Nat Struct Mol Biol. Mar.2011 18:283. [PubMed: 21317895]
24. Postila PA, Ylilauri M, Pentikainen OT. J Chem Inf Model. May 23.2011 51:1037. [PubMed: 21500800]
25. Ramaswamy S, et al. J Biol Chem. Dec 21.2012 287:43557. [PubMed: 23115239]
26. Ahmed AH, et al. J Biol Chem. Sep 20.2013 288:27658. [PubMed: 23940029]
27. Yao Y, Belcher J, Berger AJ, Mayer ML, Lau AY. Structure. Aug 20.2013
28. Stern-Bach Y, Russo S, Neuman M, Rosenmund C. Neuron. Oct.1998 21:907. [PubMed: 9808475]
29. Sun Y, et al. Nature. May 16.2002 417:245. [PubMed: 12015593]
30. Zhang W, Cho Y, Lolis E, Howe JR. J Neurosci. Jan 23.2008 28:932. [PubMed: 18216201]
31. Ahmed AH, Wang S, Chuang HH, Oswald RE. J Biol Chem. Oct 7.2011 286:35257. [PubMed: 21846932]
32. Maclean DM, Wong AY, Fay AM, Bowie D. J Neurosci. Feb 9.2011 31:2136. [PubMed: 21307250]
33. Weston MC, Gertler C, Mayer ML, Rosenmund C. J Neurosci. Jul 19.2006 26:7650. [PubMed: 16855092]
34. Carbone AL, Plested AJ. Neuron. Jun 7.2012 74:845. [PubMed: 22681689]
35. Midgett CR, Gill A, Madden DR. Front Mol Neurosci. 2012; 4:56. [PubMed: 22232575]
36. Lau AY, et al. Neuron. Aug 7.2013 79:492. [PubMed: 23931998]
37. Schauder DM, et al. Proc Natl Acad Sci U S A. Mar 25.2013
38. Hollmann M, O'Shea-Greenfield A, Rogers SW, Heinemann S. Nature. 1989; 342:643. [PubMed: 2480522]
39. Keinänen K, et al. Science. 1990; 249:556. [PubMed: 2166337]
40. Cormack BP, Valdivia RH, Falkow S. Gene. 1996; 173:33. [PubMed: 8707053]
41. Sommer B, Kohler M, Sprengel R, Seeburg PH. Cell. Oct 4.1991 67:11. [PubMed: 1717158]
42. Otwinowski Z, Minor W. Methods Enzymol. 1997; 276:307.
43. McCoy AJ. Acta Crystallogr D Biol Crystallogr. Jan.2007 63:32. [PubMed: 17164524]
44. Emsley P, Cowtan K. Acta Crystallogr D Biol Crystallogr. Dec.2004 60:2126. [PubMed: 15572765]
45. Adams PD, et al. Acta Crystallogr D Biol Crystallogr. Nov.2002 58:1948. [PubMed: 12393927]
46. Krissinel E, Henrick K. Acta Crystallogr D Biol Crystallogr. Dec.2004 60:2256. [PubMed: 15572779]
47. Sehnal D, et al. J Cheminform. 2013; 5:39. [PubMed: 23953065]
48. Chen GQ, Cui C, Mayer ML, Gouaux E. Nature. Dec 16.1999 402:817. [PubMed: 10617203]
49. Kawate T, Gouaux E. Structure. Apr.2006 14:673. [PubMed: 16615909]
50. Patneau DK, Mayer ML, Jane DE, Watkins JC. J. Neurosci. 1992; 12:595. [PubMed: 1371315]

Fig. 1. Functional characterization and structure of NOW-bound GluA2*

(A) Representative whole-cell currents recorded at -60 mV membrane potential from HEK 293 cells expressing GluA2* in response to a 500 ms application of 1 mM Glu alone, application of Glu in the continuous presence of 30 μ M of the positive allosteric modulator cyclothiazide (CTZ), application of 100 μ M NOW alone and application of NOW in the continuous presence of CTZ. Filled and open triangles indicate steady state currents at the end of NOW and Glu applications, respectively. (B) Occupancies of the closed (C), open (O) and desensitized (D) states of the receptor at the time points indicated by the triangles in (A). Subscript 'A' and '*' indicate agonist bound and ion conducting states, respectively. The occupancies are predicted using kinetic modeling (Fig. S2). (C) The 'broad' (left) and 'narrow' (right) faces of the GluA2_{NOW} structure viewed parallel to the membrane and perpendicular to the overall 2-fold axis of molecular symmetry. Inner and outer sides of membrane are indicated by parallel grey bars. Each of four subunits is in different color.

Fig. 2. Comparison of NOW-bound and ZK-bound GluA2*

(A) Superposition of the full length GluA2_{NOW} (red) and GluA2_{ZK} (blue). Red arrows show changes in the GluA2_{NOW} structure compared to GluA2_{ZK}. (B) Superposition of individual LBDs from GluA2_{NOW} and GluA2_{ZK} structures based on the upper lobe D1 and viewed along the axis of 11° rotation that brings the lower lobe D2 of GluA2_{ZK} into GluA2_{NOW}. (C–D) LBD dimers from the GluA2_{ZK} (C) and GluA2_{NOW} (D) structures. Shown are Ca's (yellow spheres) and distances between them for E487 and I633 as well as ZK and NOW molecules as stick models.

Fig. 3. LBD dimer interface crosslinking and high mobility of LBD

(A–B) Superposition of LBD dimers from GluA2_{NOW} (red) and GluA2_{ZK} (blue) structures based on the upper lobes D1 and viewed perpendicular to (A) or along (B) the axes of rotation that brings the lower lobes D2 of GluA2_{ZK} into GluA2_{NOW}. Spheres show C α 's for residues at the LBD interface substituted with cysteines. (C) Table showing LBD cross-interface distances between C α 's of the cysteine substituted residues. Each number is the average of distances for the AD and BC dimers in Angstroms. (D) SDS-PAGE analysis of spontaneous crosslinking of cysteines introduced at the LBD interface. The experiments were carried out with GluA2* receptors either in reducing conditions (2 mM DTT, left lanes) or in non-reducing conditions but in the presence of 3 mM Glu and 50 μ M CTZ (Glu +CTZ, favoring the open state) or 3 mM Glu (Glu, favoring the desensitized state) or 500 μ M NOW (NOW, favoring the desensitized state) or 100 μ M ZK (ZK, favoring the antagonist bound closed state) or in the absence of ligands (Apo, favoring the unliganded closed state). Filled and open triangles indicate positions of monomeric and dimeric bands, respectively. Note, in the absence of reducing agent, substituted cysteines can crosslink in conditions that favor different gating states, indicating high mobility of GluA2* LBDs in solution.

Fig. 4. Effects of LBD dimer interface crosslinking on gating

(A) Representative whole-cell currents recorded at -60 mV membrane potential from HEK 293 cells expressing GluA2-K493C in response to a 500 ms application of 1 mM Glu alone and applications of Glu in the continuous presence of 30 μ M CTZ or 2 mM DTT or CTZ and DTT. (B) Effects of cysteine crosslinking on activation. Shown is the ratio of initial currents recorded in the continuous presence of CTZ in the absence and presence of DTT. (C) Effects of cysteine crosslinking on GluA2 desensitization. Shown is the extent of current reduction due to desensitization measured in the absence (filled bars) or presence (open bars) of DTT.