Addendum

What Does it Take to Gate AMPA Receptors?

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AMPA receptor, gating, glutamate, linker, lurcher, TARP

ABBREVIATIONS
AMPA α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
iGluR ionotropic glutamate receptor
TARP transmembrane AMPA receptor regulatory protein
LBD ligand binding domain
TMD transmembrane domain
NASP 1-naphthylacetyl spermine
NBQX 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoloxaline-7-sulfonamide
CNQX 6-cyano-7-nitroquinoxaline-2,3-dione
DNQX 6,7-dinitroquinoxaline-2,3-dione

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ABSTRACT

In vivo, agonist binding to the open conformation of the ligand-binding domain initiates the process of gating in ionotopic glutamate receptors. Arguably, an alternative manner to gate the receptors exists, which requires a point mutation in the most-conserved sequence motif in the second transmembrane domain. Originally, this mutation occurred spontaneously in the orphan glutamate receptor subunit delta2, causing the ataxic phenotype of lurcher mice.1 In the absence of a ligand that could initiate gating at this orphan subunit, the introduction of the lurcher mutation led to spontaneous currents through delta2-lurcher channels.1 Introduction of the corresponding mutation into the AMPA receptor GluR1 induced a number of aberrant gating properties.2-5 Among those, glutamate potency was highly increased, and competitive antagonists suddenly behaved as partial agonists.2-5

We reported that the introduction of delta2 amino acids in the domain preceding the first transmembrane domain in GluR1 resulted in a mutant receptor that displayed all characteristics of lurcher-typical gating. We proposed that lurcher-like mutations work to enhance gating by destabilizing the closed state of the receptor. As a result, no or minimal conformational changes in the ligand-binding domain are sufficient for gating, explaining, respectively, why spontaneous currents occur and competitive antagonists act as partial agonists in lurcher-like channels. Strikingly, a similar conversion of antagonists upon coexpression of glutamate receptors with TARPs has recently been reported.6,7 We take this as indication that the actual mechanism of action might be very similar, and that both lurcher-like mutations and TARPs work as ‘gating enhancers’.

To initiate gating in ionotopic glutamate receptors (iGluRs), glutamate binding induces a conformational change in the ligand-binding domain (LBD) of the receptor. The two lobes of the LBD close around the agonist like the valves of a clamshell, locking the agonist in the binding cleft. The conformational change is then passed on to the three transmembrane domains (TMDs) via three short linker sequences of unknown structure. The striking subtype specificity of the three linker sequences implies that they might mediate subtype-specific gating properties and prompted us to study these regions more closely. Using a domain transplantation approach, we exchanged the three linkers of the AMPA receptor GluR1 for those of the distantly related delta2 subunit. Two effects upon exchanging the linkers were especially interesting: First, we found that the transplantation of the linker preceding the first TMD (linker A) results in a mutant GluR1 receptor that mimics all essential characteristics of the GluR1-lurcher mutant. Second, this lurcher-like effect of the linker A exchange was independent of the co-transplantation of the linker preceding the second TMD (linker B), but counteracted by the simultaneous transplantation of the linker preceding the third TMD (linker C). In this addendum we take the opportunity to examine the striking similarity between the newly described GluR1-lurcher A mutant and the original GluR1-lurcher mutant more closely. The differential positioning of the two mutations within the GluR1 subunit allowed us to combine both mutations in one mutant receptor to reinvestigate their effect on AMPA receptor gating.

GATING IN LURCHER-LIKE AMPA RECEPTOR CHANNELS

In our view, the total current mediated by GluR1-lurcher and lurcher-like mutants has two components. One is independent of the status of the LBD and only susceptible to manipulation by open channel blockers such as 1-naphthylacetyl spermine (NASP). The other component is dependent on the degree of domain closure of the LBD (Fig. 1).
would lead to gating in and slightly closed conformations, fluctuations of 3° and more arrested in a static ligand-induced gating at that more than 3° of domain closure is necessary and sufficient for NBQX, however, still retains its antagonistic effect. We interpret CNQX act as competitive antagonists at wild-type channels, both degree of domain closure, followed by DNQX (3°), CNQX (6°), ligand-dependent component more closely.

We used a set of five different ligands that induce different degrees of domain closure at wild-type AMPA receptors to examine the ligand-independent spontaneous current in turn can be subdivided into a component that is completely independent of the conformational status of the LBD (upper row) and a component that is blocked by NBQX (middle row). The ligand-independent spontaneous current can be blocked by open channel blockers, but is not influenced by antagonists that bind to the LBD (upper row). Intrinsic conformational fluctuations in the LBD are hindered by NBQX binding, which does not induce sufficient domain closure (<3°) to gate lurcher-like channels (middle row). By contrast, CNQX or DNQX binding induces enough domain closure (>3°) to gate lurcher-like channels (not shown). Binding of glutamate induces 21° of domain closure, eliciting larger current responses than partial agonists at the virtually non-desensitizing lurcher-like channels (lower row). An overlay of five structures from the apo state to the glutamate-bound state of GluR2 was used to depict the intrinsic fluctuations in the LBD (PDB codes: 1FTO, 1FTL, 1FTK, 3B7D, 1FTJ). Since NBQX-bound structure has not yet been solved, the apo state of the receptor is shown with CNQX in the binding pocket.

We used a set of five different ligands that induce different degrees of domain closure at wild-type AMPA receptors to examine the ligand-dependent component more closely.

Of these five ligands, NBQX presumably induces the smallest degree of domain closure, followed by DNQX (3°), CNQX (6°), kainate (12°) and glutamate (21°).6,8-10 While NBQX, DNQX and CNQX act as competitive antagonists at wild-type channels, both DNQX and CNQX act as partial agonists at lurcher-like mutants. NBQX, however, still retains its antagonistic effect. We interpret the differential action of the three competitive antagonists such that more than 3° of domain closure is necessary and sufficient for ligand-induced gating at lurcher-like channels. Since the LBD is not arrested in a static apo conformation but likely fluctuates between the apo and slightly closed conformations, fluctuations of 3° and more would lead to gating in lurcher-like channels. NBQX binding then arrests the LBD in a more open conformation, accounting for the ligand-sensitive component of spontaneous current (Fig. 1).

Upon combination of the GluR1-linker A mutation with the GluR1-lurcher mutation, the ligand-dependent properties of the resulting GluR1-linker A + lurcher mutant were virtually unaltered when compared to the single mutants. However, we observed an increase in the agonist-independent, spontaneous current. We evaluated this effect by calculating the relation between spontaneous current and glutamate-induced current. In the GluR1-linker A + lurcher mutant, the spontaneously gating fraction of channels was dramatically increased (from 30% to 90%), while the fraction of channels that were still agonist-sensitive was reduced concurrently.

Previously, the gating mechanism of lurcher channels had been argued to reflect either ligand-independent, true spontaneous channel openings or the activity of residual agonist present in recording solutions that gates the super-potent lurcher receptors.2-5 Since glutamate potency did not increase in concert with the huge increase in spontaneous gating, we concluded that residual glutamate cannot be the sole cause for the spontaneous currents. In our model, lurcher-like effects arise whenever mutations destabilize the closed state of the receptor. This occurs to such an extent that even without ligand-induced conformational changes in the LBD the equilibrium between closed and open states is shifted significantly in favor of the latter. Concurrently, ligand-induced conformational changes are translated into pore opening much more efficiently, and even minimal changes suffice for gating.

**WHYBother to EXAMINE ABERRANT GATING IN LURCHER-LIKE MUTANT?**

Apart from simply addressing open questions about aberrant gating, two findings add physiological relevance to the correct interpretation of lurcher-like properties. First, the crystallization of the LBD of delta2 recently revealed that D-serine and glycine bind to this orphan receptor subunit. Peculiarly though, neither substance elicits current responses at wild-type delta2 receptors; however, they decrease the spontaneous current through delta2-lurcher channels.11 Hence, a clear concept of how the lurcher mutation affects ligand-dependent properties in AMPA receptors will help the interpretation of the respective properties observed at delta2-lurcher channels.

Second, two recent reports have described that the classical competitive antagonists CNQX and DNQX act as partial agonists at AMPA receptors when transmembrane AMPA receptor regulatory proteins (TARPs) are coexpressed.6,7 This obvious similarity to lurcher raises the question whether similar mechanisms are at work. Since the exact mechanism of interaction between AMPA receptors and TARPs is currently subject to intense investigation, a detailed analysis of lurcher-like gating might provide clues as to how TARPs exert their effects on AMPA receptors.

Figure 1. Effect of lurcher-like mutations on channel gating. The total receptor-mediated current in lurcher-like AMPA receptor mutants can be divided into spontaneous current and agonist-induced current. The spontaneous current in turn can be subdivided into a component that is completely independent of the conformational status of the LBD (upper row) and a component that is blocked by NBQX (middle row). The ligand-independent spontaneous current can be blocked by open channel blockers, but is not influenced by antagonists that bind to the LBD (upper row). Intrinsic conformational fluctuations in the LBD are hindered by NBQX binding, which does not induce sufficient domain closure (<3°) to gate lurcher-like channels (middle row). By contrast, CNQX or DNQX binding induces enough domain closure (>3°) to gate lurcher-like channels (not shown). Binding of glutamate induces 21° of domain closure, eliciting larger current responses than partial agonists at the virtually non-desensitizing lurcher-like channels (lower row). An overlay of five structures from the apo state to the glutamate-bound state of GluR2 was used to depict the intrinsic fluctuations in the LBD (PDB codes: 1FTO, 1FTL, 1FTK, 3B7D, 1FTJ). Since NBQX-bound structure has not yet been solved, the apo state of the receptor is shown with CNQX in the binding pocket.
INTERACTION OF THE TARP γ2 WITH GLUR1 LINKER MUTANTS

It is intriguing to speculate that TARPs might manipulate AMPA receptors in a manner similar to lurcher-like mutations. Previous reports had indicated that the presence of the lurcher mutation in GluR1 abolished the effect of the TARP γ2 on AMPA receptor trafficking and channel gating. From this observation, it was suggested that the lurcher mutation disrupts protein association between the TARP and GluR1. It seems unlikely, though, that TARPs directly interact with the lurcher site in the highly conserved sequence motif in the second TMD of all iGluRs. Since TARPs are selective for AMPA receptors, they are likely to interact with some AMPA receptor-specific region such as the linkers. However, it could well be possible that lurcher and TARP both work by destabilizing the closed state of the receptor. Hence, we were interested in possible effects of γ2 on the GluR1-linker mutants. Interestingly, responses to both glutamate and kainate application were not significantly different in the presence or absence of γ2 for the GluR1-linker A+B mutant that carried both linker A and linker B from delta2 (Fig. 2A). This was quite unexpected since the single GluR1-linker A and GluR1-linker B mutants were potentiated by γ2. However, when the amplitudes of spontaneous currents in the absence and presence of γ2 were compared, it became apparent that γ2 increased the spontaneous current at the expense of agonist-induced current for the GluR1-linker A+B mutant (Fig. 2B). 55 ± 3% of the total receptor-mediated current was agonist-induced for the GluR1-linker A+B mutant in the absence of γ2, while only 29 ± 3% of the receptor-mediated current was agonist-induced in the presence of γ2. This TARP-mediated shift was highly significant, and was not observed for the GluR1-linker A mutant.

Hence, the TARP altered electrophysiological properties of all three linker mutants, proving that interaction between receptor and TARP still occurs. However, depending on the individual combination of linkers A and B, the properties of the respective mutants were influenced quite differently by TARP coexpression. This also fits our recent observation that TARPs influence the degree of spontaneous gating.7

TARPS AND LURCHER-LIKE MUTATIONS AS ‘GATING ENHANCERS’

From the available data it is impossible to conclude exactly how the differential effects of the TARPs are brought about (e.g., direct interaction with the receptor vs. allosteric effect). Yet the data show that the linker region of the AMPA receptor is critical for the actual TARP effect on gating. Similar to the lurcher-like mutations, TARPs might destabilize the closed state of the receptor, so that both the lurcher mutation and TARP coexpression act as ‘gating enhancers’ for AMPA receptors. Along this line, all lurcher-like mutations may offer non-physiological, artificial, quite drastic ways to enhance gating, while the TARPs constitute nature’s way to improve gating, allowing precise regional and qualitative control of this process. The data presented provide a promising starting point for a search for possible TARP interaction sites in AMPA receptors. Furthermore, it highlights that it is worthwhile to compare TARP and lurcher characteristics and their mutual interaction in more detail.

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