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Long distance effect on ligand-gated ion channels extracellular domain may affect interactions with the intracellular machinery

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Modulation of receptor trafficking is critical for controlling neurotransmission. A γ2(R43Q) point mutation on $GABA_A$ receptor subunit is linked to epilepsy in human. We recently analyzed the effect of this amino-acid substitution on $GABA_A$ receptor trafficking and showed that this mutation as well as agonist application, both affecting $GABA_A$ receptor extracellular domain, have an effect on receptor endocytosis. By comparing homology models based on ligand gated ion channels in their active and resting states, we reveal that the γ2R43 domain is located in a loop that is affected by motion resulting from receptor activation. Taken together, these results suggest that endocytosis of $GABA_A$ receptors is linked to agonist induced conformational changes. We propose that ligand or modulator binding is followed by a whole chain of interconnections, including the intracellular domain, that may influence ligand-gated channel trafficking.

Keywords: trafficking; internalization, receptor model

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Fast neurotransmission relies greatly on ligand-gated ion channels (LGICs) that are involved in many physiological functions. Plasticity of neurotransmission in physiological and pathophysiological states depend on LGIC trafficking that is modulated by a number of different mechanisms including surface targeting, mobility, and endocytosis.[1-3] $GABA_A$Rs involved in fast inhibitory neurotransmission are also regulated by the exchange between surface and intracellular compartments via a constitutive clathrin-mediated dynamin-dependent endocytosis pathway.[4] This constitutive internalization is modulated by intracellular mechanisms and is altered in pathological conditions.[5-9] Other LGIC such as AMPA or P2X receptors are also constitutively internalized by a clathrin-dependent endocytosis. In contrast, NMDA receptors are stabilized via an interaction with PDZ domain proteins and are more stable at the postsynaptic membrane.[10] Among P2X subtypes, P2X4-containing receptors are specifically and constitutively internalized.[11]

Genetic evidence has revealed a direct link between epilepsy and $GABA_A$Rs dysfunction, including trafficking alteration.[12-14] These mutations also offer an opportunity to obtain new insights into $GABA_A$R structure and function.[15] For example, an R43Q mutation located in the γ2-subunit N-terminal extracellular domain (**Fig. 1A**) is linked to childhood absence epilepsy and febrile seizure.[13,14] We recently analyzed γ2(R43Q) trafficking and showed that the γ2 subunit containing the R43Q mutation increased a clathrin- and dynamin-dependent endocytosis of $GABA_A$Rs, hindering their detection on the cell surface.[16] The γ2(R43Q)-dependent endocytosis was reduced by $GABA_A$Rs antagonists gabazine and picrotoxin, acting at a different site suggesting an allosteric effect i.e., that γ2(R43Q)-containing $GABA_A$Rs are in a conformational state that promotes internalization. Further experiments revealed that agonist exposure triggered an increase of wild-type $GABA_A$R endocytosis, both on native- and recombinant-$GABA_A$Rs.[16] Application of their respective agonists enhances also

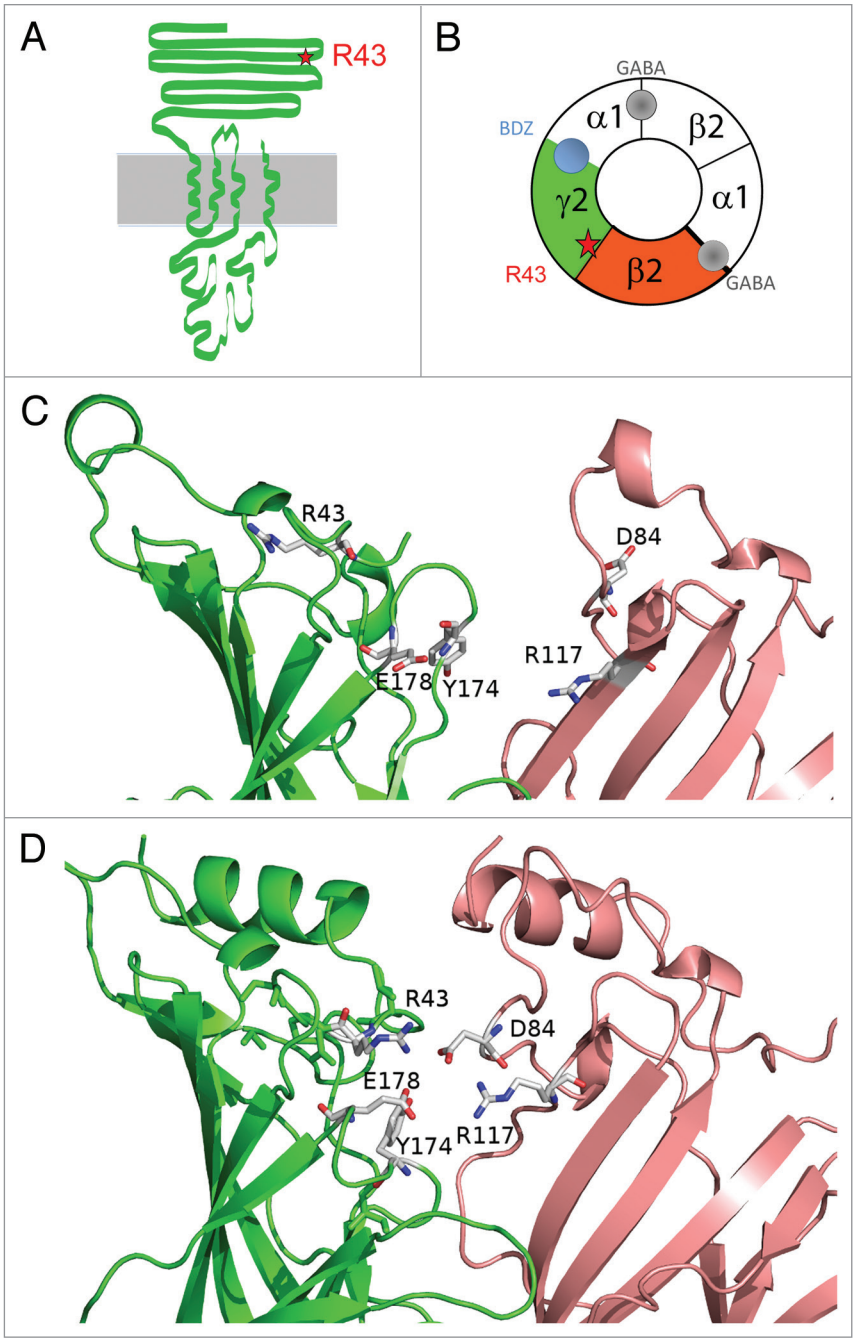

Figure 1. The γ2(R43Q) mutation destabilize the open-channel conformation. (**A**) Schematic representation of $GABA_AR$ subunits showing a large N-terminal extracellular domain, 4 transmembrane domains and a large cytoplasmic loop. The location of the γ2(R43Q) point mutation associated with childhood absence epilepsy and febrile seizure is indicated. (**B**) Schematic diagram of a α1β2γ2 $GABA_AR$ which illustrates the 5 combined subunits that form the complex, the 2 GABA active binding sites at the β2 and α1 interfaces (gray circles) and the benzodiazepine (BDZ, blue circle) allosteric binding site at the α1 and γ2 interface. In current models, γ2R43 (red star) is at the interface with β2. (**C**,**D**) Model of a α1β2γ2 $GABA_AR$ viewed from the outside. Here only the γ2 and β2 subunits are shown for clarity (γ2 in green and β2 in pink, see (**B**). γ2 (R43, Y174, E178) and β2 (D84, R117) residues are represented by sticks. These residues are within loops identified as being involved in the channel-opening motion: (**C**) resting state model, (**D**) active state model.

the rate of AMPA- or P2X4-receptor internalization suggesting that the increase might be caused by an agonist-induced conformational change in the receptor promoting an interaction with components of the endocytotic machinery.[11]

The $GABA_AR$ extracellular domain contains binding sites for agonists and allosteric modulators (**Fig. 1B**), while the intracellular domain mediates interactions with trafficking factors.[2,6,8,17] Consequently, our findings[16] suggest that $GABA_AR$ internalization is driven by a global conformational change. Molecular models show that the γ2R43 residue is at the γ2/β2 interface in the extracellular domain, on a loop positioned above the pocket, which is homologous to the GABA binding sites. Interestingly, many mutations in nicotinic receptors linked to diseases are at the interface between receptor subunits;[18] they alter the gating allosterically, i.e., from a distance.[18,19] A model indicates that γ2R43 and γ2E178 are connected through a bifurcated salt bridge: this model has been used to study the γ2(R43Q) mutation.[12,20,21] One of these studies has suggested that these positions have a long-range allosteric effect.[20] In our new $GABA_AR$ model derived from the Glutamate-gated chloride channel in the active state recently published,[22] the R43 residue of the γ2-subunit is connected to Y174 and E178 from the loop B and to β2 subunit via polar interactions that should be sensitive to the R43Q substitution and positioned on a loop thought to be involved in the channel-pore opening motion[23] (**Fig. 1D**). Here we show that these interactions are not present in our novel model of the resting state (**Fig. 1C**) which was based on the structure of a prokaryotic homolog published very recently.[24] These new models (i.e., resting and activated states) point out that the γ2R43 domain is affected by the motion resulting from $GABA_AR$ activation. This observation is consistent with an allosteric effect of the mutation. Moreover, electrophysiological recordings and kinetic analyses have shown that the long-distance effects of γ2(R43Q) substitution extend as far as the transmembrane domains.[20] Then, the extracellular domain might have an influence on receptor endocytosis in line with the current views on pentameric ligand-gated ion channels, describing a link between extracellular-, transmembrane-, and intracellular-domains.[25-27] This also suggests that allosteric drugs in wide clinical use may have an influence on receptor trafficking.

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References

1. Choquet D, Triller A. The dynamic synapse. Neuron 2013; 80:691-703; PMID:24183020; http://dx.doi.org/10.1016/j.neuron.2013.10.013
2. Luscher B, Fuchs T, Kilpatrick CL. GABAA receptor trafficking-mediated plasticity of inhibitory synapses. Neuron 2011; 70:385-409; PMID:21555068; http://dx.doi.org/10.1016/j.neuron.2011.03.024
3. Smith KR, Kittler JT. The cell biology of synaptic inhibition in health and disease. Curr Opin Neurobiol 2010; 20:550-6; PMID:20650630; http://dx.doi.org/10.1016/j.conb.2010.06.001
4. Kittler JT, Delmas P, Jovanovic JN, Brown DA, Smart TG, Moss SJ. Constitutive endocytosis of GABAA receptors by an association with the adaptin AP2 complex modulates inhibitory synaptic currents in hippocampal neurons. J Neurosci 2000; 20:7972-7; PMID:11050117
5. Bannai H, Lévi S, Schweizer C, Inoue T, Launey T, Racine V, Sibarita JB, Mikoshiba K, Triller A. Activity-dependent tuning of inhibitory neurotransmission based on GABAAR diffusion dynamics. Neuron 2009; 62:670-82; PMID:19524526; http://dx.doi.org/10.1016/j.neuron.2009.04.023
6. Jacob TC, Moss SJ, Jurd R. GABA(A) receptor trafficking and its role in the dynamic modulation of neuronal inhibition. Nat Rev Neurosci 2008; 9:331-43; PMID:18382465; http://dx.doi.org/10.1038/nrn2370
7. Kittler JT, Thomas P, Tretter V, Bogdanov YD, Haucke V, Smart TG, Moss SJ. Huntingtin-associated protein 1 regulates inhibitory synaptic transmission by modulating gamma-aminobutyric acid type A receptor membrane trafficking. Proc Natl Acad Sci U S A 2004; 101:12736-41; PMID:15310851; http://dx.doi.org/10.1073/pnas.0401860101
8. Leidenheimer NJ. Regulation of excitation by GABA(A) receptor internalization. Results Probl Cell Differ 2008; 44:1-28; PMID:17549438; http://dx.doi.org/10.1007/400_2007_039
9. Smith KR, Muir J, Rao Y, Browarski M, Gruenig MC, Sheehan DF, Haucke V, Kittler JT. Stabilization of GABA(A) receptors at endocytic zones is mediated by an AP2 binding motif within the GABA(A) receptor β3 subunit. J Neurosci 2012; 32:2485-98; PMID:22396422; http://dx.doi.org/10.1523/JNEUROSCI.1622-11.2011
10. Roche KW, Standley S, McCallum J, Dune Ly C, Ehlers MD, Wenthold RJ. Molecular determinants of NMDA receptor internalization. Nat Neurosci 2001; 4:794-802; PMID:11477425; http://dx.doi.org/10.1038/90498
11. Bobanovic LK, Royle SJ, Murrell-Lagnado RD. P2X receptor trafficking in neurons is subunit specific. J Neurosci 2002; 22:4814-24; PMID:12077178
12. Frugier G, Coussen F, Giraud MF, Odessa MF, Emerit MB, Boué-Grabot E, Garret M. A gamma 2(R43Q) mutation, linked to epilepsy in humans, alters GABAA receptor assembly and modifies subunit composition on the cell surface. J Biol Chem 2007; 282:3819-28; PMID:17148443; http://dx.doi.org/10.1074/jbc.M608910200
13. Tan HO, Reid CA, Single FN, Davies PJ, Chiu C, Murphy S, Clarke AL, Dibbens L, Krestel H, Mulley JC, et al. Reduced cortical inhibition in a mouse model of familial childhood absence epilepsy. Proc Natl Acad Sci U S A 2007; 104:17536-41; PMID:17947380; http://dx.doi.org/10.1073/pnas.0708440104
14. Wallace RH, Marini C, Petrou S, Harkin LA, Bowser DN, Panchal RG, Williams DA, Sutherland GR, Mulley JC, Scheffer IE, et al. Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. Nat Genet 2001; 28:49-52; PMID:11326275; http://dx.doi.org/10.1038/ng0501-49
15. Noebels JL. The biology of epilepsy genes. Annu Rev Neurosci 2003; 26:599-625; PMID:14527270; http://dx.doi.org/10.1146/annurev.neuro.26.010302.081210
16. Chaumont S, André C, Perrais D, Boué-Grabot E, Taly A, Garret M. Agonist-dependent endocytosis of γ-aminobutyric acid type A (GABAA) receptors revealed by a γ2(R43Q) epilepsy mutation. J Biol Chem 2013; 288:28254-65; PMID:23935098; http://dx.doi.org/10.1074/jbc.M113.470807
17. Wang H, Bedford FK, Brandon NJ, Moss SJ, Olsen RW. GABA(A)-receptor-associated protein links GABA(A) receptors and the cytoskeleton. Nature 1999; 397:69-72; PMID:9892355; http://dx.doi.org/10.1038/16264
18. Changeux JP, Taly A. Nicotinic receptors, allosteric proteins and medicine. Trends Mol Med 2008; 14:93-102; PMID:18262468; http://dx.doi.org/10.1016/j.molmed.2008.01.001
19. Sine SM, Engel AG. Recent advances in Cys-loop receptor structure and function. Nature 2006; 440:448-55; PMID:16554804; http://dx.doi.org/10.1038/nature04708
20. Goldschen-Ohm MP, et al. An epilepsy-related region in the GABAA receptor mediates long-distance effects on GABA and benzodiazepine binding sites. Mol Pharmacol 2009; PMID:19846749
21. Sancar F, Czajkowski C. A GABAA receptor mutation linked to human epilepsy (gamma2R43Q) impairs cell surface expression of alphabetagamma receptors. J Biol Chem 2004; 279:47034-9; PMID:15342642; http://dx.doi.org/10.1074/jbc.M403388200
22. Hibbs RE, Gouaux E. Principles of activation and permeation in an anion-selective Cys-loop receptor. Nature 2011; 474:54-60; PMID:21572436; http://dx.doi.org/10.1038/nature10139
23. Taly A, Corringer PJ, Grutter T, Prado de Carvalho L, Karplus M, Changeux JP. Implications of the quaternary twist allosteric model for the physiology and pathology of nicotinic acetylcholine receptors. Proc Natl Acad Sci U S A 2006; 103:16965-70; PMID:17077146; http://dx.doi.org/10.1073/pnas.0607477103
24. Sauguet L, et al. Crystal structures of a pentameric ligand-gated ion channel provide a mechanism for activation. Proc Natl Acad Sci USA 2013; PMID:24367074
25. Miller PS, Smart TG. Binding, activation and modulation of Cys-loop receptors. Trends Pharmacol Sci 2010; 31:161-74; PMID:20096941; http://dx.doi.org/10.1016/j.tips.2009.12.005
26. Peters JA, Cooper MA, Carland JE, Livesey MR, Hales TG, Lambert JJ. Novel structural determinants of single channel conductance and ion selectivity in 5-hydroxytryptamine type 3 and nicotinic acetylcholine receptors. J Physiol 2010; 588:587-96; PMID:19933751; http://dx.doi.org/10.1113/jphysiol.2009.183137
27. Tierney ML. Insights into the biophysical properties of GABA(A) ion channels: modulation of ion permeation by drugs and protein interactions. Biochim Biophys Acta 2011; 1808:667-73; PMID:21126507; http://dx.doi.org/10.1016/j.bbamem.2010.11.022

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.