Ligand-gated Channels: Glutamate Receptors

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A Mutant δ2 Ionotropic Glutamate Receptor Exhibits Dual Regulation by Phosphoinositides

Vasileios I. Petrou[1,2], Diomedes E. Logothetis[2].
[1]Graduate Program in Neurosciences, Fishberg Department of Neuroscience, Mount Sinai School of Medicine, New York, NY, USA, [2]Department of Physiology and Biophysics, Virginia Commonwealth University School of Medicine, Richmond, VA, USA.

The δ2 glutamate receptor (GluRδ2) is considered a member of the ionotropic glutamate receptor family, although a specific ligand that activates the wild-type receptor has yet to be identified. GluRδ2 is enriched in the parallel fiber-Purkinje cell (PF-PC) synapse, but the precise physiological role of the receptor is still unclear. A naturally-occurring single point mutant in the third transmembrane domain of the receptor (A654T), named Lurcher ($GluR\delta2^{Lc}$), exhibits constitutive activity.

Our previous preliminary results suggested that the δ2 glutamate receptor - Lurcher mutant is inhibited by direct interactions with phosphatidylinositol 4,5-bisphosphate (PIP_2). Here we show that pre-incubation with wortmannin affects the activity of the receptor in a concentration-dependent manner, leading to an inhibition of the channel in the low micromolar range. This suggests that phosphatidylinositol 3-kinase (PI3K) could also be involved in the regulation of the δ2 glutamate receptor. We further investigate the involvement of phosphoinositides in the regulation of δ2 glutamate receptor using more specific PI3K and PI4K inhibitors as well as soluble stereoisomers of different phosphoinositides.

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Engineering light-gated glutamate receptors

Stephanie Szobota[1], Rika Numano[1], Claire Wyart[1], Pau Gorostiza[1], Matthew Volgraf[1], Doris L. Fortin[1], Filippo Del Bene[2], Ethan K. Scott[2], Richard H. Kramer[1], Dirk Trauner[1], Ehud Y. Isacoff[1].
[1]University of CA, Berkeley, Berkeley, CA, USA, [2]University of CA, San Francisco, San Francisco, CA, USA.

Ionotropic glutamate receptors are the major neurotransmitter receptor found at excitatory synapses in the central nervous system. We have developed a light-gated ionotropic glutamate receptor (LiGluR) that, when introduced into neurons, enables remote control of their activity. Light regulation is conferred by a nanoscale photoswitch consisting of a glutamate analog that is covalently tethered to the glutamate receptor through a photoisomerizable azobenzene moiety. The photo-switch requires a single amino-acid substitution in the glutamate receptor as a point for covalent attachment; thus LiGluR can be genetically targeted to neurons of interest.

We show that optical stimulation can be structured in designed spatial and temporal patterns, with action potentials generated by 1-5 millisecond long pulses of light. By changing the location of the amino-acid substitution, modifying the properties of the photo-switch, or applying this strategy to other glutamate receptors, we show that this approach represents a toolbox of options for precisely manipulating glutamate receptors and neural activity with light.

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Design Of A Potassium Selective, Light-gated Glutamate Receptor

Harald Janovjak, Ehud Isacoff.
UC Berkeley, Berkeley, CA, USA.

A major goal in molecular neuroscience is to understand structure-function relationships of glutamate receptor ion channels (iGluRs) and their role in behavior. In the mammalian central nervous system, iGluRs form non-selective cation channels that generate depolarizing potentials in fast excitatory synaptic processes. Having earlier introduced the light-activated glutamate receptor LiGuR, we went one step further and applied two types of structure-based design to develop a potassium selective, light-gated ion channel. Simple and fast Monte-Carlo simulations allow the rational design of optically-controlled proteins in general and LiGluR variants in particular. Using homology modeling we modified the transmembrane pore-region of LiGluR to create a potassium selective channel that responds to glutamate and light. We demonstrate that rapid pulses of light can hyperpolarize HEK-293 cells and neurons expressing this novel channel, which can be used as a tool in glutamate receptor screening applications and may enable optical neuronal inhibition.

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NMDA Receptor Subunit Arrangement Probed By LRET

Anu Rambhadran, Vasanthi Jayaraman.
Univeristy of Texas Health Science Center at Houston, Houston, TX, USA.

NMDA receptors are unique among the different subtypes of the ionotropic glutamate receptors since they require two agonists, glycine and glutamate, to bind to the receptor for the channel to open. They are heteromeric receptors composed of glycine binding subunits, such as NR1 and glutamate binding subunits, such as NR2, with the NR1 and NR2 subunits forming a dimer of dimers. While the crystal structures of the isolated ligand binding domain suggest that the dimer consists of one NR1 and one NR2 subunit, the arrangement of the dimer of dimers is not known, i.e. are the NR1 subunits adjacent, or across in the dimer of dimers. Using a modified NMDA receptor with specific donor:-acceptor fluorophores at the N-terminus, and at various sites on the domain 1 of the ligand binding domain we have measured the distances between the NR1 subunits, between the NR2 subunits, and between NR1 and NR2 subunits, using luminescence resonance energy transfer. Using these distance constraints we show that the NR1-NR1 subunits and the NR2-NR2 subunits are adjacent to each other in forming the dimer of dimers.

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Activation Mechanism of Native NMDA Receptors in Cultured Rat Neurons in Culture

Jason Myers, William Borschel, Eileen Kasperek, Gabriela Popescu.
University at Buffalo, Buffalo, NY, USA.

The activation mechanisms of recombinant NMDA receptor isoforms have been established in sufficient detail to account for their responses at both single-channel and macroscopic levels. Still, the reaction mechanism of native receptors remains uncertain due to indetermination of isoforms expressed, regulatory mechanisms and difficulties in regulating channel number. To investigate the activation mechanism of native receptors we recorded single-channel currents from cultured neurons dissociated from rat cerebral cortex and hippocampus. In several cell-attached patches, the currents originated from only one channel which remained active for tens of minutes. Activity recorded from neurons maintained for less than 19 days in vitro (DIV) resembled those of recombinant NR1/NR2B isoforms (means, s.d.): Po = 0.12 ± 0.06, MOT = 3.2 ± 0.5 ms (n = 8; 783,147 events). In contrast, in all one-channel records obtained from 21 - 35 DIV neurons we observed kinetics similar to those of recombinant NR1/NR2A receptors (means, s.d.): Po = 0.33 ± 0.10 and MOT = 5.6 ± 0.9 ms, (n = 7; 890,060 events). Importantly, for both types of activity we routinely identified clusters of activity characterized by three distinct open durations consistent with modal behavior. These data support previous reports indicating that dissociated neurons in culture recapitulate the developmental pattern of isoform observed in the intact animal and show for the first time that, like recombinant receptors in heterologous cells, receptors native to rat cortical neurons can adopt modal kinetics.

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Kinetic Effects of Perturbations in the Ligand Binding Domain of NMDA Receptors

Cassandra Kussius, Jason Myers, Kevin Barnum, Gabriela Popescu.
University at Buffalo, Buffalo, NY, USA.

NMDA receptors become active only after both glycine and glutamate bind to their cognate ligand binding domain (LBD) on NR1 and NR2 subunits, respectively. It has been proposed that the conformational changes that lead to channel opening are initiated by agonist-induced closure of the LBDs and that the stability of the closed structure correlates with the agonist's efficacy. To probe this hypothesis we characterized kinetic changes in receptor gating arising from complementary perturbations at the NR2-LBD: a) NR2-specific partial agonists, L-homocysteate or SYM2081, each reported to have ~80% efficacy; and b) NR2-LBD mutations (K487C, N687C) which lock the LBD in a closed-cleft conformation. We recorded steady-state single-channel currents from cell-attached patches containing only one receptor at saturating agonist concentrations. Kinetic analyses of these data indicated that neither perturbation affected the core gating mechanism of the channel which consisted of five closed and two open states, including desensitization. Open durations were only minimally affected with most of the kinetic effects observed resulting from changes in the duration of closures. Two closed time components were significantly changed compared to Control (tau_2, 1.7 ± 0.1 ms, tau_3 = 4.6 ± 0.2 ms, n = 5). These were increased by partial agonists to 3.5 ± 0.3 ms and 9.1 ± 0.8 ms, respectively (n = 10, $p<0.05$) and decreased for the locked-LBD mutant to 0.9 ± 0.1 ms and to 3.2 ± 0.2 ms (n = 4, $p<0.05$). These results are consistent with the hypothesis that the stability of the closed NR2-LBD conformation correlates with channel gating efficacy. Further, the data show that distinct local perturbations at the NR2-LBD affect gating through the same mechanism observed as changes in the duration of two specific closed time components.

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Diversity of NR1/NR2B Receptor Gating Kinetics

Stacy Amico[1], Navjot Kaur[2], Gabriela Popescu[1].
[1]University at Buffalo, Buffalo, NY, USA, [2]Invitrogen, Frederick, MD, USA.

NMDA receptors are heteromeric glutamate-activated ion channels composed of NR1- and NR2-subunits. Controlled expression of four NR2-isoforms