studies of the peptide in a solution of small unilamellar vesicles were conducted and showed that the increase in helical content is also present in the context of close proximity to a lipid membrane. To confirm, single molecule fluorescence resonance energy transfer (smFRET) was used to examine the peptide in both the unphosphorylated state and in the PKCα-phosphorylated state, in order to gauge the distance between two native cysteines in the peptide. Phosphorylation yielded a reduced distance between these cysteines, indicative of a shift to more compressed secondary structure, that is, coil to helix.

1438-Pos Board B389

Characterization of a "Hotspot" in the AMPA Receptor Activation Pathway

George B. Dawe[1], Maria Musgaard[2], Mark R. Aurousseau[1], Philip C. Biggin[2], Derek Bowie[1].
[1]Pharmacology & Therapeutics, McGill University, Montreal, QC, Canada, [2]Biochemistry, University of Oxford, Oxford, United Kingdom.

Ionotropic glutamate receptors (iGluRs) facilitate the bulk of synaptic excitation in the mammalian central nervous system. Structures of full-length, AMPA-type iGluRs (AMPARs) have recently been reported in conformations thought to represent resting, pre-open, and desensitized states. However, it is uncertain what molecular interactions determine whether an agonist-bound AMPAR will favor channel opening or desensitization. We previously described how the activation of kainate-type iGluRs (KARs) is dependent upon occupancy, by sodium, of an electronegative pocket in the ligand-binding domain (LBD). Subsequently, we asked to what extent this pocket, conserved amongst iGluR subfamilies, regulates AMPAR activation. To investigate this subject we utilized electrophysiological (outside-out patch) recordings from iGluR subunits transiently expressed in HEK 293 cells, as well as molecular dynamics (MD) simulations. Unlike the KAR subunit GluK2, receptors comprised of the AMPAR subunit GluA2 did not require occupancy of the pocket by a positive charge to activate. Interestingly, a lithium ion has been detected in the pocket of recent crystal structures of the GluA2 LBD. The effect of lithium in the external recording solution was to dramatically slow the desensitization kinetics of GluA2. MD simulations supported an increased affinity of the site for lithium versus sodium, and predicted that lithium binding holds subunits closer together. Through disrupting an inter-subunit electrostatic bridge adjacent to the "cation" pocket, the effect of lithium was greatly attenuated. In fact, the removal of key charges at this interface produced receptors barely capable of activation, although the functional deficit was rescued by the modulator cyclothiazide or co-expression with auxiliary subunits. We propose that when electrostatic interactions at the apex of the LBD are stabilized, AMPARs are primed for activation, whereas the disruption of these interactions directs receptors to desensitized states upon agonist binding.

1439-Pos Board B390

Dynamics of the Cytoplasmic Region of an AMPA-Subtype Glutamate Receptor Revealed by State Dependent FRET

Ljudmila Katchan[1], Linda G. Zachariassen[2], Anders S. Kristensen[2], Andrew J.R. Plested[1].
[1]FMP Berlin, Berlin-Buch, Germany, [2]University of Copenhagen, Copenhagen, Denmark.

AMPA receptors (AMPARs) are glutamate-gated ion channels, which mediate fast excitatory neurotransmission in the central nervous system. Extensive crystallographic studies of the extracellular domains and, more recently, crystal structures and single particle EM reconstructions of full-length receptors have set the framework for further investigations of receptor conformational dynamics, gating mechanism and regulation. However, intracellular regions are either truncated or not resolved in these structures. Further, the conformational transitions of the intracellular domains during receptor gating have not been investigated.

In present study, we have explored single and double fusions of cyan and yellow variants of green fluorescent protein (CFP and YFP, respectively) at intracellular sites of AMPAR to enable measurement of conformational changes using Fluorescence Resonance Energy Transfer (FRET) in live cells. The fluorescent fusions retain wild-type receptor expression and kinetic properties. Fluorescence Lifetime Imaging (FLIM) showed ligand-dependent FRET efficiency. Conformational rearrangements accompanying receptor function were measured using a Patch Clamp Fluorometry (PCF) setup on live HEK 293 cells in real time. Our results suggest that FRET efficiency is dependent on the functional state of the receptor and allosteric modulation by Cyclothiazide, an AMPA receptor desensitisation blocker. Thus the intracellular sites undergo conformational rearrangements during receptor function.

1440-Pos Board B391

Partial Agonist Binding Reveals a Unique Arrangement of AMPA LBDs

Hector P. Salazar Garcia, Clarissa Ebli, Miriam Chebli, Andrew Plested.
Leibniz-Institut für Molekulare Pharmakologie & Neurocure Initiative, Charité Universitätsmedezin, Berlin, Germany.

Ionotropic glutamate receptors (iGluRs) are large tetrameric membrane proteins that transduce the chemical signal from neurotransmitters into membrane depolarization at synapses in the brain. The conformational transition induced by the association of glutamate molecules to the ligand-binding domains (LBDs) of these receptors provides the free energy that drives the opening of the transmembrane ion channel. Here, we describe the crystal structure of a GluA2 LBD tetramer in presence of the partial agonist 5-fluorowillardiine (FW) (FW sLBDs). Validation of the structure by a battery of engineered metal bridges showed that this LBD configuration corresponds to an intermediate state of receptor activation distinct from the previously publish closed-angle (CA) structure. GluA2 activation therefore, involves a combination of both intra- and inter-LBD dimer conformational transitions. The presented results provide new quantitative data supporting the idea of a dynamic LBD during activation in the context of a tetramer.

1441-Pos Board B392

Structural Mechanism of Glutamate Receptor Activation and Desensitization

Joel R. Meyerson[1], Janesh Kumar[2], Sagar Chittori[3], Prashant Rao[1], Jason Pierson[4], Alberto Bartesaghi[1], Mark L. Mayer[5], Sriram Subramaniam[1].
[1]National Cancer Institute, National Institutes of Health, Bethesda, MD, USA, [2]National Centre for Cell Science, Wellcome Trust /DBT India Alliance Fellow, Ganeshkhind, India, [3]National Institute of Child Health and Human Development,, National Institutes of Health, Bethesda, MD, USA, [4]FEI Company, Hillsboro, OR, USA, [5]National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA.

Ionotropic glutamate receptors are ligand-gated ion channels that mediate excitatory synaptic transmission in the vertebrate brain. To gain a better understanding of how structural changes gate ion flux across the membrane, we trapped rat AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) and kainate receptor subtypes in their major functional states and analysed the resulting structures using cryo-electron microscopy. We show that transition to the active state involves a 'corkscrew' motion of the receptor assembly, driven by closure of the ligand-binding domain. Desensitization is accompanied by disruption of the amino-terminal domain tetramer in AMPA, but not kainate, receptors with a two-fold to four-fold symmetry transition in the ligand-binding domains in both subtypes. The 7.6 Å structure of a desensitized kainate receptor shows how these changes accommodate channel closing. These findings integrate previous physiological, biochemical and structural analyses of glutamate receptors and provide a molecular explanation for key steps in receptor gating.

1442-Pos Board B393

Long Timescale Simulations of Ligand Binding in Glutamate Receptors

Alvin Yu, Albert Lau.
Department of Biophysics and Biophysical Chemistry, Johns Hopkins University, Baltimore, MD, USA.

Ionotropic glutamate receptors (iGluRs) are ligand gated ion channels that mediate the majority of fast excitatory transmissions in the central nervous system. They transduce chemical information upon agonist binding into electrical information at synapses. In this study, we present long timescale simulations of both ligand binding association and dissociation events. Novel intermediate states and metastable interactions are identified using potential of mean force (PMF) calculations. Kinetics are inferred using a Markov state model (MSM).

1443-Pos Board B394

Can Activation and Desensitization Properties of iGluRs Be Predicted and Understood by Studying the LBD Dimer Dynamics?

Maria Musgaard[1], Bryan Daniels[2], George B. Dawe[2], Mark Aurousseau[2], Derek Bowie[2], Philip C. Biggin[1].
[1]Department of Biochemistry, University of Oxford, Oxford, United Kingdom, [2]Department of Pharmacology and Therapeutics, McGill University, Montreal, QC, Canada.

Ionotropic glutamate receptors (iGluRs) are vital for the function of our central nervous system (CNS), e.g. in learning and memory formation, and thus implicated in many CNS disorders. The tetrameric iGluRs contain a glutamate-gated cation channel with the extracellular ligand binding domains (LBD) forming a dimer of dimers. Subsequent to channel opening,

iGluRs undergo conformational changes to a desensitized state with channel closure while glutamate remains bound. The LBD dimer interface plays important roles in activation and desensitization. The first step in desensitization is believed to involve opening and rearrangement of this dimer interface. The three major iGluR subtypes, AMPA, kainate and NMDA receptors, are similar in structure and sequence, yet show large diversity in activation and desensitization kinetics. AMPA receptors activate and desensitize rapidly whereas NMDA receptors have much slower kinetics. Sequence variation at the LBD dimer interface may play key roles in determining these properties of specific receptors. E.g., as opposed to other iGluRs, activation of GluK2 homotetrameric receptors requires ion binding to the LBD dimer interface. Using computational methods, equilibrium and biased molecular dynamics simulations at atomic resolution, we have characterized the influence of subtype differences and interface mutations on the dynamics of LBD dimers of GluA2 AMPA receptors, GluK2 kainate receptors and GluN1/GluN2A NMDA receptors. Surprising differences are revealed, e.g. that K759 of GluA2, which structurally appears to function like sodium in GluK2 by positioning the positive charge in the ''cation site'' of GluA2, dynamically has a destabilizing effect. Furthermore, interface stability has been investigated using steered molecular dynamics simulations. For the majority of cases, the amount of work required to open the dimer interface predicts whether a mutation has a destabilizing effect, thus producing a less active receptor.

1444-Pos Board B395

Free Energy Landscapes for a Kainate Receptor Ligand-Binding Domain

Tyler J. Wied, Albert Y. Lau.
Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

The kainate family of ionotropic glutamate receptors (GluK) mediate neurotransmission in pre- and post-synaptic neurons. Previously computed free energy landscapes for iGluR ligand-binding domains (LBD) revealed subtype-dependent conformational differences between NMDA and AMPA iGluR families. Here we report free energy landscapes for the GluK2 ligand-binding domain (LBD) in apo and glutamate-bound states. The GluK2 free energy landscapes reveal both apo and glutamate-bound LBDs preferentially access the closed-cleft conformation and suggest a conformational selection mechanism of binding. These results are consistent with the free energy landscapes for NMDA receptors but not AMPA receptors. Additionally, the open-cleft conformation is more accessible to the GluK2 apo state than the glutamate-bound state, consistent with previous results for both NMDA and AMPA receptors. Finally, large-scale structural dynamics are characterized by principle component analysis.

1445-Pos Board B396

Functional Coupling between the Finger and Thumb Domains of ASIC1A

Aram J. Krauson, Marcelo D. Carattino.
Renal-Electrolyte, University of Pittsburgh, Pittsburgh, PA, USA.

Acid sensing ion channels (ASICs) are neuronal cation selective channels that respond to sudden drops in extracellular pH and desensitize in the continuous presence of protons. The mechanism that allows these channels to sense and respond to changes in extracellular pH is not well understood. Here we examined the contribution to channel activation of the finger and thumb domains of ASIC1a, two areas that reside in close proximity in the periphery of the extracellular region. Residues located at the interface of these two domains were individually mutated to Cys and the reactivity of the mutant channels toward the thiol reactive reagent MTSET was assessed using the two-electrode voltage clamp technique. We identified seven sites in the thumb domain, positions 325, 327, 344, 345, 348, 351 and 352, and two in the finger domain, positions 152 and 154, where MTSET treatment reduced the magnitude of the response to extracellular acidification. Residues 325, 327, 344, 345, 348 in the thumb domain are oriented toward residues 152 and 154 in the finger domain in the solved atomic structure of ASIC1 at low pH. Our results indicate that the finger and thumb domains experience a conformational change and become closer in response to extracellular acidification. To further assess the role of the finger-thumb interactions in ASIC1a proton activation, we generated channels with substitutions at neighboring positions in the finger and thumb domains. We found that the response to extracellular acidification after MTSET treatment of double mutant channels bearing Cys substitutions at positions 154 and 325 was significantly lower than the response of channels bearing individual mutations at these positions, consistent with functional coupling between the finger and thumb domain. Taken together, our results suggest that the finger and thumb domains contribute to ASIC1a activation.

1446-Pos Board B397

Gating Mechanism and Movements in Acid Sensing Ion Channel 1A

Swarna S. Ramaswamy[1], David MacLean[1], Hugo Sanabria[2], Vasanthi Jayaraman[1].
[1]Biochemistry and Molecular Biology, University of Texas Health Science Center, Houston, TX, USA, [2]Physics and Astronomy, Clemson University, Clemson, SC, USA.

Acid Sensing Ion Channels (ASICs) are proton sensitive ion channels found in central and peripheral nervous system. They are involved in a variety of physiological processes including sensory perception, synaptic transmission and nociception. It is also involved in pathological processes like stroke and hypoxia leading to cell death. Thus understanding the mechanism of working of these ion channels are of importance in order to be able to design modulators. Here we present results from a combination of biophysical, molecular, functional and computational studies that looks at various aspects of ASIC functions. Earlier studies pointed towards possible involvement of carboxylate residues in the extracellular domain, in pH sensing. Our results indicate that there are three carboxylate pairs that are involved in gating. The carboxylates upon proton binding, induce a conformational change that causes the finger and thumb domains of ASIC come closer to each other. Our results indicate that these carboxylate pairs and the conformational change are key factors that mediate gating of the ion channel. We then proceeded to study the inter subunit conformational changes that might be involved in desensitization process. Biophysical investigations indicate that the subunits move away from each other upon desensitization. This movement is not global between the subunits. The segments closer to the central vestibule stay relatively in the same position whereas the residues towards the periphery of the ion channel moves away from each other more prominently. These results show that the ion channel undergoes a lateral flowering-like motion during desensitization in comparison with the resting state of the ion channel.

1447-Pos Board B398

Controlled Activation of Heteromeric P2X Receptors by ATP and Magnesium

Emily Harnish.
Membrane Physiology and Biophysics Section, National Institute of Neurological Disorder and Stroke, Bethesda, MD, USA.

P2X receptor channels are a family of trimeric cation channels that are activated by extracelluar ATP. Seven subtypes of P2X receptors have been identified in mammals, and they are widely distributed throughout the body, serving important roles in sensory signaling and inflammation. In physiological solutions, ATP is ionized and primarily found in complex with Mg^{2+}. We recently found (Li, Silberberg and Swartz ,2013 PNAS 110, E3455-63) that the slowly desensitizing $P2X_2$ and $P2X_4$ receptors can be activated by free ATP, but $MgATP^{2-}$ promotes opening with very low efficacy; Mg^{2+} thus acts as a competitive antagonist. In contrast, both free ATP and $MgATP^{2-}$ robustly open the rapidly desensitizing $P2X_3$ subtype, although $P2X_3$ receptor channel currents are inhibited by Mg^{2+} through an allosteric mechanism. Interestingly, both inhibitory effects of Mg^{2+} are disengaged in heteromeric $P2X_{2/3}$ channels. In the present study we investigated the properties that $P2X_6$ when forming heteromeric channels with $P2X_2$ and $P2X_4$ subunits. The $P2X_6$ subunit is abundantly expressed in the central nervous system along with $P2X_2$ and $P2X_4$, yet does not form functional homomeric channels on its own. When co-expressed in HEK cells, we find that heteromeric $P2X_{2/6}$ and $P2X_{4/6}$ channels are functional, and unlike homomeric $P2X_2$ and $P2X_4$ receptors, can be efficiently activated by $MgATP^{2-}$. Our results also suggest that Mg^{2+} allosterically regulates $P2X_{2/6}$ heteromeric channels and we are currently investigating the underlying mechanism and site of activation. Taken together, our results support the general idea that heteromulterimerization of P2X receptor channels influences the forms of ATP that can activate these channels and the regulatory influences of Mg^{2+}.

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Quantitative Measure of Ca^{2+} Current and Permeability in ATP-Gated P2X7 Receptors

Xin Liang[1], Damien S.K. Samways[2], Terrance M. Egan[1].
[1]Pharmacological and Physiological Science, Saint Louis University, St. Louis, MO, USA, [2]Department of BIology, Clarkson University, Potsdam, NY, USA.

ATP-gated P2X7 receptors are prominently expressed in inflammatory cells and play a key role in the immune response. A major consequence of receptor activation is the regulated influx of Ca^{2+}. Although the physiological