have been identified as the targets of these drugs, but it is unknown how anesthetics exert their effects. Previous simulations from our group have uncovered a membrane-dependent entrance mechanism, whereby the anesthetic partitions to the membrane prior to binding a transmembrane site identified in crystal structures. To probe for additional binding sites unbiasedly and corroborate the membrane-dependent entrance tunnel previously discovered, we have used the "flooding" technique where the system is flooded with multiple copies of the drug to increase the probability of observing binding events. Our simulations elucidated not only the binding region indicated in crystal structures, but also a novel second transmembrane binding site (TM2) observed consistently across all subunits. The TM2 site is directly between the crystal structure site (TM1) and the entrance tunnel elucidated in our simulations. Free energy calculations show binding to the TM2 site increases the affinity of anesthetic for the TM1 site in addition to acting as a physical barrier blocking the exit of anesthetic from the protein. Moreover, anesthetic binding to the TM1/TM2 sites induces an iris-like tilting of the M2 helix, as observed in our previous simulations, leading to constriction and dehydration of the conduction pore. Utilizing umbrella sampling, this conformational change significantly increases the barrier to conduction, prohibiting ion conduction in the dehydrated state. Lastly, measurements of non-equilibrium work show that desflurane only minimally perturbs the work required to induce constriction of the pore, while greatly increasing the work needed for the dehydrated to open state transition. This suggests that anesthetics stabilize the dehydrated state, but do not destabilize the open state.

2762-Pos Board B454

Loose Packing of the Extracellular Domain Core of Pentameric Ligand-Gated Ion Channels is Important for Fast Activation and Desensitization

Cosma D. Dellisanti, Cynthia Czajkowski.
University of Wisconsin - Madison, Madison, WI, USA.

Pentameric ligand-gated ion channels (pLGICs) mediate fast synaptic transmission. pLGIC activation is initiated by agonist binding to the orthosteric site in the extracellular domain (ECD), which promotes rapid opening of a membrane-embedded ion-conducting pore. Prolonged agonist exposure leads to pLGIC desensitization, which results in channel closure. Eukaryotic pLGICs open in the sub-millisecond to millisecond timescale, and transition into desensitized closed states in tens of milliseconds. To date, we have limited knowledge of the structural elements that underlie the rapid kinetics of activation and desensitization in pLGICs. We previously showed that eukaryotic pLGICs are characterized by loose packing of the ECD hydrophobic core, due to cavities created at two conserved sites by buried side-chains of either polar residues (threonine and serine in nicotinic receptors, nAChRs) or alanines (in GABA-A receptors), and that increasing the packing of the ECD core at these two sites slowed agonist-induced current activation and desensitization rates (Dellisanti et al., Nat Neurosci 2007; Dellisanti and Czajkowski, J Biol Chem 2011). Interestingly, prokaryotic pLGICs, like proton-activated GLIC, activate and desensitize slowly and have a tightly packed ECD core. Here, we generated loose packing mutants of GLIC by mutating V38 and V109 (aligned to nAChR-α1 T52 and S126) to either alanines or threonine and serine, respectively. We expressed the GLIC mutants in Xenopus oocytes and monitored the effects of these mutations on proton-elicited currents using two-electrode voltage clamp. The loose packing GLIC mutants had 2- to 3-fold faster current activation and desensitization rates compared to wild-type GLIC. The effects were larger when both valines (V38AV109A and V38TV109S mutants) were mutated. Our data suggest loose packing of the ECD core is directly implicated in fast activation and desensitization of eukaryotic pLGICs.

2763-Pos Board B455

Construction and Characterization of a Functional GLIC-GABAρ Ligand-Gated Ion Channel Chimera

Borna Ghosh, Varun Tiwari, Cynthia Czajkowski.
Neuroscience, University of Wisconsin, Madison, Madison, WI, USA.

Pentameric ligand gated ion channels (pLGICs), especially anion-selective GABAARs, are major targets of general anesthetic (GA) drugs. GAs mediate their actions by binding in the transmembrane domain (TMD) of pLGICs, which potentiates anion-selective pLGIC currents and inhibits cation-selective pLGICs. Recently, crystal structures of prokaryotic cation-selective pLGIC homologs, GLIC and ELIC, have been solved with several GAs bound. High-resolution structures that reveal how GA's interact with anion-selective channels such as the GABAAR are not available, which justifies a quest for pLGIC constructs that preserve GA positive modulation and are amenable for structural studies.

Here, we report the construction and characterization of a GLIC-GABAρ chimeric pLGIC that is positively modulated by the GA pentobarbital. We fused the extracellular domain (ECD) of the prokaryotic proton-gated homopentameric channel GLIC with the eukaryotic GABAρ subunit TMD. GABAAR ρ1 subunits form homopentameric channels making them ideal for constructing chimeras with GLIC. The GLIC-ρ chimeric construct formed functional proton-gated channels when expressed in Xenopus oocytes. The reversal potential of the chimeric channels was −31mV, which is similar to the reversal potential of GABAρ receptors suggesting that the channel is chloride-selective. GABAρ receptors are normally insensitive or inhibited by GAs but a point mutation in GABAρ TMD W328M converts it to a channel that is positively modulated by pentobarbital. When this mutation was made in the GLIC-GABAρ chimeric construct, the mutant chimeric channel was directly gated by pentobarbital and proton-mediated currents were potentiated by pentobarbital. Protocols for expressing and purifying high levels of the GLIC-GABAρ chimeric channel protein are currently being developed.

2764-Pos Board B456

Allosteric Modulation of GABA-A Receptors by Different Drugs

Borna Ghosh, Tzu-Wei Tsao, Cynthia Czajkowski.
Neuroscience, University of Wisconsin, Madison, Madison, WI, USA.

Understanding how drugs allosterically modulate GABA-A receptor ($GABA_AR$) channel function remains a challenge. Anesthetic drugs like etomidate, propofol and pentobarbital bind at transmembrane domain (TMD) intersubunit interfaces at different locations. Benzodiazepines bind at the αγ interface in the extracellular domain (ECD) and neurosteroids are believed to bind within the α-subunit TMD helix bundle. A common feature of these drugs is that they potentiate GABA-induced currents. Here, we examined whether structural mechanisms underlying their functional effects are distinct. We monitored motions induced individually by these drugs at each inter-subunit TMD interface and also tested whether modulation caused by pairs of drugs binding to distinct sites are additive or super-additive.

We individually introduced a cysteine in the M1 helices of each subunit at α_1I227, β_2L223 and γ_{2L}I238 to probe the different inter-subunit interfaces. We expressed wild-type and mutant $\alpha_1\beta_2\gamma_{2L}$ $GABA_ARs$ in *Xenopus* oocytes and measured rates of modification of the substituted cysteines in the absence and presence of GABA and different allosteric modulators: the anesthetics pentobarbital (PB) and etomidate, the neurosteroid THDOC and the benzodiazepine flurazepam. GABA activation significantly increased MTSEA modification of αI227C, βL223C and γI238C. PB activation increased modification of αI227C and γI238C but not βL223C. Modulating concentrations of PB or etomidate had no effects. THDOC significantly increased the rate of modification of βL223C whereas flurazepam increased γI238C modification. We found that potentiation of EC_{10} GABA currents by combining modulating concentrations of THDOC and flurazepam was the sum of the potentiation produced by the two drugs individually. Combining PB and THDOC produced potentiation more than the sum of the potentiation produced by the two drugs individually. Our data suggests that drugs binding to different sites act via distinct mechanisms. Further analysis is underway to determine if these mechanisms are independent or coupled.

2765-Pos Board B457

Mechanism of Inhibition of Recombinant $GABA_A$ Receptors by Pentylenetetrazole and Its Alleviation by Anticonvulsants

Akimasa Iijima, **Yongli Chen**.
Hawaii Pacific University, Kaneohe, HI, USA.

Despite the wide prescription of drugs targeting postsynaptic γ-aminobutyric acid receptors ($GABA_ARs$) for seizure and epilepsy, mechanisms by which those drugs exert their effects on $GABA_AR$ are poorly understood. This is mainly attributed to the unavailability of crystal structure of the receptor and the various assembly of 19 different subunits (α1- α6, β1-β3, γ1-γ3, δ, ε, π, θ, ρ1-ρ3). Here, we studied the effects of two anticonvulsants on the recombinant $\alpha1\beta3\gamma2$ $GABA_AR$ using whole-cell current recordings combined with rapid kinetic techniques. Our results showed that pentylenetetrazole (PTZ), a compound used to artificially induce convulsive effect in animal models, is a mixed inhibitor of the receptor. Anticonvulsant ethosuximide (ES) and the major metabolite (α-methyl-α-pheny-succinimide, MPS) of another anticonvulsant methsuximide (MS) were found to alleviate the inhibition of the receptor by PTZ, while MS itself doesn't. Those data suggest that the enhancement of postsynaptic $GABA_AR$ response is one of the mechanisms of the anticonvulsant effect of ES and MS. T-type calcium channel was suggested to be one of the targets of ES.

Acknowledgement of support: This project was supported by grants from the National Center for Research Resources (5P20RR016467-11) and the National Institute of General Medical Sciences (8P20GM103466-11) from the National Institutes of Health. The content is solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.