and the muscle nicotinic acetylcholine receptor (AChR) with pore blockers. From the comparative analysis of these results, it appears as though the muscle AChR and GLIC have very similar open-channel pore structures, whereas the open-channel pore of ELIC seems much narrower, and thus, closer to the structural model of ELIC in its closed state. Hence, it is likely that the closed-to-open conformational change of ELIC at the level of the pore domain is subtler than the one that could be predicted if ELIC were to adopt a GLIC-like structure in the open state.

3270-Pos Board B425

Role of Post-M4 in the Gating of GLIC and ELIC

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The outermost lipid-exposed M4 alpha-helix in the transmembrane domain of each subunit of the nicotinic acetylcholine receptor (nAChR) plays an important role in channel gating and may provide the allosteric link between nAChR function and lipid sensitivity. The C-terminal residues of M4 appear to be particularly important, with deletion mutations abrogating channel activity. Post-M4 may influence channel gating via direct interactions with the Cys-loop of the agonist binding domain, a structure that plays a key role allosterically translating agonist binding into channel gating. In this communication, we explore the role of post-M4 in the function of two prokaryotic homologs, the pH-activated GLIC and the cystamine-activated ELIC. using the two voltage electrode clamp apparatus, we show that single alanine mutations of each of the five C-terminal residues in GLIC has little effect on the pH_{50} for activation, with a slight loss of function only occurring upon mutation of either of the phenylalanine residues F314 and F313 that extend from post-M4 to interact with the remainder of the transmembrane domain. Sequential deletions of post-M4 residues, however, have more dramatic effects. Deletion of the three terminal residues (F316-F314) drops the pH_{50} from 4.74 down to 4.34. Further deletions lead to a complete loss of activity showing that the extended length of post-M4 plays a critical role in GLIC channel activity, as it does with the nAChR. Surprisingly, detrimental effects of post M4 deletions were not observed with ELIC. In fact, deletion of post-M4 residues in ELIC typically led to a slight gain in function, with EC_{50} values for cystamine decreasing from 0.81 mM (wild type) to ~0.50 mM. Our results highlight distinct functional characteristics of two homologous pLGICs.

3271-Pos Board B426

State-Dependent Etomidate Binding in $GABA_A$ Receptors Probed with Cysteine Substitution and Protection from Modification

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Background: A central tenet of ligand-receptor theory is that agonists bind more tightly to active than to inactive receptors. This is a difficult concept to test experimentally, because in most agonist-receptor systems, the act of assessing agonist binding affinity results in receptor activation. In $\alpha1\beta2\gamma2L$ $GABA_A$ receptors, the anesthetic etomidate is an allosteric agonist at high concentrations, and potentiates GABA activation at low concentrations. Sites mediating both actions were photolabeled with azi-etomidate, identifying the β2M286 sidechain as a contact point. We used a cysteine substitution and sulfhydryl modification to study etomidate interactions with this sidechain. **Methods:** using two-microelectrode voltage clamp electrophysiology in *Xenopus* oocytes, we characterized etomidate agonism and potentiation of GABA activation in $\alpha1\beta2M286C\gamma2L$ $GABA_A$ receptors. We studied covalent modification of the β2M286C sidechain by a sulfhydryl-selective reagent, para-chloromercuribenzenesulfonate (pCMBS) with and without GABA. Etomidate-dependent protection of the sulfhydryl was also assessed. **Results:** Oocyte-expressed $\alpha1\beta2M286C\gamma2L$ receptors displayed reduced sensitivity to GABA, and no agonism by etomidate. However, etomidate still enhanced GABA-activated currents from $\alpha1\beta2M286C\gamma2L$ receptors. Exposure of $\alpha1\beta2M286C\gamma2L$ receptors to pCMBS irreversibly increased activation by low (EC10) GABA. The apparent rate of pCMBS modification increased with addition of GABA. Etomidate in a concentration-dependent manner reduced the rate of β2M286C modification by pCMBS. Etomidate protection at β2M286C was enhanced in the presence of GABA. **Conclusions:** Etomidate, like azi-etomidate, binds next to the β2M286 residue. Based on its apparent protectant potency, the affinity of etomidate for GABA-bound receptors is greater than that for resting receptors, as expected. Our results have important implications for both designing and interpreting experiments that use mutations to map allosteric modulator/agonist sites.

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3272-Pos Board B427

Identifying the Propofol Binding Site(S) in Heterologously Expressed Human Alpha1 Beta3 Gaba-A Receptors using a Photoreactive Propofol Analog

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Propofol, a widely used intravenous general anesthetic, potentiates GABA-A receptor ($GABA_AR$) function at anesthetic concentrations, and ''knock-in'' mice expressing a single mutation at position 15 in the β3 subunit M2 helix (βM2-15) have reduced sensitivity to many of the anesthetic effects of propofol. However, the location of propofol's binding site(s) remains to be determined. Here we use a photoactive analog of propofol, [^{3}H]*m*-azipropofol(2-isopropyl-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenol), an aryl diazirine with broad amino acid side chain reactivity, to identify propofol binding site(s) in affinity-purified, expressed human $\alpha1\beta3$ $GABA_ARs$ in detergent/asolectin solution. *m*-Azipropofol acts as a tadpole anesthetic with potency (EC_{50} = 3μM) similar to propofol, and it acts as a low efficacy $GABA_AR$ potentiator (Hall et al. 2010, J Med. Chem. 53, 5667). Irradiation of $GABA_AR$ for 30 min at 365nm resulted in [^{3}H]*m*-azipropofol photoincorporation into both the α1 and β3 subunits that was inhibitable by propofol and non-radioactive *m*-azipropofol with IC_{50}s of 28 ± 3 μM and 6.7 ± 1 μM, respectively. Individual amino acids photolabeled by [^{3}H]*m*-azipropofol were identified by protein microsequencing of subunit fragments generated by enzymatic or chemical digestion. In β3, there was propofol-inhibitable photolabeling of β3Met-286, an amino acid that is photolabeled by [^{3}H]azietomidate in the etomidate binding site at the β3M3-α1M1 interface (Li et al. 2006, J Neurosci. 26, 11599, Chiara et al. 2012, Biochem. 51, 836). In α1, [^{3}H]*m*-azipropofol photolabeled α1Ile-239 within α1M1, located one helical turn below α1Met-236 that is photolabeled by [^{3}H]azietomidate. Consistent with propofol and *m*-azipropofol binding to this etomidate site, they completely inhibit [^{3}H]azietomidate photolabeling with IC_{50}s of 46 ± 3 μM and 23 ± 3 μM, respectively. Both β3Met-286 and α1Met-236 are in close proximity to βM2-15, which docking calculations suggest may also be part of this propofol binding site.

3273-Pos Board B428

Modulation of Delta Subunit-Containing $GABA_A$ Receptors by Etomidate Depends on Subunit Arrangement

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Background: $GABA_A$ receptors are allosterically modulated by a variety of compounds including etomidate and tetrahydrodeoxycorticosterone (THDOC). Extrasynaptic $\alpha\beta\delta$ $GABA_A$ receptors mediate tonic inhibition, and may be more sensitive to modulators than synaptic $\alpha\beta\gamma$ receptors. Studies of concatenated subunit assemblies suggests that the subunit arrangement of $\alpha\beta\delta$ may vary (1). We compared the modulation by etomidate and THDOC on $\alpha1\beta3\delta$ receptors with different subunit arrangements, and to $\alpha1\beta3\gamma2L$ receptors. **Methods:** Plasmids encoding concatenated $GABA_A$ receptor subunit assemblies and free subunits were obtained from Prof. Erwin Sigel (Bern, Switzerland). *Xenopus* oocytes were used to express β3-α1-δ/β3-α1 and β3-α1-δ/α1-β3 receptors as well as free subunit mixtures. Effects of etomidate (3 μM) or THDOC (1 μM) on maximal GABA-activated receptor currents were measured using two-microelectrode voltage-clamp electrophysiology. Etomidate enhancement of β3-α1-δ/β3-α1 was assessed over a broad [GABA] range, and analyzed with an allosteric model. **Results:** High GABA elicited small currents in oocytes expressing β3-α1-δ/β3-α1 and β3-α1-δ/α1-β3 receptors. THDOC produced similar large current enhancements in β3-α1-δ/β3-α1 and β3-α1-δ/α1-β3 receptors. However, etomidate produced a greater current enhancement in β3-α1-δ/α1-β3 receptors than in β3-α1-δ/β3-α1. Etomidate increased the maximal response of β3-α1-δ/β3-α1 receptors to GABA and produced a small leftward shift in GABA EC50. **Discussion:** using concatenated receptor constructs resulted in evidence of δ subunit incorporation that was not seen using free subunit mRNAs. GABA is a weak partial agonist in $\alpha1\beta3\delta$ receptors, while etomidate and THDOC modulate *via* different sites. Etomidate efficacy in $\alpha1\beta3\delta$ is similar to that in $\alpha1\beta3\gamma$ receptors with homologous subunit arrangement, and interactions at β3/β3 interfaces may be more efficacious than those at β3/α1 interfaces.

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Reference:
1) Kaur KH et al, J. Biol Chem 2009;284:7889-96