3663-Pos

Molecular Dynamics Simulations of the GABA Type A Receptor

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The type A GABA-receptors (GABARs) are chloride ion channels found in the brain and are the major inhibitory neurotransmitter receptors. Upon binding of an agonist, the GABAR opens and increases the intraneuronal concentration of chloride ions, thus hyperpolarizing the cell and inhibiting the transmission of the nerve action potential. GABARs can also be modulated by a variety of pharmacologically important drugs, such as diazepam and also other regularly abused drugs such as ethanol. Here, we present homology models (based upon the 4 Å resolution EM acetylcholine receptor structure) and simulations of the most abundant GABAR subtype ($\gamma 2\alpha 1\beta 2$), and also the alcohol-sensitive subtype ($\delta\alpha 6\beta 3$). As well as GABA itself, a variety of ligands were docked to the proteins to assess the potential binding sites, and also the affect of ligand binding on the properties of the structure. Through the modeling and simulation of the two different receptor subtypes, and comparison of the ligand effects upon each one, we are able to gain greater insight into the specific residues important in binding, and also details of the different behavior of the subtypes. This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. Release number LLNL-ABS-417208.

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An Electrophysiology Assay for the Fast Characterization of $GABA_A$ Ion Channel Modulators

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The $GABA_A$ receptors belong to a family of ligand-gated ion channels mediating fast synaptic transmission. They are drawing great attention in the pharmaceutical industry due to their potential role in the development of new therapeutics affecting anxiety, sleep disorders, and muscle relaxation. However, ligand-gated ion channel screening has been hampered by the lack of suitable high throughput electrophysiology platforms. The existing electrophysiology screening platform (IonWorks) is unable to apply ligands rapidly during current recording, which can be a major drawback for inactivating channels. While some studies have shown that it is possible to record the GABA tail current and use that information during a screen, such methods have an inherently lower signal to noise ratio and cannot be used on faster desensitizers.

Here we present the use of a novel electrophysiology screening platform integrating a microfluidics network for the study of $GABA_A$ receptor pharmacology. This platform features fast (<100ms) solution exchange coupled with simultaneous data recording. A novel assay could monitor GABA response in real time, and obtain a 3 point EC_{50} dose curve within 1 minute.

The $GABA_A$ $\alpha 1\beta 3\gamma 2$ expressing HEK cells from Millipore were used for this study. The channel was targeted with agonists, including GABA and muscimol, inhibitors (picrotoxin, bicuculline, and gabazine), and positive modulators, including diazepam, zolpidem and chlordiazepoxide. The positive modulators produced concentration dependent augmentation of the GABA EC_{20} response. The pharmacology data determined using this method was consistent with the literature values obtained using other platforms. Statistical data for inter and intra-plate reproducibility, current stability, and Z-values, is used to validate this approach.

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Characterization of the Etomidate Binding Site in Expressed $\alpha 1\beta 3$ $GABA_AR$ by Photoaffinity Labeling

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A major site of general anesthetic action is the inhibitory γ-aminobutyric acid type A receptor ($GABA_AR$), which responds to various anesthetics by an enhanced GABA response. Using a photoreactive analog of the general anesthetic etomidate ([^{3}H]azietomidate), the etomidate binding site in the bovine brain $GABA_AR$ was identified by the pharmacologically specific photolabeling of two amino acids in the transmembrane domain, βMet-286 in the M3 helix and αMet-236 ($\alpha 1$ numbering) in the M1 helix, which both project into the interface between the β and α subunits (Li et al, J. Neurosci., 2006 26:11599-605). The $GABA_AR$ preparation used in that study was heterogeneous, as it was isolated on a benzodiazepine affinity column from a detergent extract of brain membranes. To examine anesthetic interactions with $GABA_AR$ of known subunit composition, we expressed $\alpha 1\beta 3$ $GABA_AR$ in HEK cells with an N-terminal FLAG epitope tag on $\alpha 1$ and purified hundreds of picomoles of receptor by detergent solubilization, affinity chromatography, and reconstitution into lipid. When this $GABA_AR$ was photolabeled with [^{3}H]azietomidate, the ^{3}H incorporation was enhanced by GABA and inhibited by etomidate, as determined by SDS-PAGE. From a preparative scale [^{3}H]azietomidate photolabeling of $GABA_AR$ (350 pmol of muscimol binding sites), potential photoincorporation in each of the 4 transmembrane helices from both the $\alpha 1$ and β3 subunits was examined by Edman degradation. In the $\alpha 1\beta 3$ $GABA_AR$, [^{3}H]azietomidate photolabeled the two residues at the β-α interface identified previously, as well as three additional residues, two within the β3M3 helix and the third in the $\alpha 1$M3 helix. To determine if these new labeled residues contribute to an etomidate binding site or represent lipid-exposed labeling, we are currently characterizing the etomidate inhibition of [^{3}H]azietomidate photolabeling at the level of individual amino acids.

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Effect of GABA Concentration on Current-Voltage Properties of the GABA(A) Receptor

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The γ-aminobutyric acid type A ($GABA_A$) receptor functions as a chemical to voltage transducer in the central nervous system, converting neurotransmitter signals to changes in postsynaptic membrane potential. The kinetics of GABAergic currents are known to be affected by membrane potential, but the dependence on neurotransmitter concentration has not been fully explored. The present study investigates the current-voltage (IV) relationship of $\alpha 1\beta 2\gamma 2$s $GABA_A$ receptors across a range of GABA concentrations using whole-cell patch clamp of transfected HEK293 cells. We have determined and validated a voltage ramp protocol verses a more traditional stepwise procedure, in order to rapidly quantify rectification, hysteresis and the reversal potential at GABA concentrations ranging from 0.3 to 1000 μM. Results show that the rectification and hysteresis of the IV curves are dependent on GABA concentration, whereas the reversal potential is independent. Specifically, measures of rectification and hysteresis segregated into two distinct populations, at low (0.3-10 μM) and high (30-1000 μM) GABA concentrations. We provide evidence that the current-voltage properties of $GABA_A$ receptors are concentration dependent and, consequently, that the GABA concentration must be taken into context when designing experiments and interpreting results. Finally, these results have implications for a better understanding of synaptic responses where receptor location plays a role in the level of GABA exposure.

3667-Pos

Molecular Determinants in Glycine and GABA-A Receptors that Sense Intracellular Chloride Concentration

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GABA-A and glycine receptors are ligand-gated ion channels belonging to the Cys-loop superfamily. They are permeable to anions and mediate fast synaptic inhibition in the central nervous system. We recently showed that intracellular chloride can affect the time course of deactivation of these channels (1). The aim of the present work was to identify the residues that are involved in ''sensing'' the intracellular chloride concentration.

We used the fast concentration jump technique to apply brief pulses (1-3 ms) of saturating glycine or GABA to outside-out patches from transiently-transfected HEK cells expressing alpha1 or alpha1 beta glycine receptors or alpha1 beta2 gamma2L GABA-A receptors. The effect of intracellular chloride could be mediated either by residues in intracellular loops, such as M1-M2 and M3-M4, or by the residues lining the channel itself. We used a combination of deletion and Ala/Cys-scanning mutagenesis approaches to address this question.

In other nicotinic channels, the long M3-M4 intracellular loop can be replaced by the equivalent domain (7 amino acids) of the orthologous prokaryotic channel from *Gloeobacter violaceus* without losing channel function (2). We found that chimeric constructs of glycine and GABA-A receptors containing the prokaryotic M3-M4 domain retained modulation by intracellular chloride, suggesting that the major cytoplasmic domain is unlikely to mediate this effect. The results of mutagenesis of residues facing the ion permeation pathway on the contrary strongly suggests that chloride ions affect channel kinetic by binding to a site along the pore; we have identified conserved residues that reduce the differences between the deactivation rates measured at different chloride concentrations.

(1) Pitt, Sivilotti and Beato (2008) J.Neurosci 28, 11454-11467
(2) Jansen, Bali and Akabas (2008) J.Gen.Physiol. 131, 137-146

3668-Pos

Microsecond Simulations Show that Ethanol Binds between Subunits and Stabilizes the Open Form of a Glycine Receptor Model

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