cultures of human airway smooth muscle cells endogenously expressing $GABA_A$ channels composed of α4, α5 β3 γ1, γ2, δ and θ subunits. We obtained success rates above 95% for transiently or stably transfected HEK cells and frozen "ready to assay" HEK cells expressing $GABA_A$ channels. Tissue-derived immortalized cultures of airway smooth muscle cells exhibited a slightly lower recording success rate of 75% using automated patch, which was much higher than the 5% success rate using manual patch clamp. Primary cells harvested from tissue yielded 30% success on this automated electrophysiology platform. In all cases, both responses to agonist (EC_{50} measurements) and pharmacology of GABA modulators and inhibitors compared well to previously reported manual patch results. The data presented here demonstrates that both the biophysics and pharmacologic characterization of $GABA_A$ channels in a wide variety of cell formats can be performed on this automated patch clamp system. This automated approach exhibited a much higher success rate, lower variability, and higher experimental throughput as compared to manual patch clamp techniques.

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Mapping GABAA Receptor Single Nucleotide Polymorphisms (SNPs) Linked to Epilepsy: Insights into the Receptor Gating and Assembly

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Missense polymorphisms or variants that affect function and/or expression of $GABA_A$ receptors (GABARs) have been associated with idiopathic generalized epilepsies (IGEs) in *GABRA1, GABRA6, GABRB3, GABRG2 and GABRD* genes. Three separate IGE-associated mutations were identified in GABAR subunits (β3(G32R), α6(Q237R) and γ2(K328M)), which represent a large range of GABARs in the nervous system. We sought to investigate the contributions of these mutations into the assembly and function of GABARs. Homology modeling suggested that G32R is located within the N-terminal α-helix β3 subunit domain, and Q237R and K328M are located within the pre-M1 segment of the N-terminal α6 subunit domain and the M2-M3 loop of the N-terminal γ2 subunit domain, respectively. We studied gating properties and surface expression of wild type (wt) α1β3γ2, α6β2γ2, α1β2γ2 and mutant α1β3(G32R)γ2, α6(Q237R)β2γ2, α1β2γ2(K328M) receptors expressed in HEK293T cells. We found that the mutations share common gating defects, but distinctive trafficking defects. Thus, mutant β3(G32R) subunits displayed a mixed profile, causing both gating and trafficking defects of α1β3γ2 receptors, whereas mutant α6(Q237R) and γ2(K328M) subunits caused exclusive channel gating defects of α6β2γ2 and α1β2γ2 receptors. Unexpected, homology modeling predicted that the β3(G32R) mutation affects a salt bridge across the γ2/β3 subunit interface, which underlies an assembly motif reported to be essential for inter-subunit interactions in assembled receptors. In contrast, α6(Q237R) and γ2(K328M) subunit mutations are predicted to interact with residues in Pre-M1 domain, M2-M3 loop and Cys-loop of α6 and γ2 subunits that are critical for desensitization-deactivation coupling of GABARs.

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Interactions of GABAA Receptors with Steroid-Like Positive and Negative Modulators

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A new model for the gamma-aminobutyric acid class A (GABAa) receptor is presented, based on recent crystal structures of the homologous eukaryotic GluCl channel (3RHW). The models suggest an additional disulfide bridge in the transmembrane domain of both alpha and gamma (but not beta) subunits. Models for the GABAa receptor in complex with steroid or steroid-like modulators such as cholesterol, pregnenolone, and thyroid hormone have also been developed, based on ivermectin sites in PDB:3RHW and mutagenesis studies. Extended molecular dynamics simulations of these models indicate stable binding interactions between the modulator and the receptor, with moderate adjustment of modulator orientation. Hydrogen bonding patterns between modulator and receptor polar groups are shown to be modulator-dependent; these results will be compared to prevailing hypotheses for essential pharmacophores in modulation of GABAa receptors by neurosteroids.

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Optical Control of Neuronal Inhibition with Genetically Engineered Light Inhibited GABAA Receptors (Li-GABARs)

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GABA is the main inhibitory neurotransmitter in the brain, acting primarily at ionotropic $GABA_A$ receptors. The α subunit is critical in determining $GABA_A$ receptor functional properties and pharmacological regulation. There are six α subunit subtypes that are differentially expressed in the nervous system but few selective antagonists are available to dissect the functional role of those subtypes in neurons. We have therefore engineered α subunit specific light inhibited $GABA_A$ receptors (Li-GABARs). We generated α subunits with a cysteine mutation that allows attachment of a photoswitchable tethered ligand (PTL) consisting of a cysteine reactive maleimide group, a photoisomerizable azobenzene core and a variable GABAR ligand. Using cysteine-scanning mutagenesis we identified optimal photoswitch attachment sites near the GABA binding pocket for both α1 and α5 subunits, and tested multiple PTLs to produce maximal light dependent block of GABAR activity while minimally affecting basal receptor properties. We recorded from hippocampal slices expressing Li-GABARs and found evidence that α5, but not α1 containing receptors counteract NMDA receptor dependent synaptic depolarization. This novel interaction could provide a basis for the regulation of synaptic plasticity and memory by α5 receptors. This approach in general provides a strategy to study the role of specific $GABA_A$ receptor subunits and their importance in neuronal function and disease.

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A Molecular Dynamics Study on the Effect of Disulfide Bonds in Cys-Loop Ligand-Gated GABAA Receptors

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GABAA-receptors (GABARs) are chloride ion channels in the 'cys-loop receptor' superfamily that are major inhibitory neuroreceptors. Upon agonist binding, GABARs open and increase intraneuronal chloride ion concentration, hyperpolarize the cell and inhibit neural transmission. Reducing agents will break the disulfide bonds in the cys-loop of the receptor. Indeed, it has been speculated that sulfhydryl compounds modify proteins via thiol/disulfide redox reactions and serve as neuromodulators (1). Reducing reagents were found to decrease the GABA EC50 in the highly homologous GABAC-receptor, while oxidizing reagents increased the EC50 (2). Conversely, conflicting findings suggests the mechanism of action of redox modulation does not alter GABAR agonist-binding affinity (3). However, studies are complicated by the fact that mutagenesis of the cysteines commonly produces failure of subunit assembly (4). Thus, to investigate the effect of disulfide bond breakage on the receptor we generated various GABAR homology models with and without disulfide bonds. These systems were subjected to ligand docking and extensive molecular dynamics simulations to determine if the GABA affinity was modulated. This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

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Allosteric Modulators Targeting Multiple Binding Sites in Bacterial Homolog of GABAA Receptor

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GABAA receptors belong to the family of pentameric ligand-gated ion channels (LGIC), which are involved in fast inhibitory neurotransmission. GABAA receptors are allosterically modulated by benzodiazepines, general anesthetics and alcohols. In this study, we investigate the effects of a range of general anesthetics, alcohols and non-competitive inhibitors on ELIC, which is a recently characterized prokaryote homolog of GABAA receptors. We used two-electrode voltage clamp recordings to identify ELIC modulators from a library of compounds including chlorpromazine, memantine, picrotoxin, etomidate, DMCM and brominated derivatives of alcohols and chloroform. X-ray crystal structures of ELIC in complex with different modulators reveal the molecular architecture for ligand recognition. Consistent with the observation that