ion unbinding and interface opening is observed for a mutant (L783S) where the cation site is indirectly disrupted by disturbing a hydrophobic plug shielding the site. In the electrophysiological studies, this mutant appears non-functional. Overall, the results are consistent with the view that maximum conductance depends on a tightly packed dimer-interface regulated by the presence of extracellular ions.

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Details of GABA Binding to the GABA-A Receptor Revealed by Molecular Dynamics

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Through the analysis of two rare-event molecular dynamics simulations, we were able to observe the complete dissociation of a GABA molecule from the GABAA-receptor, the diffusion of that molecule into bulk water, and then the subsequent re-binding of GABA into its binding pocket.

The route for this re-binding event was not linear in fashion, but rather adopted 'bind, slide, and find' approach. We found that the GABA molecule BINDS to the protein surface outside of the binding pocket, SLIDES along the protein surface into the pocket itself, and then FINDS the correct binding orientation once it is within the binding pocket.

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$GAbA_A$ Receptor Subunit Rare Variants Identified in Patients with Idiopathic Generalized Epilepsy Alter Receptor Gating and Assembly

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Exome sequencing of ion channel genes from cases with well characterized idiopathic generalized epilepsies (IGEs) has identified many rare, nonsynonymous single nucleotide polymorphysms (nSNPs) in the human non-epilepsy genes *GABRA4*, *GABRA5*, *GABRA6*, *GABRB1*, *GABRB2*, *GABRG1* and *GABRG3*. Structural homology modeling predicts that the rare variants are mainly localized between the agonist-binding domain (N-terminus) and the channel gate (transmembrane domain, TM) of $GABA_A$ receptor subunits. We sought to investigate the effects of the rare nSNPs on assembly and function of $GABA_A$ receptors containing one of seven separate IGE-associated rare variants identified in α5 and β2 subunits (α5V204I, W280R, S402A, P453L, A459T; β2R293W, R354C), which are located within the well-defined structural $GABA_A$ receptor domains. We studied gating properties and surface expression of wild-type (wt) α5β3γ2, α1β2γ2 and mutant (mut) α5(mut)β3γ2, α1β2(mut)γ2 receptors expressed in HEK293T cells. We found that variants located within the N-terminal domain and in the juxtamembrane interface displayed gating or mixed gating and trafficking defects. Furthermore, variants within TMs displayed trafficking defects, but those within the cytoplasmic loop had no defect. Thus, variant subunits α5V204I and β2R293W displayed a mixed profile, causing both gating and trafficking defects of α5β3γ2 and α1β2γ2 receptors, whereas variant α5W280R subunit caused primarily impaired trafficking of α5β3γ2 receptors. Interestingly, homology modeling predicted that α5W280R and β2R293W variants stabilize new hydrogen bonds across the β/α subunit interface, which seems to be essential for inter-subunit interactions in assembled receptors. These findings suggest that gating and/or trafficking defects might suggest that a specific variant may be a susceptibility gene and may help to predict functional risk for loss of GABAergic function in individual IGE cases.

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Mechanistic Basis of Partial Agonism at 5-HT_3A Receptors

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Partial agonists are unable to elicit full maximal responses. The characterization of a genuine partial agonist is complex because other mechanisms, such as channel block, may also limit maximum open probability. Taking advantage of the high conductance form of the 5-HT_3A receptor, we evaluated at the single-channel level its activation by 2-Me-5HT and tryptamine, which have been classically considered as partial agonists of 5-HT_3 receptors. For all ligands, activation appears as openings in quick succession grouped in clusters showing high open probability (Popen>0.9), and open time distributions show three components. The slowest open component is 6.5- and 3.5-fold briefer for 2-Me-5HT and tryptamine, respectively, than for 5-HT. The duration of this component decreases as a function of agonist concentration due to open-channel block. For 2-Me-5HT, the forward blocking rate is 10-fold faster than for tryptamine and 5-HT. Single-channel kinetic analysis shows that 2-Me-5HT is actually a full agonist, its maximum response being limited by channel block. In contrast, tryptamine is a genuine partial agonist and its low efficacy is mainly due to a slow transition from the fully-liganded closed state to a pre-open state. After reaching the latter state, activation proceeds similarly as in the presence of 5-HT. Molecular docking shows that interactions at the binding site are similar for 2-Me-5HT and 5-HT. In contrast, the potential to form the cation-Pi interaction with W183 seems to be reduced for tryptamine. The mechanism by which ligands produce non maximal responses has implications for the design of partial agonists for therapeutic use.

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Proline Residues at the Nicotinic Acetylcholine Transmitter Binding Sites

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The two neuromuscular acetylcholine receptor (AChR) transmitter binding sites are located in the extracellular domain of the protein at the α-ε and α-δ subunit interfaces. At each site there are two vicinal prolines in the complimentary ε/δ subunit (ProD1 and ProD2). We estimated for dozens of mutations of ε/δ ProD1 and ProD2 (single-channels, HEK cells, +100mV, 23 oC) the gating energies with ACh molecules bound (G2 and G1) and without any agonist (G0). From these we calculated the energy for gating arising from the affinity change for ACh (GB1+GB2=G2-G0). Mutation of ProD2 had larger effects than of ProD1. The εProD2-L mutation, which causes a congenital myasthenic syndrome, increases αGB (+3.4 kcal/mol, throughout) but does not change G0. For the same mutation in the δ subunit the change in GB was smaller (+1.9). All of the side chain substitutions at ε/δProD2 increased both GB and G0 compared to the wild-type (which has the values −5.1 and +8.4). The largest increase in αGB was for εProD2-R (+4.7) and in G0 for δProD2-Q (+2.5). We used mutant cycle analysis to estimate αGB¬ coupling between side chains. The two ProD2 residues interact weakly with each other (−0.6), but there is a strong interaction between εProD2-αGlyB1 (+2.7) but not between δProD2- αGlyB2. It is possible that a concerted, Pro-Gly 'switch' at α-ε occurs near to the onset of channel-opening. We engineered AChRs having only one functional binding site and quantified GB(ACh) for either the α-ε (by using the δProD2-R knockout or α-δ (by using the εProD2-R knockout) site. The sites provide +5.2 and −4.7 kcal/mol with ACh. One-site AChRs will be useful for studying the energy sources for gating by agonists at the two different binding sites.

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Enhancement of α9α10 Nicotinic Acetylcholine Receptor Desensitization by Nimodipine

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Entry of calcium ions through nicotinic acetylcholine receptors hyperpolarizes outer hair cells (OHCs) by activating colocalized SK potassium channels. This process, termed efferent inhibition, plays a central role in the protective reflex by suppressing the amplification of incoming sound. OHC nicotinic receptors are composed of α9 and α10 subunits that are activated by acetylcholine (ACh) release from efferent neurons originating within the medial olivocochlear nucleus. Fast application of ACh to GH_4C_1 cells transiently transfected with plasmids encoding mouse α9 and α10 subunits, produced an inward current that displayed a concentration-dependent amplitude, with an EC_{50} of 41 ± 5.4 μM and Hill slope (n_H) of 0.89 ± 0.1 (n=5). This current was antagonized by nicotine, displaying a K_B of 9.0 ± 1.0 μM (n=8), consistent with the ACh-mediated current arising from activation of heteromeric α9α10 receptor channels. Inward ACh currents in the presence of the dihydropyridine antagonist nimodipine (10 μM) were reduced in amplitude and displayed faster decay kinetics. Exponential decay rates (τ) increased from 647.5 ± 66.0 ms (n=6) in control, to 160.6 ± 25.8 ms (n=6) in the presence of nimodipine (10 μM) ($p<0.0005$). The concentration-response relationship to ACh was not significantly affected by nimodipine, displaying an EC_{50} of 28 ± 6.7 μM and Hill slope (n_H) of 1.28 (n=5) ($p>0.05$), suggesting that the increased decay rate of ACh currents did not result from a decrease in agonist binding affinity. using a double-pulse protocol, ACh (300 μM)-mediated responses recovered from desensitization with an exponential time-course (τ = 3.81 ± 0.67 s, n=3). Recovery was slowed in the presence of nimodipine (τ = 8.88 ± 2.54 s; n=3), indicating that nimodipine stabilizes the desensitization state of the α9α10 receptor and suggests that these receptors can be modulated to fine-tune efferent inhibition.