adamantane cage reduced activity. Another showed that the adduct must be more than 2 carbons. Pre-exposing the virus to drug before inoculation showed inactivation and exposure-recovery of the virus on the ~10-minute time scale. Resistance development is dramatically reduced. For selected compounds, 10 passages (~5 weeks) in the presence of drug were required before the 2009 H1N1 developed resistance. However, the mechanism of action is unclear. Liposome assays indicate direct block of S31N M2 (22-62). But 2009 H1N1 M2-transfected HEK cells are not blocked, either on the 2- or the 30-minute time scales. Yet, the revertant (N31S) is well blocked. Solid state NMR suggests that drugs bind to the S31N transmembrane peptide domain. The resistant strains developed in the presence of these drugs show no mutations in M2, but a few mutations in hemagglutinin. It is possible that these hydrophobic amines function partly by neutralizing the endosome. However, the virus pre-exposure results indicate a direct effect on the virus, not just on the endosome. The A/WSN/33 virus is not blocked by these drugs in cytopathic effect assays, but the revertant (N31S) is, indicating for A/WSN/33 that the M2-block is the key effect. In summary, resistance-invulnerable drugs for the 2009 H1N1 influenza A virus have been identified and the mechanism of action is yet to be defined.

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Dual Regulation of G Proteins and the G Protein-Activated Potassium Channels (GIRK) by Lithium

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Cellular targets of Li^+, such as glycogen synthase kinase 3β and G proteins, have been long implicated in bipolar disorder (BPD) etiology. However, recent genetic studies link BPD to other proteins, in particular ion channels. Li^+ affects neuronal excitability, but the underlying mechanisms and the relevance to putative BPD targets are unknown. We discovered a novel, dual regulation of G protein-gated K^+ channels (GIRK) by Li^+, and determined the underlying molecular mechanisms. In hippocampal neurons, therapeutic doses of Li^+, 0.5-2 mM, increased GIRK basal current (I_{basal}) but attenuated neurotransmitter-evoked GIRK currents (I_{evoked}) mediated by $G_{i/o}$-coupled G protein-coupled receptors (GPCRs). Molecular mechanisms of these regulations were studied with heterologously expressed GIRK1/2. In excised membrane patches, Li^+ increased I_{basal} but reduced GPCR-induced GIRK currents. Both regulations were membrane-delimited and G protein-dependent, requiring both Gα and Gβγ subunits. Li^+ did not impair direct activation of GIRK by Gβγ, suggesting that inhibition of I_{evoked} results from a Li^+ action on Gα, probably through inhibition of GTP-GDP exchange. In direct binding studies, Li^+ promoted GPCR-independent dissociation of $G\alpha_i^{GDP}$ from Gβγ by a Mg^{2+}-independent mechanism. This previously unknown Li^+ action on G proteins explains the second effect of Li^+, the enhancement of GIRK's I_{basal}. The dual effect of Li^+ on GIRK may profoundly regulate inhibitory effects of neurotransmitters acting via GIRKs. Our findings link between Li^+, neuronal excitability, and both cellular and genetic targets of BPD: GPCRs, G proteins and ion channels.

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The Microglial K^+ Channels Kv1.3 and KCa3.1 as Potential Therapeutic Targets for Ischemic Stroke

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Activated microglia significantly contribute to the secondary inflammatory damage in ischemic stroke and therefore constitute attractive targets for post-infarct intervention. Microglia express the voltage-gated Kv1.3 and the calcium-activated KCa3.1 channels, both of which have been reported to be involved in microglia mediated neuronal killing, oxidative burst and inflammatory cytokine production. However, most of these experiments have been performed with cultured neonatal microglia and it has always been questioned whether these cultures accurately reflect the K^+ channel expression of activated microglia in adult brain. Following intrahippocampal LPS injection or middle cerebral artery occlusion (MCAO) with 7 days of reperfusion we observed Kv1.3 and KCa3.1 immunoreactivity on activated microglia in mouse brain. In both conditions we further detected currents exhibiting the biophysical and pharmacological properties of Kv1.3 and KCa3.1 on microglia immediately following isolated with CD11b-magnetic beads. Channel expression was significantly higher than on microglia isolated from control brains. We next investigated the effect of genetic deletion and pharmacological blockade of KCa3.1 on the reperfusion injury following ischemic stroke using reversible MCAO as a model. KCa3.1$^{-/-}$ mice and wild-type mice treated with the KCa3.1 blocker TRAM-34 exhibited significantly smaller infarct areas and improved neuronal survival and motor coordination in neurological deficit test on day-7 after MCAO. Kv1.3 blockade with PAP-1 exhibited similar beneficial effects in wild-type mice but did not further reduce infarct area or improve neurological deficit in KCa3.1$^{-/-}$ mice. In male Wistar rats combined blockade of both Kv1.3 and KCa3.1 with PAP-1 and TRAM-34 also did not further reduce infarct area compared to treatment with either TRAM-34 or PAP-1 alone suggesting that blockade of one microglial K^+ channel is sufficient to improve outcomes in ischemic stroke.

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Epilepsy-Associated Point Mutation in the Pore Domain of K_V2.1

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A large scale sequencing endeavor (Epi4K Consortium, Nature 501:217-221, 2013) recently reported discovery of several *de novo* mutations in genes encoding ion channels, neurotransmitter receptors and other proteins that may explain severe, early onset childhood epilepsy. One intriguing variant was found in *KCNB1*, encoding K_V2.1. Although K_V2.1 is a potassium channel involved in repolarization of neuronal action potentials, it has not previously been associated with epilepsy. The reported variant (T374I) was heterozygous and affects a highly conserved residue within the K_V2.1 ion selectivity filter 5 amino acids N-terminal to the GYG motif. We performed heterologous expression of wildtype (WT) and mutant K_V2.1 channels and used whole-cell patch clamp recording to define the functional consequences of the mutation and to infer the pathophysiology of the epilepsy. Wild-type or mutant K_V2.1 channels were transiently expressed under control of the CMV promoter in CHO cells. Expression of channel subunits was confirmed by co-expression of a fluorescent protein encoded by the same plasmid. Cells expressing homomeric WT-K_V2.1 exhibited large outward currents with rapid voltage-dependent activation and slow inactivation. By contrast, cells expressing homomeric K_V2.1-T374I exhibited no outward or inward current. Our findings suggest that this mutation impairs ion permeation and confers a loss-of-function as the molecular basis for epilepsy. Further studies will evaluate the impact of mutant subunits on formation of functional heteromultimeric channels to explore possible dominant-negative behavior.

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Molecular Dynamics Studies of Ion Permeation in Human Voltage-Gated Proton Channel

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The human voltage-gated proton channel (hHv1) is a transmembrane protein responsible for selective proton permeation across cell membranes in nasal mucosa, sperm, and white blood cells. Its pathological states include male infertility, allergies, and diseases such as cystic fibrosis, asthma, and lupus. Its involvement in ischemic stroke and invasiveness of breast cancer cells has substantiated hHv1 as a therapeutic target for drug designs for which require the understanding of hHv1 structure and proton conduction mechanism. We recently constructed and validated a homology model (Kulleperuma et al., 2013) of hHv1 characterized by the presence of a salt bridge between anionic D112 and cationic R208 side chains in the narrow region of the hydrated pore. Thanks to the pairing of these and other charged residues in ionic networks, the distribution of charged and polar residues in the wild-type channel is a priori compatible with permeation of either cations or anions. However, a static-field electrostatic barrier opposing cation movement arises in neutral mutants of residue D112, consistent with the observation that these mutants are selective to anions (Musset et al., 2011). Our recent experiments show that proton selectivity is restored in double mutant D112V/V116D, while D112S and D112V/D116S are anion-selective and D112V does not conduct ions. Atomistic molecular dynamics simulations of these mutants in lipid bilayers show that their structures differ in the organization of ionic side chains in the external vestibule and suggest that, consistent with the above analysis, the distribution