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The efficiency of both pharmacological manipulations of PLC was verified with the DAG-sensor PKC γ -C1 and the PI(4,5)P₂-sensor PLC δ 1-PH, monitored in total internal reflection fluorescence microscopy.

These data suggest that activation of PLC is an indispensable step in G α PCR – TASK signaling and are inconsistent with the hypothesis that direct G α_q -interaction mediates TASK current inhibition.

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675-Pos Board B444

Inward Rectification of TWIK-1 Two-Pore Domain K⁺ Channels

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Mammalian two-pore domain K⁺ channels (K2P) mediate background K⁺ conductance and play an important role in regulation of cellular excitability and electrolyte homeostasis. TWIK-1 (also known as K2P1), the first cloned mammalian K2P channel, is highly expressed in the brain, kidney, and heart. They contribute to a large passive K⁺ conductance in rat hippocampal astrocytes, conduct inward leak Na⁺ currents in human cardiac myocytes under pathological hypokalemia, and regulate phosphate and water transport in mouse proximal tubule and medullary collecting duct, respectively. TWIK-1 K⁺ channels were first characterized in *Xenopus* oocytes and defined as weakly inward rectifying K⁺ channels. However, whether TWIK-1 K⁺ channels show inward rectification is contradictory, as several reports indicate that TWIK-1 K⁺ channels do not exhibit weakly inward rectification when expressed in mammalian cells and *Xenopus* oocytes. Here we report that TWIK-1 K⁺ channels heterologously expressed in Chinese hamster ovary cells show weakly inward rectification in physiological K⁺ gradients. Such a rectification is caused by voltage-dependent blockade of intracellular blockers rather than rapid fast inactivation, as intracellular blockers bind to TWIK-1-specific sites in the inner pore of TWIK-1 K⁺ channels. These results improve current understandings of the function of TWIK-1 K⁺ channels as well as their contributions to cellular behaviors.

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K2P and Kir K⁺ Channels in Physiological Bilayers

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We are interested in understanding the mechanism of modulation of inwardly-rectifying (Kir) and two-pore (K2P) potassium ion channels by physiological and modulatory lipids. The phospholipid phosphatidylinositol 4,5-bisphosphate (PIP₂) is critical for Kir channel activity, and recent crystal structures of Kir2.2 (one of them in complex with PIP₂) have shed light on PIP₂-Kir channel interactions. However, the mechanism of PIP₂ binding and gating is not fully understood. Here, we used a multi-scale approach, consisting of sequential coarse-grained and atomistic molecular dynamics simulations of Kir2.2 embedded in a phospholipid/PIP₂ bilayer, to determine whether this computational approach leads to the same PIP₂-binding site as observed in the PIP₂-bound crystal structure. Our results correctly predict the PIP₂ binding site in Kir2.2 even when slightly different structures were used as starting coordinates for these simulations. These results therefore demonstrate the predictive power of this computational approach for the study of protein interactions with PIP₂ and possibly other modulatory lipids.

Having validated this multi-scale approach, we applied it to study both Kir and K2P channels with PIP₂ in physiological bilayers. TREK-1, the prototypical K2P channel, is modulated by PIP₂, but the exact mechanism by which it interacts with PIP₂ has not been fully elucidated. To explore these mechanisms, we have built structural models of TREK-1 based upon the recent crystal structures of the related K2P channels, TWIK-1 and TRAAK. These two different structural models of TREK-1 were validated by comparison to functional scanning mutagenesis data, which revealed that TRAAK provides the best structural template for modelling of TREK-1. This new structural model of TREK-1 now provides an opportunity to use multi-scale simulations to explore the interaction of TREK-1 with PIP₂/modulatory lipids, and to gain a greater insight into the molecular mechanisms which underlie regulation of K2P channel gating.

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Role of K⁺ Channels in Alveolar Macrophages-Mediated Inflammatory Response upon Anthrax Lethal Toxin Stimulation

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The causes of rapid death in anthrax infection both in animals and humans are unknown and concern over the use for biological warfare has renewed interest in elucidating the mechanisms of anthrax induced inflammation. Interleukin-1 β (IL-1 β) secretion is an important inflammatory response against anthrax lethal toxin (LeTx) a virulence factor of *Bacillus anthracis*. Here, we report that LeTx induces a significant increase in inwardly-rectifying K⁺ (Kir) and voltage-gated K⁺ (Kv) currents in mouse and human macrophages. Furthermore, we also show that blocking either Kir or Kv channels significantly inhibits LeTx-induced IL-1 β secretion suggesting that activation of macrophage K⁺ channels plays an important role in LeTx-induced inflammatory response. In addition, we also investigated the role of macrophage K⁺ channels in macrophage priming, a well-known macrophage infection model involving pre-exposure of the cells to a low level of antigen that augments the response to subsequent challenge. Specifically, priming of alveolar macrophages by either Lipopolysaccharides (LPS), an endotoxin of all gram-negative bacteria, or *Bacillus* spores, augments inflammatory response upon LeTx stimulation as compared to unprimed cells challenged with LeTx alone. Our study shows that pre-exposure to low levels of LPS or to the spores also significantly augments LeTx-induced activation of macrophage K⁺ channels suggesting that activation of K⁺ channels might be part of the priming mechanism.

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β -Adrenergic Receptor-Mediated Suppression of the Medium After Hyperpolarization in Rat Hippocampal Neurons Maintained in Culture

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The firing of a train of action potentials in hippocampal pyramidal CA1 neurons is regulated by both medium (mAHP) and slow (sAHP) afterhyperpolarizations. The mAHP is generated by activation SK and M-channels, and the deactivation of hyperpolarisation-activated H-current. In contrast, the sAHP is mediated by activation of an unknown calcium-dependent potassium channel. Organotypic hippocampal slices are a useful tool for studying the effects of altered protein expression, but it is not known whether neurons maintain the channel subtypes that underlie the mAHP or sAHP. The mAHP (generated by 25 action potentials initiated from a –80 mV) was inhibited 52% by apamin (100 nM) and 49% by XE991 (10 μ M), indicating that it is generated by activation of both SK and M channels. The amplitude of the mAHP was found to increase with action potential number, an effect that was blunted by apamin. These data indicate that activation of SK channels, and not M-channels, are primarily responsible for the recruitment of the mAHP in response to increasing trains of action potentials. As previously described, application of isoprenaline significantly suppressed the sAHP. Interestingly, application of isoprenaline (1 μ M) also suppressed the mAHP in organotypic slices by 52 \pm 0.05 % (n=5). The prior block of SK channels by apamin did not prevent suppression of the mAHP by isoprenaline, indicating that suppression of the mAHP by β -adrenergic receptor activation does not result from inhibition of SK channel activity. These data indicate that organotypic slices retain the channel subtypes that underlie the medium and slow AHPs. The novel effect of isoprenaline suggests that the increased excitability of hippocampal neurons observed in the presence of the β -receptor agonist is a combined effect of suppressing both the medium and slow AHPs.

Voltage-gated Na Channels

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Microsecond Molecular Dynamics Simulations of the Open State Structure of a Bacterial Voltage-Gated Sodium Channel Reveal Mechanisms of Ion Selectivity and Conduction

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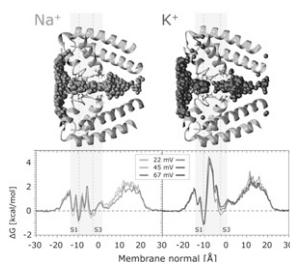
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Microsecond atomic detail equilibrium molecular dynamics simulations based on the open-state crystal structure (McCusker et al, 2012, Nature Comm) of a bacterial voltage-gated sodium channel (NavMs) have been employed to characterize the mechanisms underlying ion selectivity and conductance of the channel embedded in a lipid bilayer membrane. This approach captured the full plethora of conduction events, revealing a complex mixture of single and multi-ion phenomena, with decoupled rapid bi-directional water transport. Channel selectivity for Na over K ions was found to increase with decreasing applied membrane potential. In marked difference to K-channel simulations, no voltage lag was observed for Na⁺. Unlike in K⁺ channels, the ions are fully

hydrated at all times, even when bound. The ion positions were correlated with electron density in selectivity filter of the crystal structure. Remarkably, and in stark contrast to K-channels, ionic conduction was found to be independent of net water flux, which was zero for all applied voltages and ionic species. This zero water transport was found to result from the balance of two large and opposing water fluxes of equal magnitude.



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Investigating the Voltage Sensor Domains of Nav1.4, its Structural and Functional Properties via Histidine Scanning Mutagenesis

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Mammalian sodium channels are composed of four homologous domains (DI, DII, DIII and DIV). Each domain is composed of six helical transmembrane segments (S1-S6). Together, the folding of S5-S6 segments of all the domains form the pore domain (PD). The S1-S4 segments of each domain form the voltage sensor domain (VSD). Recently, mutations in the VSD of Nav channels have been linked to pathologies such as hypo- and normokalemic periodic paralysis and recently dilated cardiomyopathy Gosselin-Badaroudine P., et al., (2012): PlosONE, 7(5):e38331.

Here we use the histidine scanning mutagenesis technique to investigate the role of the positively charged amino acids along the S4 segments of each domain. Detecting a proton current at hyperpolarized potentials indicates that the mutated residue is located in the gating charge transfer center of the protein. Also, proton transport implicates that the mutated residue moves across the gating charge transfer center during activation. Furthermore, a shift in the Q-V curve indicate that the mutated residue plays an important role in the stabilization of the S4 segment in its activated or resting position.

The results lead to the creation of the first structural model of the VSDs of a mammalian sodium channel in its resting state. This structural model features hydrophobic septa of different dimensions. Indeed, the VSD of the fourth domain displays a hydrophobic septum much larger than the septa of the other domains. This difference of the VSD of the fourth domain provides a rationale for its late onset in the activation sequence and the fact that no gating pore current have been uncovered in the VSD of DIV of mammalian sodium channels.

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Atrial Selectivity in Sodium Channel Block by Amiodarone

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Introduction: Na⁺ channel blockers are usually applied to atrial fibrillation (AF), but may sometimes cause cardiac contractile dysfunction. However, amiodarone, a multi-channel blocker with Na⁺ channel block, does not induce cardiac dysfunction. In this study we tested the hypothesis that Na⁺ channel block by amiodarone is selective in atrial myocytes (AM) compared to ventricular myocytes (VM). **Methods and Results:** Na⁺ currents (I_{Na}) and resting membrane potentials (RMPs) were measured using whole cell patch-clamp technique in isolated rabbit AM and VM. Amiodarone inhibited I_{Na} in AM (IC₅₀: 1.4 ± 0.3 μM; n=8) much more than in VM (40.4 ± 11.9 μM; n=7; P<0.01). Amiodarone at 10 μM dramatically shifted steady state inactivation curve to hyperpolarized direction in AM (-19.6 ± 2.1 mV shift; n=12) compared to VM (-6.3 ± 0.8 mV shift; n=13; P<0.01). In mexiletine, there was no significant difference in I_{Na} inhibition between AM and VM. The shifts of inactivation curves by mexiletine at 10 μM were comparable in AM and VM. RMPs in AM (-75.0 ± 1.3 mV; n=4) were more depolarized than in VM (-82.1 ± 1.1 mV; n=9; P<0.01). In the absence of drugs, the half inactivation voltage in AM (V_{1/2}: -89.7 ± 0.9 mV; n=19) was 12.5 mV more negative than VM (-77.2 ± 0.6 mV; n=20; P<0.01). Furthermore, we evaluated the effects of amiodarone and mexiletine on conduction velocity (CV) in Langendorff-perfused rabbit hearts by optical mapping system. The decrease of CV by amiodarone at 5 μM was significantly larger in atrium (-34.3 ± 5.6%; n=5) compared to ventricle (-4.8 ± 1.0%; n=5; P<0.01). However, the reduction of CV by mexiletine at 5 μM in atrium was smaller than in ventricle. **Conclusion:** Amiodarone preferentially inhibits I_{Na} of AM compared to VM. This atrial-

selective Na⁺ channel block by amiodarone may contribute to treating AF without affecting ventricular contractility.

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Wild-Type Sodium Channels and 'Atypical' Brugada Syndrome Mutants Interact through C-Terminal Region

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Introduction: Brugada syndrome (BrS) is an inherited cardiac disorder that can be caused by mutations in the cardiac sodium channel gene resulting in a reduction of sodium currents. However, several 'atypical' sodium channel mutations identified in patients show minimal biophysical defects questioning how such mutants may produce a BrS phenotype. We have previously demonstrated that when "atypical" BrS mutants were co-expressed with wild-type channels (WT) mimicking the heterozygous patient genotype, this lead to a dramatic reduction in sodium currents thereby explaining the disease phenotype. Here we hypothesized that a direct interaction between atypical BrS mutants and WT is mediated via the calmodulin-binding IQ domain of the channel protein. **Methods:** 'Atypical' BrS mutations with minimal biophysical defects were co-expressed with either WT or WT channels containing a mutated calmodulin binding IQ motif (IQ/AA) in HEK293 cells. BrS mutations from all intracellular loops were studied. Biophysical properties of mutants were investigated by patch clamp. Co-immunoprecipitation and cell surface biotinylation were performed to assess interaction and channel location. **Results:** BrS mutants co-expressed with IQ/AA had current amplitudes restored to control levels. This abrogated any loss-of-current phenotype we observed when the "atypical" mutants were co-expressed with WT. Cell surface biotinylation showed a significant reduction of both WT and mutant channels at the plasma membrane on co-expression, while surface expression was restored for both channels when mutants were co-expressed with the IQ/AA construct. Importantly, while co-immunoprecipitation experiments demonstrated interactions between WT and "atypical" mutant channels, IQ/AA failed to interact with atypical BrS mutants. **Conclusions:** Our data suggest that 'atypical' BrS mutations suppress both mutant and WT channels expression at the cell surface via interactions mediated by the calmodulin-binding (IQ) domain of the cardiac sodium channel.

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The β₁-Subunit of Nav1.5 Cardiac Sodium Channel is required for a Dominant Negative Effect through α-α Interaction

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Brugada syndrome (BrS) is an inherited autosomal dominant cardiac channelopathy. Several mutations on the cardiac sodium channel Na_v1.5 which are responsible for BrS lead to misfolded proteins that do not traffic properly to the plasma membrane. In order to mimic patient heterozygosity, a trafficking defective mutant, R1432G was co-expressed with Wild Type (WT) Na_v1.5 channels in HEK293T cells. This mutant significantly decreased the membrane Na current density when it was co-transfected with the WT channel. This dominant negative effect did not result in altered biophysical properties of Na_v1.5 channels. Luminometric experiments revealed that the expression of mutant proteins induced a significant reduction in membrane expression of WT channels. Interestingly, we have found that the auxiliary Na channel β₁-subunit was essential for this dominant negative effect. Indeed, the absence of the β₁-subunit prevented the decrease in WT sodium current density and surface proteins associated with the dominant negative effect. Coimmunoprecipitation experiments demonstrated a physical interaction between Na channel α-subunits. This interaction occurred only when the β₁-subunit was present. Our findings reveal a new role for β₁-subunits in cardiac voltage-gated sodium channels by promoting α-α subunit interaction which can lead to a dominant negative effect when one of the α-subunits shows a trafficking defective mutation.

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Proton Modulation of Ranolazine Effects on Slow Inactivation in Sodium Channels

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Ranolazine is a clinically approved anti-anginal drug with potential antiarrhythmic, antiepileptic, and analgesic applications. The therapeutic effects of ranolazine are dependent on its ability to preferentially inhibit persistent currents in a variety of voltage-gated sodium channels. Extracellular acidosis, as