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Quantum Calculations: A Test of Accuracy on a Solvated Crown Ether with an Ion, a System Large Enough to Model a Useful Section of A Protein

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A quantum calculation (DFT) has been carried out on the crown ether (CE) 14-C-4, together with up to 14 methanol molecules, or 27 water molecules, plus one ion. The free energy of complex formation is known experimentally for both Na^+ and K^+ ions, allowing comparison for methanol; neither ion can be complexed from bulk water. We calculate that the ions could be complexed from a more limited water solvent shell. In order to avoid leaving an unrealistic CE-vapor interface, 8 water molecules or 4 methanol molecules were placed on the side of the CE opposite the ion. Thus ''bulk solvation'' was 10 methanol or 19 water molecules. In addition to optimizing the geometry at B3LYP/6-311++G** level, we did frequency calculations to obtain the thermodynamic quantities. The errors average approximately 2 kcal/mole. The methanol complex formation values are, for K^+, −2.40kcal/mole (calculated), −1.80 kcal/mole (experimental); for Na^+, −5.4 kcal/mole(calculated), −2.2 or-3.0 kcal/mole (experimental, from 2 laboratories). For water, there is only the qualitative observation that complexes do not form; however, in the calculation, removing 7 water molecules results in only one shell of solvation. The free energy of complex formation is then negative, so that a complex could form. Since the system has over 100 atoms, it is large enough to model the interaction of a protein with an ion at the surface of the protein (see abstract of Kariev and Green: ''Quantum calculations on the KcsA channel 1/4rdquo;), suggesting that quantum calculation is useful in a case where polarizability and charge transfer, neither present in standard molecular dynamics, are important. (Acknowledgement for calculations: W.R. Wiley supercomputer facility of the EMSL at PNNL).

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Quantum Calculations on the KcsA Channel Cavity: N_A^+ and K^+ Solvation, and the Energetics of the Transfer of the Ion to the Selectivity Filter

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We have calculated the energy of the Na^+ and K^+ ions as solvated in the cavity of the KcsA channel, using the DFT method B3LYP, with basis set 6-31+G**. This method in a comparable calculation on a crown ether system for which the free energy of complex formation is known experimentally turned out in calculations (using frequency calculation for room temperature values) to be accurate to ±2 kcal/mole (but with a slightly larger basis set: see Kariev and Green abstract ''Quantum Calculations: A test of accuracy 1/4''). The transfer free energy of the two ions to the S4 (lowest) position of the selectivity filter can be calculated, and should therefore be approximately this accurate. The geometry of the hydrated ion in the center of the cavity agrees with the experimental X-ray structure (Zhou and MacKinnon, 2004). We are also calculating the barrier to the transfer from the center to the S4 position. We observe that the Na^+ ion can move slightly to the side of the S4 position, which helps in understanding the observation that Na^+ ion can block the channel. The solvation in the S4 position comes from the hydroxyl and carbonyl groups on the threonines that constitute the site, and we have determined the geometry of this structure as well. We will also show a first step toward a calculation of the salt bridges that we postulate are responsible for the open structure of the voltage sensing domain of a voltage sensitive K^+ channel. (Acknowledgement for calculations: W.R. Wiley supercomputer facility of the EMSL at PNNL).

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Immunomodulation of Voltage-Dependent K^+ Channels in Macrophages: Molecular and Biophysical Consequences

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Voltage-dependent potassium channels play a pivotal role in the modulation of macrophage physiology. Macrophages are professional antigen-presenting cells and produce inflammatory and immunoactive substances that modulate the immune response. Blockage of K_v channels by specific antagonists decreases macrophage cytokine production and inhibits proliferation. Numerous pharmacological agents exert their effects on specific target cells by modifying the activity of their plasma membrane ion channels. Investigation of the mechanisms involved in the regulation of potassium ion conduction is, therefore, essential to the understanding of potassium channel functions in the immune response to infection and inflammation. Here we demonstrate that the biophysical properties of voltage-dependent K^+ currents are modified upon activation or immunosuppression in macrophages. This regulation is in accordance with changes in the molecular characteristics of the heterotetrameric $K_v1.3/K_v1.5$ channels, which generate the main K_v in macrophages. An increase in K^+ current amplitude in LPS-activated macrophages is characterized by a faster C-type inactivation, a greater percentage of cumulative inactivation and a more effective Margatoxin inhibition than control cells. These biophysical parameters are related to an increase in $K_v1.3$ subunits in the $K_v1.3/K_v1.5$ hybrid channel. In contrast, DEX decreased the C-type, the cumulative inactivation and the sensitivity to Margatoxin concomitantly with a decrease in $K_v1.3$ expression. Neither of these treatments apparently altered the expression of $K_v1.5$. Our results demonstrate that the immunomodulation of macrophages triggers molecular and biophysical consequences in $K_v1.3/K_v1.5$ hybrid channels by altering the subunit stoichiometry.

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Functional KCNH1 Potassium Channels in Danio Rerio are Essential for Early Development

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The physiological role of KCNH1 channels has not been clarified in detail yet, but the predominant neuronal expression in mammals indicates a role in electrical signaling. The only described physiological function of KCNH1 so far is the promotion of myoblast fusion. Moreover, KCNH1 channels apparently enhance proliferation of cancer cells, thus exhibiting oncogenic potential. Many genes involved in cancerogenesis play a physiological role during embryonic development. To study KCNH1 channel function in this respect, we surveyed genomic databases of *Danio rerio*, a widely used vertebrate-development model organism, and found two putative *kcnh1* paralogs located on chromosomes 17 (*kcnh1a*) and 22 (*kcnh1b*). The corresponding amino acid sequences show >80% identity to that of human KCNH1. We observed both *kcnh1* genes to be endogenously expressed in different organs of adult fish and at early developmental stages, most prominently in neuronal tissues. We electrophysiologically analyzed the encoded channels by two-electrode voltage-clamp and patch-clamp techniques on *Xenopus* oocytes, showing the gene products to form functional potassium channels. The *Danio rerio* channels exhibited typical characteristics known from other species, such as dependence of activation kinetics on prepulse potential and extracellular Mg^{2+} concentration, as well as current inhibition by intracellular Ca^{2+}/calmodulin. After morpholino-mediated knockdown of the individual *kcnh1* paralogs in zebrafish embryos we observed severe effects on development. The morphants were retarded in growth, showed abnormal body curvature, exhibited complete body malformation or even died within less than 24 hrs after fertilization, depending on the degree of knockdown. The most remarkable phenotypes were alterations of brain structures like head edemas, incomplete brain growth and necrotic degeneration. Given the electrophysiological similarity of *Kcnh1* channels to the human homolog, we suggest that human KCNH1 potassium channels play a similar role during early embryonic development in humans.

Voltage-gated K Channels-Gating I

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Microtubule Dependent Mechanisms Regulate the Trafficking Deficient Phenotype of hERG Mutations Linked to Long QT Syndrome

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Type 2 Long QT syndrome (LQT2) is caused by mutations in the *human Ether-a-go-go Related Gene (hERG)*, which encodes the voltage-gated K^+ channel α-subunit that underlies the rapidly activating delayed rectifier K^+ current in the heart. Most LQT2 missense mutations decrease hERG trafficking to the plasmalemma. The trafficking-deficient LQT2 (tdLQT2) phenotype is characterized by a loss of hERG glycosylation and current. The purpose of this study was to test the hypothesis that multiple cellular mechanisms underlie the tdLQT2 phenotype. We studied the tdLQT2 mutations G601S- and R752W-hERG in stably expressed HEK293 cells, and found that G601S-hERG selectively colocalized with the Endoplasmic Reticulum (ER) and Golgi apparatus proteins calnexin and 58K. Nocodazole is an antimicrotubule agent that is used to study microtubule dependent trafficking of proteins between the ER and the Golgi apparatus. Nocodazole treatment (20 ∈ 1/4M, 18-24 hours) did not alter the functional expression of G601S-hERG or R752W-hERG, but it did alter the immunostaining and glycolytic processing

of G601S-hERG. After nocodazole treatment the G601S-hERG immunostaining pattern changed from perinuclear to one that consisted of focal aggregates located throughout the cytosol. Nocodazole treatment also increased the glycolytic processing of G601S-hERG similar to that of wild-type hERG. In contrast, nocodazole treatment did not alter the immunostaining or glycolytic processing of R752W-hERG. These data suggest that the tdLQT2 phenotype for G601S-hERG but not R752W-hERG is regulated by microtubule function. We conclude that microtubule dependent and independent mechanisms may regulate the tdLQT2 phenotype.

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Mink Dictates the Alpha Subunit Composition of Surface-Expressed N-Type Potassium Channels

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Homomeric and heteromeric complexes formed by members of the Kv3 subfamily of voltage-gated potassium (Kv) channel alpha subunits generate currents essential for the high-frequency firing of mammalian neurons. Kv3.1 and Kv3.2 alpha subunits generate delayed rectifier currents, whereas Kv3.4 generates fast-inactivating currents. This 'N-type' fast-inactivation occurs via an N-terminal 'ball' domain, which blocks the channel pore directly after opening, preventing K^+ ion flux. Heteromeric channels containing Kv3.4 with either Kv3.1 or Kv3.2 exhibit N-type inactivation with a rate dependent upon the number of Kv3.4 alpha subunits in the tetramer. As Kv channel inactivation and inactivation recovery rates are important determinants of excitable cell action potential morphology and refractory period duration, the stoichiometry of these heteromeric complexes is expected to be tightly regulated. Here, using channel subunits cloned from rat and transiently expressed in CHO cells, we show that Kv3.4 current is significantly suppressed (>90%) by the ancillary beta subunit MinK (KCNE1) and that the suppression can be rescued by co-expression of Kv3.1. Through use of dominant-negative pore mutants and N-terminal A and B box (NAB) intra-subfamily binding domain mutants, we demonstrate that MinK ensures that Kv3.4 alpha subunits can only reach the surface as part of a heteromeric complex with Kv3.1. Thus, by acting as a molecular matchmaker, MinK governs Kv channel inactivation rate and, potentially, cellular excitability and refractory periods.

622-Pos

Pharmacological-Induced Increase in the Functional Expression Of hERG Current

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The human Ether-a-go-go Related Gene (hERG) K^+ channel is linked to type 2 Long QT Syndrome (LQT2), and most LQT2 mutations decrease hERG current (I_{hERG}). Since LQT2 follows an autosomal dominant inheritance pattern, increasing the functional expression of WT-hERG may have therapeutic potential. The goal of this study was to identify ways to increase I_{hERG} without altering gating. We tested the hypothesis that nocodazole (noc), an antimicrotubule agent, and cytochalasin D (cytoD), an antimicrofilament agent, would increase I_{hERG} because they can alter the kinetics of protein trafficking to and from the membrane. We cultured HEK293 cells stably expressing WT-hERG in noc (20∈1/4M) or cytoD (5∈1/4M) for 18-22 hours and measured I_{hERG} using the whole-cell patch clamp technique. Using a holding potential of −80 mV, cells were pre-pulsed to 50 mV in 10 mV increments for 5 seconds, followed by a test-pulse to −50 mV for 5 seconds for control, noc, or cytoD treated cells. The peak I_{hERG} measured during test-pulse was plotted as a function of the pre-pulse. The data were described using a Boltzmann equation to calculate the maximal current density (I_{MAX}), midpoint potential for activation ($V_{1/2}$), and the slope factor (k) for I_{hERG} activation. Noc treatment did not alter any of these parameters. CytoD treatment increased I_{MAX} (control=93 ± 8 pA/pF, n=5; cytoD=156 ± 15 pA/pF, n=7) but did not alter not $V_{1/2}$ or k. CytoD also did not alter the voltage-dependent rates of I_{hERG} deactivation (n=6, per group). These data suggest that the functional expression of WT-hERG is increased by inhibition of microfilaments but not microtubules. We conclude that targeting microfilaments and/or microfilament-dependent proteins may represent a novel strategy for increasing the functional expression of WT-hERG without altering I_{hERG} function.

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hERG Heteromeric 1A/1B and Homomeric 1A Channels Exhibit Differential Pharmacological Sensitivities

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The majority of hERG screens aiming to minimize the risk of drug-induced long QT syndrome have been conducted using heterologous systems expressing the hERG 1a subunit, yet both hERG 1a and 1b subunits contribute to the channels producing the repolarizing current I_{Kr}. Previous studies show that differences in gating in heteromeric 1a/1b vs. homomeric 1a channels markedly increase repolarizing current during the ventricular action potential and protect against QT prolongation in computational models. We conducted a pharmacological analysis of 50 compounds targeting hERG channels and selected for their chemical diversity to evaluate differences in sensitivity that may influence safety margins or contribute to a stratified risk analysis. Experiments were carried out using the IonWorks™ plate-based electrophysiology device. Non-cumulative, 8-point concentration effect curves were generated, with each point representing data from 20 to 30 cells. Potency was determined as IC_{50} values (∈1/4M) obtained from data normalized to vehicle and 100% blocking levels and fitted to the Hill equation. To minimize possible sources of variability, compound potency was assessed using test plates arranged in alternating columns of 1a and 1a/1b cells. Although most compounds had similar potencies at both variants, some surprising differences were observed. For example, fluoxetine (Prozac) was 6-fold more potent at blocking hERG 1a/1b compared to 1a channels. The results were robust when compounds were tested against the hERG 1a and 1a/1b cell lines in parallel, but statistical analysis encompassing longitudinal variation indicates such differences may not be sufficient to warrant routine use of hERG 1a/1b in preclinical high throughput screens. However, our findings have uncovered several important candidates for further risk evaluation as we learn more about native subunit composition in different populations or changes in subunit composition during development.

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A New Mechanism for Long QT Syndrome: Polypeptides Encoded by hERG1a Non-Sense Mutations Regulate hERG1a/1b Channels

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hERG potassium channels are critical for cardiac action potential repolarization. Disruption of channel function by inherited mutations in the gene encoding hERG have been shown to cause type 2 long QT syndrome (LQT2) by perturbing trafficking, assembly, selectivity and activation gating. To date, most mutations have been studied in heterologous systems expressing the hERG 1a subunit, yet both hERG 1a and 1b subunits contribute to the channels producing the repolarizing current IKr. hERG 1a and 1b subunits are structurally identical except for the N terminal region, which is unique and much shorter in the 1b subunit. Differences in gating result in markedly increased repolarizing current in heteromeric 1a/1b vs. homomeric 1a channels during the ventricular action potential and protect against QT prolongation in computational models. We examined nonsense mutations giving rise to truncations at different points in the amino terminus of hERG 1a. Surprisingly, these fragments had little or no effect on maturation of hERG 1a and 1b subunits expressed in HEK-293 cells. Instead, they altered gating and increased rectification as if the channels were homomers of hERG 1a subunits. Thus, by ''complementing'' the hERG 1b subunit (and its short N terminus), the mutant 1a fragments reduce the repolarization capability of the channel and mediate a novel mechanism of type 2 long QT syndrome.

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The Eag Domain Regulates Outward Current Density and Recovery from Inactivation of the hERG K^+ Channel Through a Non-Covalent Interaction

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The Human Ether-á-go-go Related Gene (hERG) encodes a voltage-activated K+ channel. hERG contributes to repolarization of the ventricular action potential as the primary component of the cardiac delayed rectifier K^+ current (I_{Kr}) and has also been shown to modulate neuronal firing frequency. hERG gating is characterized by rapid inactivation upon depolarization and rapid recovery from inactivation and slow closing (deactivation) upon repolarization. These factors combine to create a resurgent hERG current, where the amplitude of the current is paradoxically larger with repolarization than with depolarization. These gating transitions also determine the timing and amplitude of the resurgent current. Previous data has suggested that the hERG N-terminus regulates gating kinetics, however the molecular mechanisms are not fully understood. Deletion of the N-terminus (amino acids 2-354) has been shown to speed channel deactivation and recovery from inactivation compared to that of wild-type hERG. Relative outward current amplitude is also increased during the depolarization phase of N-truncated channels, leading to reduced current