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Molecular Basis of Coupling Between the DIII Voltage-Sensor and Pore of a Sodium Channel

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The activation of voltage-sensors, upon depolarization, leads to the opening of pore gates in a voltage-dependent sodium channel. To elucidate the molecular principles underlying this conformational coupling, we have investigated a putative gating interface in domain III of the sodium channel using voltage-clamp fluorimetry and tryptophan-scanning mutagenesis. Most mutations have similar energetic effects on voltage-sensor activation and pore opening. However, several mutants stabilized the activated voltage-sensor while concurrently destabilizing the open pore. When these mutants were mapped onto a homology model of the sodium channel, most of them were localized to hinge regions of the gating interface. Our analysis shows that these residues are involved in energetic coupling of the voltage-sensor and the pore when both are in the resting or activated conformation. These results support the notion that electromechanical coupling in a voltage-dependent ion channel involves movement of rigid helical segments connected by elastic hinges.

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The Biophysical Costs Associated With Tetrodotoxin Resistance in the Garter Snake, Thamnophis Sirtalis

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Tetrodotoxin (TTX) is a potent toxin that specifically binds to voltage gated sodium channels (NaV). TTX binding physically blocks the flow of sodium ions through NaV, thereby preventing action potential generation and propagation. Populations of the garter snake, Thamnophis sirtalis, have evolved TTX resistance by substituting amino acid residues in the highly conversed domain IV P-loop of NaV1.4, allowing them to feed on tetrodotoxic newts. Different populations of the garter snake have different degrees of TTX-resistance closely related to the number of amino acid substitutions. Here, we tested the voltage dependence, kinetics, and ion selectivity of NaV1.4 containing sequences from different garter snake populations. Using Xenopus oocytes under cut-open voltage clamp, we observed significant changes in voltage dependent gating properties of the TTX resistant NaV. The most TTX-resistant channel had hyperpolarized activation midpoint (−6 mV) compared to TTX-sensitive channels. Fast inactivation of the TTX-resistant NaV had significant depolarizing shifts of 2.2 mV in mildly TTX-resistant channels, and 4.5 mV in strongly TTX-resistant channels. Depolarizing shifts in slow inactivation were also observed in TTX-resistant channels with 20 mV and 16 mV shifts in mildly resistant and strongly resistant channels, respectively. In addition, ion selectivity and permeability of TTX-resistant channels were significantly different from those of the TTX-sensitive channel. These results suggest TTX resistance comes at a cost to channel performance caused by changes in the gating properties and ion selectivity of TTX-resistant sodium channels.

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A Cation-Pi Interaction in the Cardiac Sodium Channel Local Anesthetic Receptor Discriminates Between Antiarrythmics

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Voltage-gated sodium channels generate the upstroke of the cardiac action potential. Anti-arrhythmic and local anesthetic compounds inhibit these channels and are widely employed to modulate cellular excitability. Previous studies have shown that two conserved aromatic residues in DIVS6 are crucial for binding of antiarrythmics as their mutation to non-aromatic side-chains reduces drug affinity. Furthermore, it has been shown that in the skeletal sodium channel isoform, the aromatic residue Phe1579, but not Tyr1586, forms a cation-pi interaction with lidocaine or QX-314. However, it remains unclear if this cation-pi interaction is conserved in the cardiac sodium channel isoform and if it is a general trait of anti-arrhythmic drugs. In this study we employ in vivo nonsense suppression to incorporate a series of fluorinated phenylalanine derivatives at two homologous positions in the cardiac sodium channel Nav1.5, Phe1760 and Tyr1767, to determine if the functionally and structurally related compounds lidocaine, mexiletine, flecainide and ranolazine bind to Nav1.5 via a cation-pi interaction. Our results show that Phe1760 forms a cation-pi interaction with lidocaine and mexiletine, but not flecainide or ranolazine. Furthermore, none of the compounds tested formed a cation-pi interaction with Tyr1767. These findings present a significant step towards deciphering the subtle differences that determine the binding modes of functionally and structurally related anti-arrhythmic compounds to cardiac sodium channels.

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Use-Dependent Block of Nav1.4 Human Paramyotonia Congenita Mutation By Ranolazine

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Ranolazine, an anti-anginal drug, has been shown to bind to inactivated state(s) of the cardiac (Nav1.5) and peripheral neuron (Nav1.7 and Nav1.8) Na^+ channels and cause use-dependent block (UDB).The drug also reduces persistent (late) current caused by Nav1.5 gain-of-function Na^+ channel mutations. We tested the hypothesis that ranolazine would have similar effects on the skeletal muscle wild-type (WT) Na^+ channel, Nav1.4, and on the paramyotonia congenita gain-of-function mutation, R1448C, which is associated with sustained firing of action potentials and long-lasting involuntary contractions leading to muscle stiffness. Whole-cell I_{Na} was recorded from HEK293 cells transiently expressing the human Nav1.4 WT and R1448C channels. At a holding potential of −140 mV, ranolazine (3-100 μM) caused UDB (10 and 30 Hz) of WT and R1448C I_{Na} with half-maximal inhibitory concentrations (IC_{50}) of 63.9 ± 4.8 (n=3-5 cells) and 20.3 ± 2.1 μM (n=4 cells) for WT and 63.7 ± 5.4 (n=3-5 cells) and 20.9 ± 2.4 μM (n=3-7 cells) for R1448C, respectively. UDB (10 Hz) of R1448C I_{Na} increased when the holding potential was changed from −140 to −90 mV (IC_{50}= 10.8 ± 1.7 μM, n=5-6 cells). Ranolazine (10-100 μM) caused a concentration-dependent hyperpolarizing shift in the voltage-dependence of intermediate and slow inactivation of WT (n=3-7 cells) and R1448C (n=3-7 cells) I_{Na}. Ranolazine (30 μM) slowed the recovery from inactivation of both WT (n=3 cells) and R1448C channels (n=5 cells). An increase of depolarizing pulse duration from 2 to 20 msec did not affect the UDB of WT (n=4 cells) or R1448C (n=4-5 cells) $I_{Na}$by 30 μM ranolazine. The data suggest that ranolazine blocks the open state and interacts with the inactivated states of Nav1.4 WT and R1448C channels. The state and UDB of Nav1.4 channels by ranolazine may be useful to inhibit sustained action potential firing, as seen in paramyotonia congenita.

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Cardiac Dynamics In-Silico: Pharmacological Targeting of Long QT3 Syndrome

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The treatment of cardiac arrhythmia is largely empirical due to incomplete understanding of antiarrhythmic drug-receptor dynamics. However, our laboratory has recently determined that drug receptor interactions of commonly prescribed local anesthetics (LAs) can be described using theoretical models based on a limited number of experimentally determined parameters. Here, we use experimental function data from cardiac Na^+ channels harboring disease-linked mutations to build models that simulate measured properties of drug interactions with mutant channels. We developed computational models of 3 closely linked LQT3 mutants: deltaKPQ, D1790G, and Y1795C that account for 1) mutant-specific biophysical channel gating characteristics including mean open time and persistent Na^+ current; 2) drug partitioning that underlies situation-dependent blockade; and 3) drug sensitivity to LAs that matches experimental data for steady-state inactivation (SSI), tonic block (TB), concentration and frequency dependence of use-dependent block (UDB), and recovery from UDB. Experimental 30υM flecainide block of D1790G induces a 5.5 ± 1.4mV hyperpolarizing shift in SSI, similar to our simulations (6.25mV). Experimental fits to TB reveal an IC_{50} of 48υM (simulations: 53.5υM), and for UDB, an IC_{50} of 1.7υM (simulations: 2υM). Frequency dependence of UDB is in excellent agreement with experimental data over a broad range of pacing frequencies (1 - 10Hz). We have built similarly predictive models of LA interactions for deltaKPQ and Y1795C. The *in-silico* platform that we have developed to predict drug-receptor interactions can be used to predict how subtle alterations to gating resulting from the mutations, as well as differential sensitivities to LAs, predispose patients to under- or overly-effective therapy with commonly prescribed antiarrhythmic drugs.