III and IV (DIII-IV) and their putative receptor in the pore region of the channel. Introduced or acquired mutations in this complex lead to defective channel inactivation, yet the precise structural basis for these interactions has not yet been fully described. Using the photocrosslinking (PC) unnatural amino acid p-benzoyl-L-phenyl alanine (pBpa) it is possible to trap close structural interactions (~3 Å) during transient conformational rearrangements thus mapping structural reorganisations associated with different states. The rates at which crosslinking reactions occur can report on the affinity of such interactions. We have incorporated the pBpa in mammalian cells at the putative inactivation peptide (IFMT) motif of the DIII-IV linker and demonstrated state dependent trapping of the transient conformations associated with fast inactivation, with different phenotypes and rates of trapping revealing distinct environmental changes at each site. Incorporation of pBpa at two tyrosine residues, Y1494 and Y1495 of NaV1.5, which have previously been implicated in the coupling of activation and inactivation and to interact with Calmodulin produced channels with normal gating and robust expression. Interestingly, the observed state dependent trapping upon irradiation with UV light during patch clamp experiments was functionally distinct from the state-dependent cross-linking interactions observed within the IFM locus. Thus, trapping at these sites suggests a more extensive involvement of the DIII-DIV linker in fast inactivation gating beyond the canonical IFMT motif.

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Differential Lipid Dependence of Function of Bacterial Sodium Channel Homologues

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The lipid bilayer is important for maintaining the integrity of cellular compartments and plays a vital role in maintaining the hydrophobic/charged interactions necessary for structure, conformational flexibility and function. Despite the intimate relationship between ion channels and the membranes in which they are embedded, challenges resulting from the dynamic and complex nature of cellular membranes have limited our ability to address the functional role of these interactions. To directly assess lipid dependence of activity, we examined channel function ofthree purified bacterial sodium channel orthologues (NaChBac, NavMs, and NavSp) by cumulative $^{22}Na^+$ uptake into proteoliposomes containing a 3:1 ratio of POPE and another glycerophospholipid (POPC, POPG, POPS, Cardiolipin (CL), POPA, or PI). We observed a unique lipid dependence for each homologue tested. Common to each was a low level of activity above background (uptake into protein free liposomes) when the second lipid was a zwitterionic lipid such as POPE and POPC. Maximal activity for full-length NaChBac and NavMs proteins was observed in POPE + POPG liposomes. On the other hand, full-length NavSp channels possessed a different lipid dependence, with maximal activity in liposomes containing POPE + PI. No strong lipid dependence was observed for pore-only constructs of NavMs or NavSp, that lacked the S1-S4 segments, suggesting that the lipid dependence of sodium channels may arise from their abilities to affect the voltage-sensing domains. The effect may be maximized by specific lipid-protein interactions that are uniquely favourable in each homologue, giving rise to differing lipid dependences.

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Calcium-Mediated Tailspin of Calmodulin on the IQ Motif of the Neuronal Voltage-Dependent Sodium Channel $Na_v1.2$

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$Na_V1.2$ is regulated by calmodulin (CaM), an essential calcium sensor with two homologous domains (N and C). CaM binds tightly to an IQ motif in the intracellular C-terminal tail of the pore-forming alpha subunit of $Na_V1.2$ as well as to the inactivation gate. The IQ motif interacts with the ''semi-open'' cleft of apo CaM: I inserts into the cleft, while Q contacts the FG-turn of CaM (2KXW). To learn how CaM-IQ responds to increases in intracellular $[Ca^{2+}]$, we determined equilibrium constants for apo and calcium-saturated CaM binding to biosensors containing mutated IQ motif sequences sandwiched between YFP and CFP. Their quantum yields permit resolution of K_d values close to 1 nM from equilibrium titrations. Changes of $Na_V1.2$ residues making close contacts with apo CaM were anticipated to diminish binding of both apo and calcium-saturated CaM. However, the quantitative effects differed by orders of magnitude. The affinity for calcium-saturated CaM dropped by factors of 10-100, while effects on apo CaM binding were more severe. Thus, the CaM-IQ interface differs dramatically depending on calcium-saturation of CaM. NMR studies of a complex of $(Ca^{2+})_2$-CaM-C-domain bound to the IQ motif showed that calcium binding opens CaM but also causes it to pivot by 180° so that it binds to the IQ motif in the opposite direction. The I of the IQ motif contacts the ''open'' hydrophobic cleft of $(Ca^{2+})_2$-CaM, but the Q points towards the linker between the N- and C-domains of CaM. In conjunction with 2KXW, this new structure provides the first pair of high resolution structures for apo and calcium-saturated CaM bound to a single IQ motif. *Support: NIH R01 GM57001 and Carver Charitable Trust Grant 01-224.*

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Molecular Determinants for the Genesis of the Action Potential

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In nerves and muscles, action potentials are initiated by the rapid activation of voltage-gated sodium (Nav) channels and terminated by the delayed activation of voltage-gated potassium (Kv) channels. This sequential activation, which is the prerequisite for the genesis of the action potential, requires faster activation kinetics of the voltage-sensor domains (VSD) in Nav as compared to Kv channels. Despite on decades of investigations, the molecular determinants and mechanisms underlying this phenomenon remain elusive. Here, we show that this differential gating is mostly imparted by six conserved hydrophilic residues Thr or Ser located in the S2 and S4 segments of the VSD in domains I-III of Nav channels while these positions are commonly occupied by hydrophobic residues in the VSD of Kv channels. Hydrophilic substitutions at the S2 position in the Shaker Kv channel accelerate the gating charge transfer by decreasing its energy barrier while hydrophilic substitutions at the S4 position speed up VSD activation by destabilizing its resting conformation. Interestingly, these hydrophilic residues are present in a Nav-related gene expressed in an evolutionary-distant unicellular choanoflagellate, suggesting that rapidly-gated Nav channels evolved before the emergence of metazoans and their nervous systems. We also show that the physiological co-expression of the ubiquitous regulatory β1 subunit further accelerates VSD movement in both a neuronal and a muscular Nav, providing a molecular basis for the β1-dependent fast gating mode previously detected from the ionic conductance. Our study uncovers the fundamental molecular determinants and possible mechanisms that enabled the differential gating in sodium and potassium channels and the emergence of the action potential. This work was supported by NIH grant GM030376.

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Development and Characterisation of Cardiomyocytes Derived from Murine Embryonic Stem Cells of a DCM Caused by a SCN5A Mutation

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Introduction: Mutations on Nav1.5 sodium channels have been reported recently in patients with complex cardiac arrhythmias associated with Dilated cardiomyopathy (DCM). Nav1.5 channels are encoded by SCN5A gene and allow the action potential generation in the heart. Its involvement in structural heart disease such as DCM is not well elucidated. We recently identified a novel mutation (R219H) on this channel protein to cause DCM associated with a complex arrhythmic phenotype Gosselin-Badaroudine P., et al., (2012): PlosONE, 7(5):e38331. The biophysical characterisation in Xenopus oocytes expression system revealed a proton (H+) leak at hyperpolarised voltages through the mutant protein. The aim of this study is to develop a cellular model based on the differentiation of murine stem cells carrying the R219H mutation to characterise the H+ leakage in physiological conditions.

Methods: Murine stem cells differentiated to cardiomyocytes were dissociated and the H+ leak current is measured by the patch clamp method. The morphology of the derived cardiomyocytes was investigated by immunocytochemistry. Intracellular pH and acidification is also observed through fluorescence techniques (BCECF).

Results and conclusion: The patch clamp experiments confirmed the presence of a H+ leak at hyperpolarized voltages due to the R219H mutation. After maturation, the use of typical sarcomere markers (myosin light chain 2v