metabolite, had a higher affinity than fluoxetine, with an IC50 of 29 μM. Fluoxetine inhibited currents in a frequency-dependent manner, shifted steady-state inactivation to more hyperpolarized potentials, and slowed the recovery of $Na_v1.5$ channels from inactivation. Mutating a phenylalanine (F1760) and a tyrosine (Y1767) in DIV S6, two amino acid residues known to be essential for class 1 antiarrhythmic drug binding, significantly reduced the affinity of fluoxetine and its frequency-dependent inhibition. We used a non-activating $Na_v1.5$ mutant to show that fluoxetine displays open-channel block behavior. We concluded that fluoxetine blocks $Na_v1.5$ channels by binding to the class 1 antiarrhythmic site. The blocking of cardiac Na^+ channels should be taken into consideration when prescribing fluoxetine alone or in association ith other drugs that may be cardiotoxic or for patients with conduction dsorders.

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Protein-Protein Interactions Based Drug Discovery Against the Voltage-Gated Sodium Channel
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The pore-forming α subunit of the voltage-gated Na^+ (Nav) channels (Nav1.1-Nav1.9) provides the basis for electrical excitability in the brain. These Nav channels are regulated by a number of accessory proteins. One of the important regulator of Nav channels is intracellular fibroblast growth factor 14 (FGF14), a member of not-secreted intracellular FGFs (iFGFs; FGF11-13). This iFGF14 acts as an allosteric modulator of the Nav channel and regulates neuronal excitability by controlling Nav channel gating properties and expression.
In pursuit of drug discovery against the FGF14-Nav1.6 channel complex, we are combining molecular modeling to design peptide-fragments of FGF14, split-luciferase complementation assay (LCA) to evaluate the potency of these peptides, and electrophysiology to further validates these peptides as disrupters of the FGF14/Nav1.6 channel complex. We already have created a FGF14 dimer homology model based on the FGF13 dimer crystal structure and have showed with in-cell LCA that the FGF14 monomer: monomer interface overlaps with the FGF14/Nav channel interface. We have also identified key amino acids in proximity to the FGF14/Nav1.6 interface and show that mutations at these sites affect the Nav channel assembly with the FGF14 monomer and control the access of small fragment peptides to the interface. Based on our preliminary data, we hypothesize that FGF14-peptide fragments mimicking the FGF14 protein will compete with FGF14 for binding to Nav channels and serve as potential chemical leads for drug development against Nav channels. The proposed small peptides based on the FGF14:Nav1.6 protein-protein interactions act as allosteric modulators for the Nav channel functions. These results will provide fundamental new knowledge for the design of new leads targeting the FGF14/Nav channel complex as a novel drugs target interface.

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Specificity of Calmodulin Recognition of Human Voltage-Gated Sodium Channels
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Voltage-dependent sodium channels (Nav1.X) control the rising phase of action potentials in excitable cells. Mutational pathologies include epilepsy, Long QT syndrome, familial autism, and pain insensitivity. This family of ion channels is regulated by calmodulin (CaM), a small, essential eukaryotic calcium sensor that contains two highly homologous domains whose affinities for calcium differ by an order of magnitude. CaM is known to recognize at least two regions of NaV: the highly conserved inactivation gate between transmembrane domains III and IV, and an IQ motif, located in the intracellular C-terminus of the Nav. The IQ motif, which may serve as a CaM "sink", holds CaM available to quickly re-associate with the inactivation gate upon calcium binding. High-resolution structures of apo CaM bound to the IQ motifs of NaV1.2, 1.5 and 1.6 show many similarities. However, the free energies of CaM binding to these motifs are markedly different. In our studies of CaM binding to the IQ motif of NaV1.2, the C-domain of both apo (calcium-free) and calcium-saturated CaM bind and calcium binding to CaM lowers its affinity for the IQ motif. To better understand calcium-mediated feedback control, we conducted thermodynamic analyses of CaM binding to the IQ motif sequences representing all 9 members of the human Nav family and the consensus inactivation gate. CaM binding was detected by monitoring the loss of FRET intensity of a biosensor containing the IQ motif sandwiched between two YFP and CFP. The nine sodium channels split into two classes based on their binding affinity for apo CaM at the IQ motif. We are exploring the roles of individual residues to determine the positions that are necessary and sufficient to confer preferential binding of apo or calcium-saturated CaM. Support: NIH R01 GM57001.

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Block of Na^+ Currents and Suppression of Action Potentials in Cultured Hippocampal Neurons by Gs-458967
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Voltage-gated Na^+ channels (NaChs) are common targets for anti-epileptic drugs due to their role in the initiation and propagation of action potentials in central neurons. NaCh blocking agents, such as phenytoin and lamotrigine, act by blocking peak Na^+ current (INa) in voltage- and frequency- dependent manner. A recent study showed that GS-458967 (GS967) is a potent blocker of cardiac INa. However, the effects of GS967 on neuronal INa and action potentials were not examined. Thus, using isolated rat cultured hippocampal neurons, effects of GS967 on neuronal INa and excitability were determined. GS967 (10-1000 nM) caused a voltage- (−60 mV) and frequency (10 Hz)-dependent block of INa with IC50 values of 40.6 ± 4.0 (n=4, at each concentration) and 477.0 ± 53.0 nM (n=4, at each concentration), respectively. However, tonic block of INa was minimal (7.6 ± 0.5 % at 1000 nM, n= 4). GS967 decreased the sustained repetitive firing (SRF) in response to a 1-sec depolarizing current injection with an IC50 value of 47.8 ± 2.8 nM (n=3-4, at each concentration). Consistent with minimal tonic block of INa, the compound had no effect on the amplitude of the first action potential during SRF (2.2 ± 0.8 % at 300 nM). Furthermore, GS967 reversibly reduced the epileptiform activity induced by NMDA activation (removal of extracellular Mg^{2+}) with an IC50 value of 50.7 ± 1.4 nM (n=3-5, at each concentration). In addition, the effects of GS967 on ligand-gated ion channels that are essential for synaptic inhibition or excitation were tested. GS967 (1000 nM) had no effect on GABA-activated currents (4.2 ± 3.4%, n=8). At 1000 nM, GS967 inhibited NMDA-activated currents by 18.2 ± 6.2% (n=5). Taken together, these findings provide preliminary evidence that GS967 could be effective in controlling abnormal neuronal firing.

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Cardiac Sodium Channel Display Coupled Gating
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Objective: Mutations in *SCN5A*, the gene encoding the cardiac sodium channel, have been linked to many inherited cardiac arrhythmias. We have shown the existence of dominant-negative mutations in Brugada Syndrome due to interactions between alpha-subunits. Here we investigated whether cardiac sodium channel alpha-subunits also display coupled gating in addition to interacting.
Methods: Biophysical properties were studied by patch-clamp analysis in the whole-cell configuration.
Results: We used a non-trafficking mutant which can be rescued with mexiletine, unveiling a +15 mV shift in activation compared to WT. When this non-trafficking mutant was co-expressed with WT, we still saw a shift in activation compared to WT alone, even though the mutant is not conducting. Therefore these results demonstrate that the presence of the mutant, affects the activation of the WT suggesting a gating cooperation between the 2 channels. Then we wanted to address whether the inactivation gating process was also synergic between two sodium channel subunits. We used a channel where the C-terminus was deleted and where inactivation was affected. Expression of this delta-Cter alone showed altered inactivation kinetics. Surprisingly, when co-transfected with the trafficking-competent but gating-deficient mutant channel R878C, the inactivation properties of the mutant were partially rescued. Altogether these data suggest that the presence of the full C-terminal region provided by the nonfunctional channel R878C could rescue the inactivation defect of the delta-Cter channel.
Conclusions: Mutations impairing either activation or inactivation of one channel can affect the gating properties of the other WT alpha-subunit demonstrating coupled gating properties between sodium channel alpha-subunits.

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The Trpa1 Agonist Cinnamaldehyde Acts as a Local Anesthetic Inhibiting Voltage-Gated Sodium Channels in Sensory Neurons
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Cinnamaldehyde (CA) is a highly reactive compound that has been used in experimental models of neurogenic inflammation as specific agonist of the