produced a-10 mV shift in the availability curve, slowed recovery from inactivation and increased the late Na current ($I_{Na\text{-}L}$). These changes were prevented by inclusion of the CaMKII inhibitor peptide, AIP in the pipette or partially prevented by exposure to the PKA inhibitor PKI. $Na_V1.5$-S571A, $Na_V1.5$-S526H and $Na_V1.5$-S528A channels eliminated the CaMKII induced shift in inactivation, slowing of recovery and increase in $I_{Na\text{-}L}$ S571D or S528D mimicked the effects of phosphorylation. In $Na_V1.5$-S1969A channels exposure to CAMKII increases $I_{Na\text{-}L}$ compared to $Na_V1.5$-WT with a further increase in $I_{Na\text{-}L}$ in the presence of CaMKII; however, the steady state inactivation curve is not shifted. These data suggest the presence of functional phosphorylation sites in the CT and the interaction of CaMKII and PKA in the I-II linker. The cross talk between CaMKII and PKA modulation of the channel may have important implications for electrophysiological properties of the heart.

2917-Pos Board B347
Recruitment of Calmodulin to the Tail of the Voltage-Gated Sodium Channel Nav1.2
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Voltage-gated sodium channels (NaV) found in excitable cells are responsible for the rising phase of action potentials. These multi-domain transmembrane proteins are regulated by calmodulin (CaM), a highly conserved eukaryotic protein that mediates many calcium-triggered signaling events. Inactivation of sodium channels depends on CaM-mediated feedback during repolarization. In the neuronal sodium channel NaV1.2, CaM binds at least two well-separated sites: an intracellular ''inactivation'' loop between domains DIII and DIV, and an IQ motif [IQRAYRRYLLK] in the cytosolic C-terminal tail. The IQ motif is hypothesized to recruit calcium-free (apo) CaM, making it available to move to the III-IV linker after an influx of calcium. Despite a high degree of sequence identity, the equilibrium constants for CaM binding to nine human NaV IQ motifs span more than 3 orders of magnitude. Apo CaM binds to the NaV1.2 IQ motif with a dissociation constant (Kd) of ~6 nM, while the Kd for binding the NaV1.9 IQ motif is ~ 4 μM. Mutational analysis within the IQ motif has not been sufficient to explain the full range of CaM-binding affinities observed for human NaV sequences. Thus, we hypothesized that isoform-specific differences in upstream sequences were making energetically significant contributions to the free energy of binding CaM to NaV1.2. The roles of these residues are being investigated by monitoring CaM binding to biosensors containing mutant sequences of sodium channels bracketed by auto-fluorescent proteins YFP and CFP. Residue-specific information obtained by NMR will provide structural insight into the contributions of residues in the binding interface formed by NaV IQ motif sequences binding to calmodulin from multiple eukaryotes. NIH R01 GM57001.

2918-Pos Board B348
Coupling Compartmental Models to Live Neurons to Investigate Action Potential Mechanisms
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In mammalian central neurons, action potentials are initiated in the axonal initial segment (AIS) by Nav1.2 and Nav1.6 channels, and shaped and terminated by other voltage-gated ion channels. From the AIS, the AP travels down the axon towards the presynaptic site, but also back-propagates towards the soma. The role of axonal sodium channels in AP initiation and propagation is still incompletely understood, mostly because it is difficult to record from these channels at the AIS. Instead, most experimental evidence of axonal activity is obtained indirectly, from electrical recordings at the soma. To better understand these mechanisms, we developed a real-time computational procedure where a compartmental model of the axon is coupled to a live neuron using dynamic clamp. The properties of this computational model (e.g., spatial distribution and kinetics of ion channels) are varied until the firing activity of the hybrid construct (neuron + axon compartmental model) best matches the normal activity of the neuron.

2919-Pos Board B349
Optimizing a Nav1.5 Markov-Model with a Genetic Algorithm
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Background: Markov models of cardiac voltage-gated Na^+ (Na_V) channels have been widely used for cell and tissue simulations of cardiac electrophysiology. These $Na_V1.5$ models are of varying complexity. Simple models with few states cannot account for all of the kinetics observed with multiple protocols. Increasingly complex models become computationally infeasible for multi-scale simulations and their results are difficult to interpret.
Methods: Following previously published work for the neuronal Na^+ channel, we have implemented a genetic algorithm to optimize model topology and rate parameters for the cardiac sodium channel. The advantage of this approach is a flexible topology, with unnecessary states and edges removed in favor of model plausibility and computational speed. We improved this model by parallelizing the computation and including subpopulations with random mutation rates. Patch-clamp data for model parameterization was collected by recording transiently transfected HEK293T cells expressing $Na_v1.5$ channels 24 hours post-transfection. The temperature of the recordings was controlled at various temperatures ranging from room temperature to physiological temperatures. Models were successfully fit to patch-clamp protocols, consisting of activation curves, inactivation, recovery from inactivation, and activation current traces.
Results: The final optimization employed 32 subpopulations each with 50 members and was run for 2000 generations. After 2 x 10^6 models were evaluated, the algorithm yielded a model composed of 8 states and 10 interconnecting edges. The model was able to reproduce 20ms, 100ms, and 1000ms inactivation holds, activation, and recovery from inactivation protocols with high fidelity. Fast kinetic data was reproduced by fitting traces for −30mV, −10mV, and 10 mV directly. This novel model fits a wide range of experimentally collected data and contains significantly fewer parameters than current, widely used models.

2920-Pos Board B350
Rate Constant Models cannot Describe Movement of Charged Atoms or Molecules
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Rate constant models built on laws of mass action are used widely to describe movement of ions and electric current through channels, and chemical reactions of charged substrates. But mass action laws are derived from conservation of mass and say nothing about electric charge. Laws of electricity (Maxwell's equations: a generalization of Kirchoff's current law) are about conservation of charge, not mass. In classical rate models, flows of ions are not correlated by laws of electricity. But Maxwell's equations strongly correlate flows of charge (including displacement current), **without known exception**, within one part in 10^{18} or so. Correlation coefficients (describing correlated flows of charges in rate models) should then be nearly one, something like 0.999 999 999 999 999 999. Correlations of charge movement are ignored in classical rate models, so **classical rate models cannot describe movements of charged atoms or molecules with one set of rate constants over a range of conditions**.
A proof goes like this:
Consider the spatial series of reactions
X <==>**Y** <==>**Z**
Currents in a series of reactions analyzed by mass action are not (in general) equal:
$I_{XY}/F = z_X k_{XY}$ **[X]**-$z_Y k_{YX}$ **[Y]**; $I_{YZ}/F = z_Y k_{YZ}$**[Y]**-$z_Z k_{ZY}$**[Z]**
Kirchoff's current law requires $I_{XY} = I_{YZ}$ under all circumstances and conditions.
Details can be found at http://arxiv.org/abs/1409.0243 on the physics archive. The artifactual difference I_{XY} - I_{YZ} can have large effects**.** It can produce net charge and electric fields strong enough to break down membranes, proteins, chemical bonds, and even ionize atoms, because of the enormous strength of the electric field, as described unforgettably in p.1-1, of "***Feynman's Lectures on Physics, Vol. 2, Mainly Electromagnetism***…" http://www.feynmanlectures.caltech.edu/II_toc.html.

Voltage-gated Ca Channels

2921-Pos Board B351
Targeting T-Type Channels with Protxii-Like Toxins
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Few gating-modifier toxins have been reported to specifically target T-type calcium channels, and the structural basis of toxin sensitivity remains incompletely understood. Unlike the homotetrameric Kv channels, voltage-gated calcium channels are comprised of four different domains, presenting the possibility of multiple toxin binding sites. Studies of Kv channels identified a S3b-S4 helix-turn-helix motif, termed paddle motif, which moves at

the protein-lipid interface to drive activation of the voltage-sensors. This motif is an important pharmacological target for amphipathic neurotoxins and it has been suggested that it is conserved in other voltage-gated ion channels. Here we show that the four S3b-S4 paddle motifs within the T-type calcium channel could be transplanted into four-fold symmetric Kv channel to individually examine their contributions to the kinetics of voltage sensor activation and pharmacology. Using these chimeric constructs, we screened existing gating-modifier toxins against the putative paddle motif from each domain of T-type calcium channel, Cav3.1. We found that the four individual paddle motifs of Cav3.1 channels display unique toxin binding capabilities, suggesting that gating-modifier toxins can bind to T-type calcium channels in a domain-specific fashion. Comparing ProTx-II-like toxins effect on T-type calcium channels and chimeras suggests potential amino acids involved in the direct interaction between toxin and channels.

2922-Pos Board B352

Engineering Selectivity in RGK Protein Inhibition of Cav1/Cav2 Channels

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High-voltage activated calcium channels ($Ca_V1.1$–$Ca_V1.4$; $Ca_V2.1$–$Ca_V2.3$) link electrical signals to vital physiological responses in excitable cells. Molecules that block Ca_V1/Ca_V2 channels are important therapeutics. Rad/Rem/Rem2/Gem (RGK) proteins are small Ras-like G-proteins that potently and indiscriminately inhibit all Ca_V1/Ca_V2 channels. The practical utility of RGKs as genetically-encoded Ca_V channel blockers would be vastly improved if it were possible to engineer versions that display selective inhibition of distinct Ca_V1/Ca_V2 isoforms. Ca_V1/Ca_V2 pore-forming α_1 subunits require binding to auxiliary $Ca_V\beta$s to generate functional channels. All RGKs bind wild-type (wt) $Ca_V\beta$ and this interaction is disrupted in a mutated $Ca_V\beta$ ($Ca_V\beta_{TM}$). We compared the ability of Rem and Gem to inhibit four distinct Ca_V1s ($Ca_V1.2$, $Ca_V1.3$, $Ca_V2.1$ and $Ca_V2.2$) reconstituted with either $wtCa_V\beta$ or $Ca_V\beta_{TM}$ in HEK293 cells. While both Rem and Gem blocked all channels reconstituted with $wtCa_V\beta$, Rem uniquely suppressed $Ca_V1.2+\beta_{2aTM}$ channels, a signature of $Ca_V\beta$-binding-independent inhibition. Using FRET analyses, chimeric Rem/Gem proteins, and electrophysiology we show that $Ca_V\beta$-binding-independent inhibition of $Ca_V1.2$ involves direct interaction of Rem C-terminus with $Ca_V1.2\alpha_{1C}$ N-terminus, and additionally requires the Rem nucleotide-binding domain. A mutant Rem that no longer interacts with $Ca_V\beta$ ($Rem_{\beta\text{-null}}$) selectively inhibited $Ca_V1.2+wtCa_V\beta$ channels. We further profiled the prevalence of β-binding-dependent and -independent mechanisms of inhibition by Rem2 and Rad across Ca_V1/Ca_V2 channel families. While Rem2 relied on $Ca_V\beta$ binding to inhibit all four Ca_Vs tested, Rad displayed β-binding-independent inhibition of $Ca_V1.2$ and $Ca_V2.2$. Consistent with this, $Rem_{\beta\text{-null}}$ selectively inhibited $Ca_V1.2$ and $Ca_V2.2$ channels reconstituted with $wtCa_V\beta$. In summary, our results have revealed a latent capability of distinct RGK proteins to block particular Ca_V1/Ca_V2 channels in an α_1-subunit-specific manner. We have exploited this feature to generate genetically-encoded Ca_V-isoform-selective inhibitors.

2923-Pos Board B353

Ventricular L-Type Ca^{2+} Channels and Expression of RGK Proteins in Mouse Models Associated with Diabetes

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Background: In a diabetic mouse model (db/db) we have shown reduced I_{CaL} density with unchanged single-channel activity and reduced expression of the LTCC pore $Ca_v1.2$ (Pereira et al., Diabetes 2006;55:608-15). Of note, LTCC expression and function can be decreased by RGK proteins, including the diabetes-associated protein Rad. **Aim of the study:** In the present study, we investigate the association between cardiac Rad expression with the expression and function of ventricular LTCC in two mouse models with diabetes-related metabolic disturbances (leptin-deficient obese ob/ob mice and insulin receptor substrate 2 deficient IRS2-k.o. mice). **Methods:** We obtained expression of Rad and $Ca_v1.2$ protein (Western-blot) and mRNA (qRT-PCR) in murine ventricles and recorded whole-cell I_{CaL} in freshly isolated ventricular myocytes. **Results:** The only significant change at the mRNA level was an increased Rad expression in IRS2-k.o. mice at 16 weeks of age ($206 \pm 17\%$). In line with this finding I_{CaL} density was significantly decreased (IRS2-k.o.: -7.8 ± 0.8 pA/pF; wildtype: -10.9 ± 0.9 pA/pF). At an age of 28 weeks, we found expression of both Rad and $Ca_v1.2$ protein to be significantly increased in ventricles from ob/ob mice compared to age-matched widtypes ($273 \pm 23\%$ and $159 \pm 14\%$, respectively) while I_{CaL} density was unchanged (ob/ob: -8.4 ± 0.4 pA/pF; wildtype: -8.9 ± 0.5 pA/pF). **Summary and discussion:** Our data support the idea of Rad being involved in regulation of I_{CaL} in diabetes. Regarding I_{CaL} Rad seems to act either causal (I_{CaL} decrease in IRS2-k.o. at 16 weeks) or compensatory (increased $Ca_v1.2$ expression but unchanged I_{CaL} in ob/ob at 28 weeks). Differences observed between the two investigated diabetic mouse models might be explained by differences in the underlying pathomechanisms (lack of IRS2 vs. leptin deficiency, respectively).

2924-Pos Board B354

Inhibition of Human $Ca_V2.3$ Channels via μ-, δ- and κ-Opioid Receptor Activation

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Neuronal voltage-gated $Ca_v2.3$ channels are widely expressed in the central and peripheral nervous system where they contribute to neurotransmission and pain sensation. However, modulation of the $Ca_v2.3$ channel through G protein-coupled (GPC) μ- and δ-opioid receptors is poorly defined and has not previously been reported for κ-opioid receptors. We hypothesized that activation of human μ-, δ- or κ-opioid receptors modulates $Ca_v2.3$ channels via G protein signaling. Whole-cell Ba^{2+} currents were recorded in HEK293T cells co-expressing human $Ca_v2.2$ or $Ca_v2.3$ channels and μ-, δ- or κ-opioid receptors. Selective opioid receptor agonists and antagonists were used to study receptor modulation. The involvement of intracellular signaling pathways was investigated using specific inhibitors of GPC receptor-G protein coupling. Activation of μ-, δ- or κ-opioid receptors inhibited $Ca_v2.3$ and $Ca_v2.2$ channel current amplitude by ~45% and ~60%, respectively. Inhibition of $Ca_v2.3$ was not dependent on the type of subunit co-expressed. Inhibition of the $Ca_v2.3$ channel was primarily voltage independent, as depolarizing prepulses could not relieve the inhibited current. This was in marked contrast with the primarily voltage-dependent modulation of $Ca_v2.2$ channels that showed nearly complete recovery of the inhibited current with depolarizing prepulses. For all three types of opioid receptors, the pathway leading to $Ca_v2.3$ channel inhibition was sensitive to pertussis toxin and intracellular application of GDP-β-S. Similarly, the overexpression of a G protein subunit scavenger, myristoylated-phosducin, significantly reduced the magnitude of $Ca_v2.3$ channel inhibition. Here we demonstrate that $Ca_v2.3$ channels are efficiently inhibited by activation of μ-, δ- or κ-opioid receptors. Inhibition occurs via voltage-independent G protein signaling mechanisms. These results suggest opioid receptor controls specific members of the Ca_v2 channel family via differential signaling pathways. Neuronal $Ca_v2.3$ channels are therefore potential targets for opioid analgesics.

2925-Pos Board B355

Control of Functional Targeting of Cav1.2 Channels by the γ6

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γ6 subunit of voltage-gated calcium channels is expressed in the heart and in the brain. It modulates gating of T-type channels (Hansen et al., 2004; Lin et al., 2008). It also associates with CaV1.2 channels and enhances their inactivation in the presence of the β1b, but not β2b, subunit (Yang et al., 2010). We found that γ6 subunit dramatically reduces the number of functional α1/β2a channels expressed in tsA-201 cells. Channels with the γ6 are trapped in the Golgi complex. Deletions of N-termini of α1 and γ6 subunits restore functional targeting to the plasma membrane.

We propose that the γ6 regulates functional expression of CaV1.2 channels by interacting with the pore-forming α1 subunit or with another protein in the complex.

2926-Pos Board B356

L-Type Ca^{2+} Channel Cavb Subunits Associate with and Differentially Regulate the Cardiac Cav3.2 T-Type Ca^{2+} Channel Currents

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Low voltage activated T-type calcium channels (TTCC) play a pivotal role in the developing heart. Although the TTCC isoforms, $Ca_v3.1$ and $Ca_v3.2$, underlie cardiac TTCC current ($I_{Ca,T}$) and are expressed in atrial and ventricular myocytes during development, their expression and roles recede in the adult heart. However, previous studies have demonstrated the re-expression of $I_{Ca,T}$ in pathological cardiac hypertrophy, suggesting that TTCCs contribute to the altered Ca^{2+} cycling and signaling in these pathological conditions. In addition, the reported altered expression of some $Ca_v\beta$ subunits (specifically β_1 and β_2 subunits) of the high voltage activated L-type calcium