The conformational propensity of amino acids in the unfolded state of peptides and proteins is the subject of ongoing deliberation. Recent research has mostly focused on alanine, owing to its abundance in proteins and its relevance for the understanding of helix coil transitions. We have analyzed the amide I' band profiles of the IR, isotropic and anisotropic Raman, and VCD profiles of a series of GXG peptides, X representing a subset of the naturally occurring amino acids, in terms of a conformational model which explicitly considers the entire ensemble of possible conformations rather than representative structures. The distribution function utilized for satisfactory simulations of the amide I' band profiles was found to also reproduce a set of seven J-coupling constants reported by Graf et al[1]. The results of our analysis reveal a PPII fraction of 0.91 for the central alanine residue in GAG, which strongly corroborates the notion that alanine has a very high PPII propensity. We performed a similar analysis for X=E, F, S, V, K, L and M. Preliminary indication is that E, F, K and L exhibit a substantial PPII propensity, whereas S, V and M exhibit a less pronounced PPII propensity with an increased propensity for β-strand. We also used distributions from coil libraries and MD simulations to model amide I band profiles and J-coupling constants for alanine and valine. We found most of them to be inconsistent with our experimental data. Thus, these results clearly demonstrate that caution must be taken in using coil libraries and MD simulations to describe the unfolded state of peptides and proteins, and that experimental data are a prerequisite for quantitative evaluation of amino acid residues.

1140-Plat

Intrinsic Disorder and the Evolution of Viral Overlapping Genes

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Overlapping genes result from frameshifts over pre-existing genetic sequences by a process called overprinting, yielding two or more different proteins encoded by the same nucleotides. In this situation, single base changes would frequently alter the amino acids in two or more proteins simultaneously, but such mutations would need to fit two or more sets of structural constraints. One way around such double or multiple constraints would be for overlapping genes to be enriched in structural disorder, which is more tolerant of mutations than is structure. To test this idea, we predicted structure/disorder in the protein products of manually curated overlapping genes from unspliced RNA viruses. Overlapping regions were found to be significantly more disordered than non-overlapping regions and to have a sequence composition biased towards disorder-promoting amino acids. For a subset of the overlapping genes in our dataset, the genetic sequences that were created *de novo* by overprinting of ancestral genes were determined. Most of the protein products of these novel genes are disordered and have unusual amino acid compositions. Furthermore, almost all of these gene products are accessory proteins rather than replicases or other proteins fundamental to viral replication or structure, and these proteins are orphans without homologues. Proteins that have been created by overprinting different homologues of the same genes display a diversity of functional and structural features, facts that are consistent with their *de novo* origin. Our results offer a glimpse of the structural and functional characteristics of protein regions encoded by genes created *de novo* by overprinting events in viruses. In most cases, intrinsically disordered gene products seem to help alleviate both the difficulty of generating structured proteins *de novo*, and the increased evolutionary constraints expected for multiple-coding genetic sequences.

Platform Y: Voltage-gated Ca Channels

1141-Plat

Persistent Increases In Ca^{2+} Influx Through Cav1.2 (I_{CaL})Induce Cardiac Conduction Disturbances And Sarcoplasmic Reticulum Ca^{2+} Overload To Induce Cardiac Arrhythmia And Sudden Death

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Increases in Ca^{2+} influx through Cav1.2 has been observed in cardiovascular disease (CVD) and associated with cardiac arrhythmias. **Methods**: To mimic the enhanced Cav1.2 activity in CVD, we overexpressed the Cav1.2 β2a subunit in a transgenic mouse model. In-vivo ECGs, ion currents and intracellular Ca^{2+} were measured in transgenic (TG) and control (CTR) mice. **Results**: I_{CaL} was greater in TG myocytes (23.9 ± 2.5pA/pF, CTR 13.8 ± 1.6pA/pF). TG mice had enhanced cardiac performance (EF: TG 72.7 ± 1.3%, CTR 66.7 ± 1.5%) but died suddenly (TG 50% vs CTR 100% alive at 6 months), suggesting cardiac arrhythmias. In conscious mice, there was no difference in heart rate in CTR (571 ± 29bpm) and TG (541 ± 24bpm) mice but the QT interval was significantly shorter in TG (44.0 ± 5.5ms, CTR 58.2 ± 3.4ms) mice. Second degree AV block and ectopic premature ventricular beats were observed in all 4 TG mice but not in CTR mice. In anesthetized mice, there was no difference in heart rate (CTR 513 ± 20bpm, TG 526 ± 13bpm) but the PR interval (CTR 32.4 ± 1.4ms, TG 49.8 ± 6.2ms) and QRS duration (CTR 11.4 ± 0.8ms, TG 14.5 ± 0.8ms) were significantly prolonged in TG mice, indicating conduction defects. A significantly greater % of TG myocytes (28.5%) had early (EADs) and delayed (DADs) afterdepolarizations than CTR (0.0%) due to enhanced SR load (caffeine spritz and Fluo-4 F/F_0: TG 4.7 ± 0.4 vs. CTR 3.2 ± 0.3) and I_{NCX} (TG 2.15 ± 0.6pA/pF vs CTR 1.12 ± 0.3pA/pF at +60mV). However, action potential duration (APD) was significantly shorter in TG myocytes (APD90%: 40.0 ± 5.7ms vs. CTR: 100.6 ± 15.2ms) resulting from an increase of Ito (TG vs. CTR: 60.2 ± 0.8pA/pF vs. 18.7 ± 3.0pA/pF at +50mV). **Conclusion**: Persistent increases in Ca^{2+} influx through Cav1.2 cause both conduction disturbances and SR Ca overload, and induce cardiac arrhythmias with shortened APDs and QT intervals.

1142-Plat

Rem Selectively Abolishes β1-adrenergic Regulation Of $Ca_V1.2$ Channels In Heart

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β-adrenergic modulation of cardiac $Ca_V1.2$ channels is critical for sympathetic regulation of the heartbeat, and its disruption is a harmful hallmark of heart failure. RGK (Rem, Rem2, Rad, Gem/Kir) GTPases potently inhibit Ca_V channels by interacting with their auxiliary β subunits. Intriguingly, RGK proteins are present in heart, and their levels are elevated in heart failure. We examined the impact of the RGK GTPase, Rem, on $Ca_V1.2$ channels in heart cells and assessed whether there was crosstalk with the β-adrenergic modulation of the channel. Cultured adult guinea pig ventricular myocytes expressed robust $Ca_V1.2$ currents ($I_{Ca,L}$) (15.08 pA/pF) and responded to β1-adrenergic activation (1 μM isoproterenol + 1 μM ICI118,551) with a sharp, three-fold increase in current density. Isochronal cardiac cells expressing YFP-Rem, achieved through adenovirus infection, displayed a markedly lower basal current density (5.85 pA/pF). Nevertheless, the effect of Rem in heart is quantitatively smaller than seen in recombinant channels expressed in HEK 293 cells, which feature a virtual ablation of $I_{Ca,L}$. Surprisingly, the remaining Rem-insensitive $I_{Ca,L}$ in guinea pig heart cells was essentially unresponsive to β1-adrenergic stimulation. This was not due to disruption of the signaling pathway because isoproterenol-mediated increase in cardiac I_{Ks} remained unchanged. Intriguingly, the Rem insensitive $I_{Ca,L}$ remained responsive to forskolin. These results reveal an unexpected crosstalk between RGK GTPases and β-adrenergic signaling pathway at the level of cardiac $I_{Ca,L}$, and suggests that Rem selectively inhibits spatially distinct $Ca_V1.2$ channels in single heart cells.

1143-Plat

The Timothy Syndrome Mutation In Cav1.2 Causes Dendritic Retraction Through Calcium-independent Activation Of The Rhoa Pathway

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L-type voltage-gated calcium channels (LTCs) play a key role in neuronal development by activating signaling pathways that regulate neuronal gene expression and morphology. A point mutation in the LTC CaV1.2, which blocks voltage-dependent inactivation (VDI), causes autism in Timothy Syndrome (TS) patients. While it is known how VDI influences the current through LTCs, it is not known how alterations in VDI affect the signalling function of CaV1.2 in neurons and ultimately cause developmental defects that lead to autism. Here we show that CaV1.2 channels containing the TS mutation (TS-CaV1.2) cause dendrite retraction and reduced dendrite branching in cortical neurons in vitro and in vivo. Surprisingly, we found that TS-CaV1.2 causes dendritic retraction independently of Ca2+ influx through the channel suggesting that the voltage-dependent conformational changes associated with VDI play an important and unexpected role in CaV1.2 signaling. In addition, we found that TS-CaV1.2 causes dendrite retraction by activating the RhoA signalling pathway. We found that the small GTP-binding protein Gem with the channel beta subunit play a critical role in mediating the calcium-independent activation of RhoA by TS-CaV1.2. Our results provide new insight

into how LTCs are coupled to cytoskeletal signaling pathways in neurons and shed light on the molecular mechanisms underlying the generation of TS and other Autism Spectrum Disorders.
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1144-Plat
End-stage Mechanisms Underlying Voltage and Ca^{2+}/Calmodulin-Dependent Inactivation (VDI and CDI) of $Ca_V1.3$ Channels
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The past decade has witnessed many discoveries about the early events that underlie calmodulin (CaM) regulation of Ca^{2+} channels. Much is known about the positioning of apoCaM (Ca^{2+}-free) on channels, and the initial Ca^{2+}/CaM interaction sites. Beyond this, precious little is clear about the eventual actions of this central genre of Ca^{2+} channel modulation. Does CDI involve hinged-lid occlusion, selectivity filter collapse, or allosteric inhibition of activation gating? Do CDI and VDI reach the same ultimate conformation? All these proposals remain in flux. Here, we deduce that mutations within the S6 activation gates would produce discriminating effects on activation and inactivation, depending on which mechanism holds true. For the first two end-stage mechanisms (hinged-lid occlusion and selectivity filter collapse), S6 mutations that enhance channel opening are predicted to strengthen channel inactivation. By contrast, for an allosteric mechanism, such mutations would actually weaken inactivation. These predictions motivated exhaustive mutagenesis of the S6 segments of all four homologous domains of $Ca_V1.3$. We find that S6 mutations affect VDI and CDI in strikingly different ways, indicating a fundamental divergence of their end-stage mechanisms. The pattern seen for CDI agrees remarkably well with that predicted for an allosteric mechanism. By contrast, VDI effects cannot be fully explained by any previously described end-stage mechanism. Instead, mapping the functional VDI effects onto a structural homology model of $Ca_V1.3$ reveals a telling structural pattern, suggestive of a novel 'hinged-lid-shield' mechanism. In this scheme, $Ca_V1.3$ channels feature a specialized distal S6 'shield' that repels lid closure. We validate this proposal with experiments in which the integrity of the shield and the mobility of the hinge-lid are independently modified. In all, these advances furnish a rich mechanistic backdrop for the many Ca^{2+} channelopathies involving S6 domains.

1145-Plat
Single Channel Conductance Of CaV2.2 At Physiological $[Ca^{2+}]_{Ext}$
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Ca^{2+} that enters through voltage-gated CaV2.2 channels and binds to a calcium sensor at the transmitter release site links membrane depolarization to activation of synaptic vesicle discharge. Recent evidence supports the hypothesis that the release site calcium sensor is within the single CaV2.2 channel domain. Thus, modeling presynaptic nanophysiology requires knowledge of the channel transport rate at physiological $[Ca^{2+}]_{ext}$. However, this value has only been determined previously for the non-presynaptic CaV1.x (L type) channel with a conductance of ~2.4 pS at $[Ca^{2+}]_{ext}$=2 mM (Church and Stanley, JP 1996). Since at $[Ba^{2+}]_{ext}$=100 mM CaV1.x has a conductance of ~24 pS while CaV2.2 has one of ~14 pS we predicted that at $[Ca^{2+}]_{ext}$=2 mM the latter channel would have a conductance of ~1.2 pS.
Single calcium channels were recorded using low noise, quartz electrodes from freshly isolated chick dorsal root ganglion neurons which express virtually entirely CaV2.2 current. In the presence of $[Ca^{2+}]_{ext}$=2 mM and 2 μM nifedipine, to block CaV1.x, and 0.1 mM Ni^{+} to block CaV3.X, together with standard Na^{+} and K^{+} channel blockers and n-methyl-D glucamine^{+} as the primary cation, we noted two single inward channel conductances: ~1.4 pS and ~2.5 pS (*N=4*). The larger channel was identified as CaV2.2 since it was absent in 4 of 4 patches with ω-conotoxin GIVA (2.5 μM), a specific CaV2.2 blocker, but was present in 7 out of 8 patches with 2 mM $[Ca^{2+}{}_{ext}]$ or $[Ba^{2+}{}_{ext}]$ whereas the small channel remained (*N=3*). Thus, our data indicate that at physiological $[Ca^{2+}]_{ext}$, CaV2.2 has a much higher conductance, and hence larger single channel domain, than predicted.

1146-Plat
Gating Charge Movement Is Prevented By Open State Occupancy Of N-type (CaV2.2) Calcium Channels
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L-type calcium channels show a loose coupling between channel closing and gating charge movement. There are significant gating differences between N-type and L-type channels and we wondered if some of these differences were linked to the relationship between charge movement and channel opening. This was accomplished by comparing the time constant (τ) for channel closing (τ_{Deact}) with that for Off-gating charge movement (τ Q_{Off}) over a range of voltages. Ionic currents were recorded in 5 mM Ca^{2+}, while gating currents were recorded in 0.1 mM La^{3+} and 5 mM Mg^{2+} (La-Mg) from N-channels expressed in HEK 293 cells. τ Q_{Off} was larger than τ_{Deact} and the voltage dependence of the τ Q_{Off} was less steep than that for τ_{Deact}, which suggests that gating charge relaxation does not limit channel closing. To determine if the reverse was true, we used roscovitine, which slows N-channel closing by holding the channel in a high P_o open state. We found that τ Q_{Off} was identical to τ_{Deact} in roscovitine. There was a risk that residual ionic tail current could contaminate Off-gating current, so we used an envelope protocol to measured the recovery time course of Q_{On} (no ionic current contamination), and found the same τ as for both τ Q_{Off} and τ_{Deact} in roscovitine. This coincidence of τ Q_{Off} with τ_{Deact} suggests that transition out of the roscovitine-bound high Po open state becomes rate limiting to both Q_{Off} and channel closing. We conclude that, unlike L-channels, the high P_o N-channel open state places the channel into a confirmation that locks gating charge into the activated position.

1147-Plat
Cardiac $Alpha_{1a}$-adrenoceptor Stimulation Inhibits L-type Ca^{2+} Current In The Presence Of Beta-adrenoceptor Stimulation Through Tyrosine Kinase
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Introduction: We previously showed that cardiac α_1-adrenoceptor (AR) stimulation alone potentiates L-type Ca^{2+} current (I_{Ca}) through α_{1A}-AR-PLC-PKC pathway (O-Uchi J et al. ***PNAS.***, 2005 and ***Circ Res.***, 2008). However, the interaction of α_1- and β-AR signalings for I_{Ca} regulation was not fully clarified. In the present study, we examined the effect of α_1-AR stimulation on I_{Ca} when β-AR is stimulated. **Methods**: Perforated patch-clamp was used for recording I_{Ca} from isolated adult rat ventricular myocytes. Cells were at first treated with β-AR agonist (100 nM isoproterenol) for 5 min and then α_1-AR agonist (100 μM phenylephrine) was applied in the continuous presence of isoproterenol. Holding potential was set at -40 mV and depolarization pulse to 0 mV was applied every 10 sec. **Results**: Phenylephrine significantly inhibited I_{Ca} in the presence of isoproterenol by 19.6 ± 7.6%. The α_{1A}-AR selective antagonist (WB4101) blocked this inhibitory effect by phenylephrine, but α_{1B}-AR selective antagonist (L-765,314) did not, confirming that only α_{1A}-AR is involved in this inhibitory effect. Phenylephrine had no effect on I_{Ca} activated by forskolin. In addition, inhibition of G_q signaling by PLC inhibitor (U73122) or inhibition of $G_{i/o}$ signaling by pertussis toxin did not blocked the phenylephrine-induced inhibition of I_{Ca}. The tyrosine kinase inhibitor (lavendustin A) attenuated the response of phenylephrine during β-AR stimulation. **Conclusion**: α_{1A}-AR stimulation inhibits I_{Ca} in the presence of β-AR stimulation, which is opposite to the effect observed in the absence of β-AR stimulation. This effect is not mediated through G_q and $G_{i/o}$ but through tyrosine kinase activity, which inhibits the upstream of β-AR signaling (at the level of β-AR or Gs). The inhibitory effect of α_{1A}-AR stimulation could serve as one of the regulatory feedback mechanisms when catecholamine level increases under pathophysiological conditions.

1148-Plat
Calmodulin Regulates Calcium Sparklet Activity in Vascular Smooth Muscle
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Calcium influx through L-type calcium channels (LTCCs) influences numerous physiological processes in excitable cells ranging from contraction, memory and gene expression. Clusters of LTCCs can operate in a PKCalpha-dependent, high open probability mode that generates sites of sustained calcium influx called "persistent calcium sparklets". In vascular smooth muscle, persistent calcium sparklets contribute to local and global calcium. Calcium sparklets activity varies regionally within smooth muscle cells. At present however, the mechanisms underlying heterogeneous sparklet activity are incompletely understood. Here, we use TIRF microscopy and whole-cell patch clamp electrophysiology to investigate the role calmodulin in the modulation of calcium sparklet activity. We found that inhibition of calmodulin increases calcium sparklet activity in wild type (WT) smooth muscle cells. Inhibition of calmodulin in PKCalpha KO cells, which are devoid of persistent calcium sparklets, increased calcium influx by evoking new persistent calcium sparklet sites and by increasing the activity of previously low activity sites in these cells. On the basis of these finding, we hypothesize that calmodulin plays a critical role in determining the activity of calcium sparklet sites in arterial smooth