($InsP_3$). Recently, perturbations in the $InsP_3R1$ receptor have been linked to a human neurodegenerative disorders. The slow, progressive neurological disease, Spinocerebellar Ataxia type15 (SCA15), is inherited through an autosomal dominant gene and causes degeneration of the cerebellum. A missense mutation P1059L in the regulatory and coupling domain of the receptor (P1073L in mice) has been suggested by linkage analysis to result in SCA15 in humans. A further mutation, Itpr1Δ18/Δ18, causes the deletion of 6 amino acids in $InsP_3R$-1 and results in an ataxic phenotype in mice. We have created stable cell lines expressing corresponding mutations in the rodent $InsP_3R1$ gene in DT40-3KO cells, an unambiguously $InsP_3R$ null background. Immunoblotting confirmed expression of the mutant channels at comparable levels to wild-type. In both "on-nuclear" single channel patch clamp experiments and Ca^{2+} imaging both mutated $InsP_3R1$ receptors are functional Ca^{2+} channels. A comparison of the activity of the mutated receptors with wild type $InsP_3R1$ will be presented.

2661-Pos

Regulation of Inositol 1,4,5 Trisphosphate Receptors by InsP3 Receptor-Associated cGMP Kinase Substrate (IRAG)

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Various factors interact with IP_3R, regulating Ca^{2+} release and thus serve to define the spatial and temporal characteristics of the cytosolic Ca^{2+} signal. IP_3R-associated cGMP kinase substrate (IRAG) has been reported to bind IP_3R type-1 and inhibit Ca^{2+} mobilization in smooth muscle cells. No information is however available as to whether IRAG interacts or has functional effects on other IP_3R family members. In this study, we examined whether IRAG binds to and regulates Ca^{2+} release via IP_3R type-2 or type-3. cDNA encoding IP_3R type-1, IRAG-GFP, and protein kinase G1β (PKG1β) were transiently transfected into COS cells. Following immunoprecipitation from cell lysates with an anti-GFP antibody, IP_3R type-1 was detected by immunoblotting. In contrast, an IRAG-GFP construct (IRAGΔE12-GFP) in which 40 amino acids required for binding with IP_3R was deleted, failed to interact with IP_3R type-1, but was still capable of binding to PKG1β , an additional cognate binding partner of IRAG. Similarly, IP_3R type-2 or IP_3R type-3 could be shown to interact with IRAG-GFP but not IRAGΔE12-GFP in COS cells. Next, we investigated if IRAG regulates IP_3-induced Ca-release using DT40-3KO cell lines stably expressing mammalian IP_3R type-2 or type-3 in isolation. In DT40-3KO cells stably expressing IP_3R type-2, and transiently expressing Muscarinic M3 receptor, IRAG-GFP and PKG1β, cell permeable PKG activators reduced the muscarinic agonist carbachol (CCh)-induced Ca^{2+}-release. Ca^{2+} oscillations induced by low concentrations of CCh or by stimulating the endogenous B cell receptor were similarly attenuated. No inhibitory effect was evident in cells expressing IRAGΔE12-GFP or in the absence of IRAG-GFP. Similar results were obtained with DT40-3KO cells stably expressing IP_3R type-3. These results indicate that Ca^{2+} release through all Inositol 1,4,5 trisphosphate receptors are inhibited by an interaction with IRAG and PKG1β.

2662-Pos

CaMKII-Mediated Phosphorylation of $InsP_3R2$ at Serine-150 Results in Decreased Channel Activity

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$InsP_3$ mediated calcium release through the type-2 inositol 1,4,5-trisphosphate receptor ($InsP_3R2$) in cardiac myocytes results in the activation of associated CaMKIIδ (Bare et al, 2005, *JBC*; Wu et al, 2006, *JCI*), enabling the kinase to act on downstream targets, such as histone deacetylases 4 & 5 (HDAC4 & HDAC5) (Zhang et al, 2007, *JBC*). The CaMKII activity also feedback modulates $InsP_3R2$ function by direct phosphorylation and results in a dramatic decrease in the receptor-channel open probability (P_o). The results of this study show that in planar lipid bilayers the channel activity of $InsP_3Rs$ can be inhibited by CaMKII-mediated phosphorylation, and that effect can be reversed by addition of protein phosphatases. Furthermore, we have used fragments of the $InsP_3R2$ and site-directed mutagenesis to determine that Serine at residue 150 is the CaMKII phosphorylation site responsible for modulation of channel activity. Non-phosphorylatable (S150A) and phospho-mimetic (S150E) mutations were constructed in the full-length $InsP_3R2$, expressed in COS cells and studied in planar lipid bilayers. Upon treatment with CaMKII, the non-phosphorylatable channel showed no decrease in activity. Conversely, the phosphomimetic channel displayed a very low P_o under normal recording conditions in the absence of CaMKII (2μM $InsP_3$ and 250nM $[Ca^{2+}]_{FREE}$), thus mimicking a channel that has been phosphorylated by CaMKII. The results of this study show that Serine-150 of the $InsP_3R2$ is phosphorylated by CaMKII and results in a decrease in the channel's open probability. The mechanism for the regulation of the $InsP_3R2$ appears to be a consequence of altered affinity for $InsP_3$ at the ligand binding site and/or perturbation of the receptor amino to carboxyl-terminal interaction and is currently being examined.

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2663-Pos

Excitement Over Automated Patch Clamp: Action Potentials from Cardiac Myocytes

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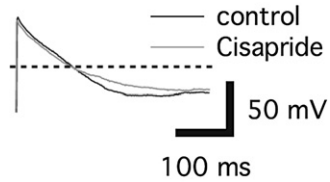
The use of cardiac myocytes is becoming increasingly important for drug safety testing. Unique features of certain planar patch clamp workstations, coupled with ease-of-use and higher data throughput, make these devices ideal tools for ion channel screening and safety testing. Using stem cell derived cardiac myocytes, recordings could be made not only in the voltage-clamp mode but also in the current-clamp mode on a planar patch clamp workstation. This demonstrates for the first time parallel current-clamp recordings on a planar patch clamp workstation. Ion channels important in drug discovery, such as hERG and voltage-gated Na+, Ca2+ and K+ channels in the voltage-clamp mode from stem cell derived cardiac myocytes will be shown. In addition, action potential recordings in the current-clamp mode at 35°C, and modulation of the action potentials by hERG active compounds, will also be shown.



2664-Pos

***In Silico* Studies of Cardiac Inotropy using a New Model of Force Generation**

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An improved model of force generation was incorporated into a complete mathematical description of action potential (AP), ionic currents and Ca^{2+} transient of the rabbit ventricular myocyte (LabHEART 5.0). This new model reproduces the main events involved in Excitation-Contraction Coupling, namely the AP (excitation), the shortening (contraction) and the Ca^{2+} transient as the link between them. LabHEART 5.0 was able to reproduce isotonic and isometric contractions and the classical curves of Force vs. Ca^{2+} and Force vs cell length. Cardiac inotropy was investigated by simulating the application of isoproterenol (ISO). This effect was achieved by altering L-type Ca^{2+} current, the slowly activating delayed rectifier K^+ current, sarcoplasmic reticulum (SR) Ca^{2+} pump, SR Ca^{2+} leak, myofilament Ca-sensitivity and cross-bridge cycling. The latter modification was essential for replicating the ISO-induced increase in force generation/shortening development experimentally observed. AP duration (APD, for 90% of repolarization) adaptation to pacing frequencies was also examined. ISO shortened APD at all frequencies with respect to control and flattened the adaptation curve, thus allowing an APD compatible with short cycle length (up to 5 Hz). ISO also increased the Ca^{2+} transient dynamic range by keeping a low diastolic level while increasing the peak Ca^{2+} at all the simulated frequencies (0.5 to 9 Hz). This model provides a useful framework to study cardiac inotropy and constitutes a starting point to investigate the electromechanical feedback in cardiac performance. The new version LabHEART 5.0 is freely available online at www.labheart.org.

Voltage-gated Ca Channels I

2665-Pos

Monte Carlo Simulation of Free Energy Components: Energetics of Selective Binding in a Reduced Model of L-Type Ca Channels

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A reduced model of voltage-gated L-type Ca channels is used to study the energetics of selective binding of Ca^{2+} versus monovalent and divalent cations. Widom's particle insertion method is combined with Grand Canonical Monte Carlo simulations to compute the electrostatic and excluded volume components of the free energy difference between channel and bath. We have shown (in ~ 30 papers) that selectivity of the L-type Ca channel and voltage activated

Na channel can both be described (over a wide range of solutions and concentrations) by the same reduced model with the same unchanging two parameters (dielectric coefficient and diameter) in which side chains are spheres (Ca channel = EEEE or EEEA; Na channel = DEKA). In the EEEE channel, Ca^{2+} selectivity is driven by charge/space competition in which selectivity arises from a balance of electrostatics and the excluded volume of ions in the crowded selectivity filter. Electrostatics selects Ca^{2+} over monovalent cations. Excluded volume selects Ca^{2+} over larger divalent cations. All these combine to create depletion zones in the ionic density profiles that are crucial determinants of the current carried by each ionic species.

2666-Pos

Energetic Variational Analysis *EnVarA* of Ions in Calcium and Sodium Channels

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Selective binding in both calcium and sodium channels can be described (in many solutions and concentrations: Biophysical Journal (2007) 93:p.1960) by the same reduced model and unchanging two parameters (dielectric coefficient and diameter) despite the very different primary structure of the two proteins (Ca channel EEEA/EEEE; Na channel DEKA) and properties, even though amino-acid side-chains (E, D, etc.) are represented only as charged spheres. Monte Carlo *MC* simulations, reported in ~30 publications, work well (we think) because they do not specify structure as an input, independent of conditions. Rather, *MC*calculates the structure as an output, as a self-organized, induced fit of side-chains to ions (and vice-versa). **Structure is different in different solutions** in self-organized systems. Self-organized systems can be powerfully analyzed using the calculus of variations, specifically, energetic variational analysis (*EnVarA*). We optimize *both* action and dissipation integrals (Least Action and Maximum Dissipation Principles), motivated by Rayleigh, then Onsager who optimized just one, or the other. The resulting systems of coupled partial differential equations automatically satisfy the First and Second Laws of Thermodynamics and electrostatic Poisson equations, with physical boundary conditions that can produce flow. *EnVarA* extends Navier-Stokes equations to complex fluids containing deformable droplets (Journal of Fluid Mechanics (2004) 515:p.293). ***EnVarA* provides a seamless extension of conservative Hamiltonian systems** (perhaps at thermodynamic equilibrium) **to dissipative systems.***EnVarA* **is a** field theory of ions in channels and solutions with entropy, friction, and flow. *EnVarA* computes current where *MC* computes only binding. *EnVarA* applied to EEEE/DEKA channels gives binding like real calcium/sodium channels. Time dependent currents computed with *EnVarA* resemble time dependent currents in either voltage activated sodium or potassium (squid axon) channels (depending on parameters), **although the *EnVarA* model has only one unchanging conformation.**

2667-Pos

3D Structure of a Recombinant DHPR Expressed in Mouse

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The dihydropyridine receptor (DHPR) is an L-type Ca^{2+} channel that acts as the voltage sensor for excitation-contraction coupling in skeletal muscle by tightly controlling the intracellular Ca^{2+} channel RyR1. Because previous 3D electron microscopic studies have largely not resolved the spatial organization of the DHPR subunits (α1s, α2-δ, β1a, and γ), we constructed mice expressing a β1a subunit with YFP and a biotin acceptor domain attached to its N- and C- termini respectively. This engineered β1a sustains a functional DHPR-RyR1 interaction and viable animals in a β1a null background. DHPRs were purified from mice by means of the (biotinylated) biotin acceptor domain, negatively stained and imaged with electron microscopy. 8,662 individual DHPRs were analyzed using single-particle image processing algorithms. Multivariate statistical analysis, classification, and multi-reference alignment yielded distinct 2D class averages corresponding to different orientations of the macromolecule. The 3D reconstruction, with 25 Å resolution, shows two distinct parts: a main body shaped like an irregular pentagon (~150x125x75 Å) with distinct corners, and a hook-shaped feature that extends ~60 Å from the main body. Consistent with the considerable conservation of membrane topology among voltage-gated channels, a good part of the main body can be closely fitted with an atomic structure of a full-length potassium channel, suggesting that the main body contains the α1s subunit. Besides the fitted potassium channel the main body has extra volume that can accommodate the YFP atomic coordinates and other subunits.

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2668-Pos

$Ca_v2.3$ Calcium Channels Inactivate from the Open State with Partial Charge Immobilization and Altered Deactivation Kinetic

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Voltage-dependent ion channels undergo inactivation following activation. In the sodium and potassium channels, the molecular determinants that govern the mechanism of inactivation involve pore blocking by a cytoplasmic particle. In calcium channels the consensus model is that the intracellular loop joining the first two homology domains of the pore forming subunit contributes to the inactivation gate or hinged-lid. To investigate key features of this model, we expressed $Ca_V2.3$ channel in *Xenopus oocytes* without auxiliary subunits and recorded ionic and gating currents using the cut-open voltage-clamp technique. Ionic current were recorded in either Ba^{2+} or Ca^{2+}. Here we report that consistent with a hinge-lead mechanisms, charge movement at the end of a depolarizing pulse decreases up to a 50% with channel inactivation. In contrast with a previous report by Patil et al (1998, Neuron 20:1020) that co-expresses auxiliary subunit with $Ca_V2.3$, trains of pulse elicited minimal inactivation. It appears then that when expressed alone, $Ca_V2.3$ channels inactivate mostly from the open state. We also found that as channels inactivate, a slow component emerges in tail-current recordings. This component contributes to about 20% of the tail currents in 80% inactivated currents and can be accounted for with a classic allosterically-coupled model provided that channels can re-open multiple times from the last closed state and that some closed-inactive channels reopen during membrane repolarization.

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2669-Pos

Action Potential Hyperpolarization Kinetics Abets the Modulation of Alpha1h T-Type Calcium Channels by KLHL1 Mutants Lacking the Actin-Binding Domain (ΔKelch)

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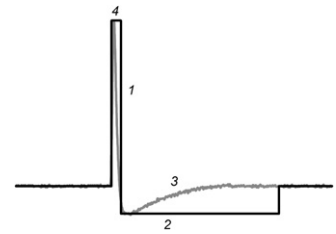
The Kelch-like 1 protein (KLHL1) is a neuronal actin-binding protein that increases the current density and channel number of $Ca_v3.2$ calcium channels *via* actin-F mediated increases in recycling endosomal activity. Removal of the actin-binding kelch motif (ΔKelch) prevents the increase in α_{1H} current density seen with wild-type KLHL1 when tested with square pulse protocols but not the increase in calcium influx seen during action potentials (AP).

Here we set out to dissect the kinetic properties of AP that confer the mutant kelch the ability to interact with α_{1H} and induce an increase in calcium influx. Square waveforms (black trace) following the AP did not significantly increase calcium influx (25%, $p>0.05$) compared to the AP (red). We investigated the effects of altering the slope of the repolarization (1), the length of hyperpolarization (2), the slope of repolarization from hyperpolarization (3) and the duration of depolarization (4) on the modulation of α_{1H} by ΔKelch. Our results show that the slope of repolarization from hyperpolarization induces the conformational changes that allow the channel to properly interact with ΔKelch, leading to increased Ca influx.



2670-Pos

C-terminal Alternative Splicing Modulates Single-Channel Gating of $Ca_v1.3$ L-Type Calcium Channel

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We have recently discovered a novel mechanism of channel modulation in $Ca_V1.3$ channels enabling cells to tightly control gating by C-terminal alternative splicing. The absence of a C-terminal modulatory motif (CTM) within a short splice form facilitates $Ca_V1.3$ channel activation at lower voltages and induces pronounced Ca^{2+} dependent-inactivation (CDI) (Singh *et al*., JBC 2008). Intriguingly, whole-cell measurements revealed a significant