about 30% of the I_{Ca} and I_{Ba}. Administration of anoxic solution did not further inhibit I_{Ca} in cells previously exposed to ZnPP-IX or SnPP-IX. We conclude that both anoxia and HO suppress I_{Ca} and I_{Ba} with the same intensity and kinetics and O_2 sensing effect becomes negligible in cells exposed to HO inhibitors.

3605-Pos

A New Paradigm for Gem Regulation of Voltage-Gated Ca^{2+} Channels

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The RGK (Rem, Rem2, Rad, Gem/Kir) family of Ras-related monomeric small GTP-binding proteins has emerged as potent inhibitors of high-voltage activated (HVA) Ca^{2+} channels. All RGK proteins bind all four subfamilies of HVA Ca^{2+} channel β subunits (Ca_vβs), and Ca_vβ is required for RGK-induced inhibition. Two modes of RGK action have been reported: (1) RGKs interfere with channel trafficking to the plasma membrane and hence reduce the number of surface channels; (2) RGKs inhibit channels already on the plasma membrane. It is generally believed that both forms of inhibition absolutely rely on the RGK/Ca_vβ interaction. However, this central hypothesis has not been tested directly. We investigated the molecular mechanism of Gem inhibition of P/Q-type Ca^{2+} channels expressed in *Xenopus* oocytes and HEK 293T cells. Gem inhibited P/Q channels without affecting their surface expression. Application of a purified Gem protein domain in inside-out membrane patches acutely inhibited P/Q channels. This acute inhibition was completely abolished when Ca_vβ was removed from surface P/Q channels, but it was fully restored after the channels regain Ca_vβ. These results unequivocally demonstrate that Ca_vβ is indispensable for Gem inhibition of surface P/Q channels. Surprisingly, however, complete disruption of the Gem/Ca_vβ interaction, as shown biochemically, did not affect Gem inhibition. On the other hand, we discovered that Gem associated with Ca_v2.1 in a Ca_vβ-independent manner. Finally, we identified a 12-amino acid region in the C-terminus of Gem that was sufficient to produce inhibition in inside-out patches and another site in the core region of Gem that was also involved in Gem inhibition. Based on these findings, we propose that Gem directly binds and inhibits Ca_Vβ-primed HVA Ca^{2+} channels on the plasma membrane.

3606-Pos

The β Subunit of Voltage-Gated Ca^{2+} Channels Acts as a Transcriptional Regulator

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Ca^{2+} channel β subunits (Ca_vβs) are essential for the surface expression and proper gating of high-voltage activated (HVA) Ca^{2+} channels. In yeast two-hybrid screens aimed at discovering novel Ca_vβ-interacting proteins, we identified a new splicing isoform of Pax6, a transcription factor crucial for the development of a variety of organs and tissues, especially the eye. Pax6 contains two DNA-binding domains (paired domain and homeodomain), a glycine-rich linker connecting these two domains, and a carboxyl (C)-terminal proline, serine and threonine (PST)-rich transactivation domain. The newly isolated isoforms, named Pax6(S), retains the paired domain, linker and homeodomain of Pax6, but its C-terminus is composed of a truncated classic PST domain and a unique S tail. In contrast to Pax6, which is 100% conserved from rodent to human and is expressed in both embryo and adult, Pax6(S) is completely conserved only in human and chimpanzee, and it is expressed only at early stages of development, suggesting that Pax6(S) has a noncanonical function. Pax6(S) retained strong transcriptional activity, although its C-terminus showed less transactivity compared with the canonical PST domain. The interaction between Pax6(S) and Ca_vβ was mainly endowed by the S tail of Pax6(S). Co-expression of Pax6(S) with a HVA Ca^{2+} channel complex containing the β_3 subunit in *Xenopus* oocytes did not affect channel properties. However, the transcriptional activity of Pax6(S) was markedly suppressed by β_3. Furthermore, in the presence of Pax6(S), β_3 was translocated from the cytoplasm to the nucleus. These results suggest that full length Ca_vβs may function as transcription regulators, independent of their role in regulating Ca^{2+} channel activity.

3607-Pos

Increased Intracellular Magnesium Attenuates β-Adrenergic Stimulation of the Cardiac Cav1.2 Channel

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Increases in intracellular Mg^{2+} (Mg_i^{2+}), as observed in transient cardiac ischemia, decrease L-type Ca^{2+} current of mammalian ventricular myocytes. Transient cardiac ischemia is also associated with an increase in sympathetic tone, which could stimulate the L-type Ca^{2+} current. Therefore, the effect of Mg_i^{2+} on L-type Ca^{2+} current in the context of increased sympathetic tone is unclear. We hypothesized that increased Mg_i^{2+} in ventricular myocytes might alter the β-adrenergic stimulation of L-type Ca^{2+} current. Using the whole-cell patch clamp method to study ventricular myocytes from C57BL6 mice, we tested the impact of increased Mg_i^{2+} on the stimulatory effect of the β-adrenergic receptor (β-AR) cascade activation on L-type Ca^{2+} current. We observed that exposure of myocytes to higher Mg_i^{2+} concentration decreased isoproterenol stimulation of the L-type Ca^{2+} current from 75 ± 13 % with 0.8 mM Mg_i^{2+} (n=11) to 20 ± 8 % with 2.4 mM Mg_i^{2+} (n=7) ($p < 0.01$). Because Mg_i^{2+} could act at multiple sites in the β-AR cascade, we activated this signaling cascade at different steps using pharmacological tools to determine the site(s) of Mg_i^{2+} action. We found that exposure of ventricular myocytes to increased Mg_i^{2+} attenuated the stimulation of L-type Ca^{2+} current mediated by isoproterenol (β-AR stimulation), forskolin (adenylate cyclase stimulation), and IBMX (phosphodiesterase inhibition). These experiments rule out significant effects of Mg_i^{2+} on the β-AR, Gs protein, adenylate cyclase, and phosphodiesterase (I-V). Taken together, our results suggest that, in transient ischemia, increased Mg_i^{2+} reduces the entry of Ca^{2+} via the L-type Ca^{2+} current by directly acting on the Ca_V1.2 channel in a cell-autonomous manner, effectively decreasing the metabolic stress imposed on ventricular myocytes until blood flow can be re-established.

3608-Pos

Localized Calcineurin in Calcium- Dependent Inactivation of L-type Calcium Channels

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The open probability of Ca_V1.2 L-type Ca^{2+} channels is enhanced by cAMP-dependent protein kinase (PKA), which is scaffolded to Ca_V1.2 channels by A-kinase anchoring proteins (AKAPs). Ca_V1.2 channels also undergo negative autoregulation via Ca^{2+}-dependent inactivation (CDI). CDI relies upon binding of Ca^{2+}/calmodulin (CaM) to an IQ motif in the carboxy tail of Ca_V1.2 L-type channels, a mechanism seemingly unrelated to phosphorylation-mediated channel enhancement. In neurons, AKAP79/150 anchors both PKA and the Ca^{2+}-activated phosphatase calcineurin (CaN) to Ca_V1.2 channels. Using transfected tsA201 cells or neurons, and tools such as the isolated calcineurin autoinhibitory peptide, over-expression of the catalytically-inactive CaN_{H151A} mutant, and RNAi suppression of AKAP79, we have found that channel-linked CaM serves as a Ca^{2+} sensor for CaN, and that Ca^{2+}/CaM-activated CaN participates in CDI by reversing channel enhancement by kinases such as PKA. We have also observed that I→E substitution in the IQ motif produces a mutant $Ca_V1.2_{I/EQ}$ channel that - when co-expressed with AKAP79 in tsA201 cells - unexpectedly exhibits ultra-fast inactivation. Ultra-fast inactivation is eliminated in Ca^{2+}-free Na^+ external solution, as well as by over-expression of the CaN_{H151A} mutant or stimulation of PKA with forskolin. One interpretation is that the intact IQ motif's affinity for Ca^{2+}/CaM limits the speed of CDI, and that reducing IQ affinity for Ca^{2+}/CaM via I→E substitution allows CDI to proceed at a greatly speeded rate. FRET results with the $Ca_V1.2_{I/EQ}$ mutant or with AKAP79 lacking the CaN anchoring motif suggest that, during periods of elevated channel activity, the IQ-CaM and AKAP79-CaN interactions are both necessary for CaN-mediated reversal of current enhancement by PKA. In sum, our work supports a synthetic view fusing previous ideas regarding CaM and phosphorylation signaling in CDI.

3609-Pos

Sumoylation of Voltage-Gated Alpha1a Calcium Channels

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The trinucleotide repeat disorder Spinocerebellar ataxia type 6 (SCA6) results form alterations in the *CACNA1A* gene coding for the α_{1A} subunit of the neuronal voltage-dependent P/Q-type calcium channel (Ca_V2.1). We have previously reported that the α_{1A} subunit is susceptible to proteasomal and lysosomal-mediated degradation, and that ubiquitin-mediated degradation is abnormal in SCA6 α_{1A}, consistent with a glutaminopathy component in SCA6. The Small Ubiquitin-related Modifier (SUMO) is only distantly related to ubiquitin (18% homology), but shares many similarities with it, including a similar protein size, tri-dimensional structure, a C-terminal glycine-glycine motif for substrate conjugation and many PEST motif-containing substrate targets. SUMO's primary functions involve nuclear events and also include

cytosolic functions such as modulation of protein levels and activity, stability and subcellular distribution.
Here we show that α_{1A} is also a target for SUMOylation. Co-expression of α_{1A} with SUMO-1 (stoichiometry of 1:5) in HEK 293 cells led to decreased in current density of 56-59% compared to control in two different WT α_{1A} isoforms (Δ47 and +47 CAG_{11}) without further changes in other biophysical properties; whereas co-expression of the SUMO-1 ΔC6 mutant did not alter current density, demonstrating that covalent-binding of SUMO-1 is necessary for its action. In contrast, the SCA6 mutants CAG_{23} and CAG_{72} were not affected by SUMO-1 suggesting this alteration could play a role in disease. Alteration of the C-terminal PEST motif in WT α_{1A} (Ax4 and ΔPEST) produced channels resistant to SUMO's effect, similarly to the SCA6 mutants, highlighting this region's role in the process. Immunoprecipitation experiments from mouse brains show that a fraction of endogenous α_{1A} is sumoylated *in vivo.*

3610-Pos
Rem2 Redistributes in Response to Neuronal Stimulation
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Rem2 is a small GTP-binding protein of the RGK family. It is targeted to the cell membrane where it interacts with the beta subunit of calcium channels and abolishes or reduces endogenous or exogenous calcium currents, and also has known interactions with calmodulin and 14-3-3. Rem2 is unique in the RGK family, being found predominantly in the brain and upregulated in response to stimulation. Knockdown of Rem2 in neuronal cultures results in fewer glutamatergic synapses. We have found that fluorescent-labeled Rem2 changes its subcellular localization in neurons from a diffuse to a punctuate distribution after neuronal stimulation or after activation of NMDA receptors. This rearrangement is calcium dependent and involves the C-terminal 30 residues, suggesting the presence of a self-association domain as well as an autoinhibitory domain that keeps Rem2 diffusely distributed until stimulation. A calmodulin-binding deficient mutant shows very little rearrangement upon stimulation, supporting a role for calcium in this phenomenon.

3611-Pos
Plasma Membrane Targeting of High-Voltage Activated Calcium Channels
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High-voltage activated Ca_V1 and Ca_V2 channels arise from the multimerization of the pore-forming $Ca_V\alpha1$ subunit, the cytoplasmic $Ca_V\beta$ subunit, the mostly extracellular $Ca_V\alpha2b\delta$ subunit, and the intracellular calmodulin protein constitutively bound to the C-terminus of $Ca_V\alpha1$. High-affinity $Ca_V\beta$ binding onto the I-II linker is required for $Ca_V\beta$ modulation of HVA channel gating and plasma membrane targeting of HVA $Ca_V\alpha1$ subunits. However, the role of the $Ca_V\alpha2b\delta$ in the targeting of HVA Ca_V channels remains to be established. In order to gauge the role of auxiliary subunits in the steady-state plasma membrane expression of HVA Ca_V, the $Ca_V\alpha1$ subunits from $Ca_V1.2$ and $Ca_V2.3$ channels were each labeled with an extracellularly hemagglutinin (HA) epitope inserted in the first extracellular loop located in Domain I. Protein expression was confirmed by immunoblotting of cell lysates with an anti-HA antibody after expression either in stably transfected $Ca_V\beta3$ or in stably transfected $Ca_V\alpha2b\delta$ cells. Membrane-bound HA-tagged $Ca_V1.2$ and HA-tagged $Ca_V2.3$ proteins were quantified in intact cells using a fluorescent-activated sorting assay. The number of HA-tagged $Ca_V\alpha1.2$ subunits increased by a 10-fold factor when co-expressed with $Ca_V\beta3$. Similar results were obtained with the HA-tagged $Ca_V2.3$ channel. In contrast, transient co-expression of the HA-tagged subunits with the auxiliary $Ca_V\alpha2\delta$ did not significantly increase the population of fluorescent cells. More importantly, we did not observe a significant increase in the fluorescent signal in the combined presence of the two auxiliary subunits suggesting altogether that $Ca_V\beta$ is the key auxiliary subunit for membrane targeting of HVA Ca_V channels. Supported by grants from the Canadian Institutes of Health Research and the Heart and Stroke Foundation of Canada to LP.

3612-Pos
Association of Voltage-Gated Calcium Channel Subunit $\alpha_2\delta$-3 with Lipid Rafts: Structural and Functional Implications
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The High Voltage-Activated (HVA) subgroup of voltage-gated calcium channels contain an $\alpha1$ subunit, which forms the selective pore and determines the main functional properties of the channel. The $\alpha1$ subunit is associated with auxiliary subunits including intracellular β and $\alpha2\delta$, which modulate trafficking and functional properties of the channels.
$\alpha2\delta$ subunits consists of two peptides: $\alpha2$ which is entirely extracellular is disulfide-bonded to a δ subunit that links the protein into the plasma membrane. There are four genes encoding $\alpha2\delta$ subunits, which are believed to have similar structure. We have shown previously that $\alpha2\delta$-2 subunits associate with lipid rafts, that are sub-domains of the cell membrane enriched in cholesterol and glycosphingolipids.
We have addressed the ability of $\alpha2\delta$-3 to associate with lipid rafts in both native tissues (it is highly expressed in brain) and in overexpression systems. We have generated mutations which reduced expression of the subunit in lipid rafts as well as the surface expression of the protein. These mutations reduced the enhancing effect of $\alpha2\delta$-3 on calcium channel currents.
The $\alpha2$ and δ peptides are product of a single gene, and they are encoded as an uninterrupted $\alpha2\delta$ pre-protein, which is further processed post-translationally. In native tissues we observed exclusively the mature form of the protein, which was strongly associated with the lipid rafts. However, in several overexpression systems we could also detect unprocessed $\alpha2\delta$-3 pre-protein coexisting with the mature $\alpha2\delta3$. The unprocessed form was localized both in the rafts and non-raft protein fractions, suggesting that maturation of the protein might occur in localized membrane domains.
These results further demonstrate the role of lipid rafts in the regulation of Ca channel currents by $\alpha2\delta$ and their involvement in the maturation of the $\alpha2\delta$ protein.

3613-Pos
CaBP1 Regulates Both Ca and Ba currents through Ca(v)1.2 (L-type) Calcium Channels
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The main goal of this work was to study the mechanism of inactivation and gating of the L-type voltage-dependent calcium channel (L-VDCC) - Ca(v)1.2 - by calcium-binding protein 1 (CaBP1).
Previously it was shown that Ca^{2+} dependent inactivation (CDI) is calmodulin (CaM)-dependent, while CaBP1 totally prevents the process. It has been suggested that the amino terminal of the pore forming subunit of the channel - Ca(v)1.2-NT plays a crucial role in mediating the effects of CaBP1 on inactivation.
Electrophysiological assay was done in *Xenopus* oocyte expression system, using two-electrode voltage clamp (TEVC) that monitors whole cell currents. Interactions between different radiolabeled and GST- fused proteins was studied *in vitro* by pull down assays.
We mapped the interaction sites of both CaM and CaBP1 on the Ca(v)1.2-NT, and discovered that these are separated sites. The functional study showed an opposite effect of CaBP1 on Ca(v)1.2 inactivation: it abolished CDI but enhanced the voltage-dependent inactivation (VDI). CaBP1 shifted the current-voltage (IV) curve of Ca^{2+} and Ba^{2+} currents to positive values. Surprisingly, removing CaBP1 binding site on the Ca(v)1.2-NT, reduced but did not fully eliminate the changes caused by CaBP1. However, we found an essential contribution of the β subunit in both inactivation and CaBP1 effect. These findings suggest that multiple determinants influence the regulation of Cav1.2 by Ca^{2+} binding proteins.

3614-Pos
Molecular Basis of a C Terminal Modulatory Mechanism in $Ca_v1.3$ Voltage-Gated Ca^{2+} Channels
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We have previously discovered an intramolecular interaction between proximal- (PCRD) and distal C-terminal (DCRD) modulatory domains in human $Ca_v1.3$ L-type Ca^{2+} channels (LTCCs) which affects channel activation and inactivation gating properties (Singh et al 2008). This is present in the long ($hCa_v1.3_{42}$) but not a short ($hCa_v1.3_{42A}$) splice variant. Interestingly, this regulation has not been reported for rat $Ca_v1.3$ channel analogues (Xu and Lipscombe 2001). We systematically compared the functional properties of long and short $Ca_v1.3$ splice variants of mouse and rat with human channels after expression in tsA-201 cells using the whole-cell patch-clamp technique. The