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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 ($Ca_v\beta$s), and $Ca_v\beta$ 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/$Ca_v\beta$ 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 $Ca_v\beta$ was removed from surface P/Q channels, but it was fully restored after the channels regain $Ca_v\beta$. These results unequivocally demonstrate that $Ca_v\beta$ is indispensable for Gem inhibition of surface P/Q channels. Surprisingly, however, complete disruption of the Gem/$Ca_v\beta$ interaction, as shown biochemically, did not affect Gem inhibition. On the other hand, we discovered that Gem associated with $Ca_v2.1$ in a $Ca_v\beta$-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 $Ca_V\beta$-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 ($Ca_v\beta$s) 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 $Ca_v\beta$-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 $Ca_v\beta$ 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 $Ca_v\beta$s 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