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