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

C-terminal modulation was also present in mouse ($V_{0.5act}$[mV]: $mCa_v1.3_{42}$ -4.1 ± 0.4 n=36; $mCa_v1.3_{42A}$ -12.0 ± 0.5 n=25; p<0.0001, Mann-Whitney-test) and indistinguishable from human ($V_{0.5act}$[mV]: $hCa_v1.3_{42}$ -3.9 ± 0.6 n=33; $hCa_v1.3_{42A}$ -11.2 ± 0.7 n=12; p<0.0001, Mann-Whitney-test) but was absent in rat. Exon 11, which is only present in rat $Ca_v1.3$, is not responsible for the difference suggesting single amino acid exchanges in other transmembrane domains or within the N- or C-terminus to account for the species difference. Additionally, we report a new short $Ca_v1.3$ splice variant ($hCa_v1.3_{43S}$) identified in human and mouse brain tissue. The voltage-dependence of $hCa_v1.3_{43S}I_{Ca}$ activation and inactivation was significantly shifted to more hyperpolarized potentials ($V_{0.5act}$[mV]: $hCa_v1.3S$: -12.4 ± 1.0, n=10, p<0.0001; $V_{0.5inact}$[mV]: $hCa_v1.3_{42}$: -2.7 ± 0.6, n=12, p<0.0001, Mann-Whitney-test) and channel inactivation was significantly faster compared to the long form (% inactivation during 250ms at V_{max}: $Ca_v1.3_{42}$: 63.6 ± 2.4; $Ca_v1.3_{43S}$: 87.0 ± 1.5; p<0.0001, Mann-Whitney-test). These gating differences are due to the lack of the DCRD. In contrast to $hCa_v1.3_{42A}$, $hCa_v1.3_{43S}$ still contains the PCRD. We therefore hypothesize that this short form can be modulated by binding the DCRD domain of adjacent LTCCs or the free C-terminal peptide derived from $Ca_v1.2$. Support: FWF (P-20670, JS), University of Innsbruck (AK).

3615-Pos

The Regulation of N-Type ($ca_v2.2$) Voltage-Gated Calcium Channels by $Ca_v\beta$ Subunit N- and C-terminal Variable Domains

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Auxiliary β subunits of voltage-gated calcium channels (Ca_v) promote the trafficking of pore-forming α1 subunits to the plasma membrane (PM) and modulate channel properties.$Ca_v\beta$s contain a conserved Src homology 3 (SH3) and guanylate kinase (GK) core,linked by a flexible hook and flanked by variable N-(V1) and C-termini (V3).Structural similarity with membrane-associated GK (MAGUK) proteins suggests that $Ca_v\beta$s may act as molecular scaffolds.Thus,the variable N- and C-termini of β subunits may be important for discrete sub-cellular targeting and modulation of Ca_v signalling as well as channel gating.To address this question, full length CFP-β1b and CFP-β1b constructs lacking the N-terminus (deltaV1), C-terminus (deltaV3),or both (β1b-core) were co-expressed with $Ca_v2.2$ α1/α2δ-1 in COS-7 cells,and their effects on $Ca_v2.2$ localization,function and modulation by ERK1/2,examined.When expressed alone,CFP-β1b exhibited strong nuclear/cytoplasmic localisation whereas loss of the C-terminus enhanced expression at the PM. Whilst all CFP-β1b constructs facilitated trafficking of $Ca_v2.2$ to the PM,lower levels of $Ca_v2.2$ expression occurred with all mutants,suggesting the importance of both N- and C-termini in membrane targeting of $Ca_v2.2$.In spite of this,$Ca_v2.2$ current density was uniform for all β1b constructs,implying that not all α1:β complexes at the PM are functional.In contrast to most reports,we observed little influence of N-terminal deletion on the biophysical properties of $Ca_v2.2$.However,C-terminal deletion enhanced the rate of current activation and reduced channel availability,highlighting a role for this region of β1b in channel gating.Modulation of $Ca_v2.2$ by ERK1/2, which is dependent on the presence of β subunit,was however unaffected by N-/C-terminal deletion.Together,these findings support a role for the N- and C-terminal variable domains of β1b in membrane targeting of Ca_v and highlight the importance of the C-terminus of β1b in gating of N-type $Ca_v2.2$ channels.

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3616-Pos

Gamma1 Subunit Renders Cav1.2 Channels Dependent on Cell Cycle

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Auxiliary γ subunits are known to enhance inactivation of L-type calcium channels. When co-expressed in tsA-201 cells with α_{1C} and β_{2a} subunits, the γ_1 subunit shifts the voltage-dependence of inactivation by about −20 mV. The voltage of half-maximal inactivation, $V_{1/2}$, was determined with 5 s long conditioning pre-pulses. On average, addition of γ_1 to α_{1C}/β_{2a} channels changed $V_{1/2}$ from -24 ± 5 mV (n=30) to -44 ± 12 mV (n=92). We noticed that $V_{1/2}$, but not the steepness of the voltage-dependence of inactivation, varies greatly in cells with γ_1 and set up to find the cause of the cell-to-cell variability. Serum starvation and ''ER shock'' by the N-glycosylation blocker tunicamicin further shifted inactivation to negative voltages. The average $V_{1/2}$ was -59 ± 12 mV (n=12) in serum-free and -69 ± 13 mV(n=32) in tunicamicin treated cells. These treatments altered inactivation only when γ_1 was present and the effects were similar when β_3 substituted for β_{2a}. Mutations of γ_1 that remove consensus N-glycosylation sites had only partial effect ($V_{1/2}$=-60 ± 18 mV, n=29) and did not reduce the cell-to-cell variability, indicating that N-glycosylation of γ_1 was not its primary cause.
Serum starvation and tunicamicin are known to produce cell-cycle arrest in the G0/G1 phase and, therefore, could act on γ_1 indirectly by interfering with a cell-cycle dependent pathway. We characterized inactivation in cells expressing fluorescent probes visualizing cell-cycle activity. In support of our hypothesis, $V_{1/2}$ was -55 ± 16 mV, n=32, in G1 and -36 ± 7 mV, n=20, in S/G2/M cells. Therefore, we propose that a novel cell-cycle dependent regulatory pathway controls voltage-dependent inactivation and functional availability of L-type calcium channels in the presence of γ_1 subunit.
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3617-Pos

Remodelling Ca^{2+} Responsiveness of $Ca_v2.3$ by Ca_vb Subunits: Role of an N-Terminal Polyacidic Motif

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Ca^{2+}-dependent inactivation of Ca_v2 channels is highly sensitive to intracellular Ca^{2+} buffers. Therefore, it seems likely that the cytoplasmic Ca^{2+} buffering scenario will have a large impact on the activity of $Ca_v2.3$ channels, which mediate Ca^{2+} influx associated with medium to slow neurotransmitter release. Using the whole-cell patch-clamp technique, here we show that the kinetics of the fast and slow components of macroscopic inactivation, τ_f and τ_s, of $Ca_v2.3$ are significantly slower when the cell is dyalized with 0.5 mM EGTA than when is dyalized with a solution containing no intracellular chelators. Rat $Ca_v\beta_3$ and a $Ca_v\beta$ subunit from the human parasite *Schistosoma mansoni* ($Ca\beta_{Sm}$) eliminate the sensitivity of τ_f, but not of τ_s, to 0.5 mM EGTA. Interestingly, $Ca_v\beta_{Sm}$also eliminates the sensitivity of τ_f to 5 mM BAPTA, whereas $Ca_v\beta_3$ does not. Differently from mammalian $Ca_v\beta$'s, $Ca_v\beta_{Sm}$ contains a long N-terminal poly-acidic motif (NPAM). Does this motif interfere with responsiveness of τ_f to BAPTA? Coexpression with a $Ca_v\beta_{Sm}$ subunit without NPAM increased the sensitivity of τ_f to 5 mM BAPTA and enhanced the sensitivity of τ_s to EGTA and BAPTA. Coexpression with a chimaeric $Ca_v\beta_3$ subunit that contains an NPAM suppressed the sensitivity of both τ_f and τ_s to intracellular buffering. Thus, we conclude that presence of NPAM in $Ca_v\beta$ subunits reduces or suppresses the sensitivity of $Ca_v2.3$ inactivation to intracellular chelators. Perhaps NPAMs compete for Ca^{2+} with cellular buffers in the microdomains associated with Ca_v channels. We propose that the NPAM is a built-in buffer within the architecture of the $Ca_v\beta_{Sm}$ subunit with a function in modulating inactivation of schistosome Ca_v channels. Recombinant mammalian $Ca_v\beta$ subunits containing NPAMs could potentially offer a novel therapeutic strategy for diseases associated with enhanced Ca^{2+} entry.

3618-Pos

Oligomerization of $Ca_v\beta$ Subunits is an Essential Correlate of Ca^{2+} Channel Activity

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Voltage gated calcium channels conduct Ca^{2+} ions in response to membrane depolarization. The resulting transient increase in cytoplasmic free calcium concentration is a critical trigger for the initiation of such vital responses as muscle contraction, secretion and transcription. The $Ca_v1.2$ calcium channel pore is formed by the α_{1C} subunit that is associated with auxiliary $\alpha_2\delta$ and cytosolic $Ca_v\beta$ subunits. All four major $Ca_v\beta$s share a highly homologous membrane associated guanylate kinase-like (MAGUK) domain that binds to α_{1C} at the α-interaction domain (AID) situated in the linker between transmembrane repeats I and II. In this study we show that $Ca_v\beta$ form multimolecular homo- and hetero-oligomeric complexes in human vascular smooth muscle cells expressing native $Ca_v1.2$ calcium channels and in Cos7 cells expressing recombinant $Ca_v1.2$ channel subunits. $Ca_v\beta$s oligomerize at the α_{1C} subunits residing in the plasma membrane and bind to the AID. However, $Ca_v\beta$ oligomerization occurs independently on association with α_{1C}. Molecular structures responsible for $Ca_v\beta$ oligomerization reside in three regions of the GK module of MAGUK. Augmentation of $Ca_v\beta$ oligomerization does not change the voltage-dependence and kinetics of the channel, but significantly increases the current density. Thus, oligomerization of $Ca_v\beta$ subunits represents a novel and essential aspect of Ca^{2+} signal transduction.

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