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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3619-Pos

Interaction of the Calcium Channel α_2/δ_1 Subunit with an ATP Synthase in the Plasma Membrane of Muscle Cells

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The calcium channel α_2/δ_1 subunit is expressed prior to $Ca_v1.1$ and is localized at the leading edges of young skeletal myotubes, suggesting that it may interact with other cellular components. To address this issue, multi protein complexes were isolated from skeletal muscle of P0 mice and analyzed with blue native PAGE and Western blots. These studies showed that the α_2/δ_1 subunit is found in a large complex of ~670 kDa, which was also present in cultures of skeletal myotubes and C2C12 cells. This complex was isolated and further analyzed with mass spectroscopy. Mascot search results identified the β subunit of the ATP synthase (ATP5β) in the ~670 kDa complex, which is normally present in the mitochondrial membrane. However, the presence of ATP5β and α_2/δ_1 subunit in the plasma membrane was confirmed by biotin labeling of membrane proteins. Immunocytochemistry analysis of skeletal myotubes in culture showed co-localization of α_2/δ_1 subunit and ATP5β in some cells. To determine a functional significance of the interaction between α_2/δ_1 subunit and ATP5β, calcium transients were electrically evoked and recorded from myotubes using confocal microscopy. Exposure of myotubes to an ATP5β monoclonal antibody resulted in a significantly faster decay of calcium transients after a train of stimuli. This effect was less pronounced when myotubes were stimulated with a single pulse. These experiments provide the first direct evidence suggesting that the α_2/δ_1 subunit may be able to form complexes with proteins other than the Cav1.1 and suggest new signaling roles of α_2/δ_1 in muscle cells. In addition, they show that a molecule involved in the synthesis of ATP in the inner mitochondrial membrane can be also localized in the plasma membrane. Supported by the Muscular Dystrophy Association.

3620-Pos

Activity-Dependent Cav1.2 Cluster Surface Expression in Insulin-Secreting Cells

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Background
L-type Calcium channels evoke insulin secretion, and are particularly important for an initial rapid burst of hormone secretion in both rodents and human. The magnitude of this secretory burst depends on the number of Cav1.2 in plasma membrane (PM). However, the regulation of Cav1.2 dynamics in the PM has previously not been elucidadted.
Methods
4D confocal imaging performed in Cav1.2-GFP transfected INS-1 cells. The distance (R) between geometrical cell center and each Cav1.2 cluster, was determined for assessment of Cav1.2 surface dynamics. Flourescence Recovery After Photobleaching (FRAP) and Fluctuation Correlation Spectroscopy (FCS) were used for quantifying Cav1.2 kinetics in PM.
Results
Stimulation with 20mM glucose or 70mM KCl for >15min lead to Cav1.2 cluster internalization as revealed by 4D confocal imaging. After 30min, the average R value decreased from 7.093 ± 0.176 μm and 6.214 ± 0.1082 μm to 5.76 ± 0.202 μm and 5.23 ± 0.156 μm, respectively ($P<0.0001$ and 0.001). The internalized Cav1.2 clusters co-localized with EEA-1, an early endosome marker. Cav1.2 internalization was also detectable by FCS. Stimulation with glucose decreased the number of Cav1.2 clusters in the PM by ~ 40%. In addition, after glucose stimulation Cav1.2 clusters were loss mobile and diffusion times increased from 0.4 ± 0.2s to 0.6 ± 0.2s.
eIF3e (eukaryotic translation initiation factor 3, subunit E) has been implicated in the regulation of activity-dependent Cav1.2 surface expression in neurons. Silencing of eIF3e counteracted Cav1.2 cluster internalization. FRAP experiments demonstrated that ablation of eIF3e increased the time constant of Cav1.2 recovery from 0.79s to 1.50s under resting conditions and from 0.21s to 0.63s in stimulated cells. These effects were associated with reduced insulin secretion, slower cell proliferation and increased apoptosis rates.
Conclusion
Cav1.2 cluster surface distribution in insulin-secreting cells is activity-dependent. Cav1.2 traffic is regulated by eIF3e and affects both cell function and survival.

3621-Pos

Vascular Cav1.2 Expression is Increased in Atherosclerosis

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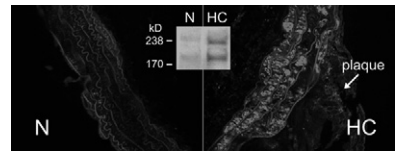
Vascular Ca^{2+} channels play an important role in the pathogenesis of hypertension, but little is known about their role in atherosclerosis. We studied the expression and function of $Ca_V1.2$ in aorta from LDLR -/- mice fed normal or high cholesterol (HC) diet for 26 weeks. HC diet markedly increased serum triglyceride, total cholesterol and phopholipid levels and generated atherosclerotic plaques, but had no significant effect on blood pressure. Immunohistochemistry (Figure) and Western blot analysis (inset) revealed that aortic $Ca_V1.2$ expression was significantly upregulated in mice fed HC diet compared to normal (N) diet. The majority of the $Ca_V1.2$ expression was in vascular smooth muscle cells (VSMCs), rather than atherosclerotic plaques. Despite overexpression of $Ca_V1.2$ subunits, aortic rings from the HC mice had a diminished constrictor response to $Ca_V1.2$ channel activator FPL64176, perhaps a result of reduced expression of the contractile protein α-actin. Inclusion of 1 mg/kg/d rosuvastatin or amlodipine in the HC diet ameliorated all observed changes. These data suggest that during atherosclerosis: (1) VSMCs develop a phenotype switch involving upregulation of $Ca_V1.2$ subunits but downregulation of contractile protein α-actin, and therefore reduced contractility; (2) $Ca_V1.2$ channels may be involved in atherosclerosis.



3622-Pos

Expression of Calcium Channel Subunits in Mesenchymal Stem Cells Undergoing Muscle Differentiation

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Mesenchymal stem cells (MSC) have the potential to generate multiple lineages including muscle. We examined expression of the $Ca_v1.1$ and α_2/δ_1 subunits of the L-type calcium channel and muscle-specific genes in Lin^- bone marrow MSC (BM-MSC) isolated from mouse. BM-MSC were initially cultured in Mesencult media with serum (StemCell Technologies). Multiple passaged and adherent in culture BM-MSC were enriched for c-kit^+ and Sca-1^+ and treated for myogenic differentiation in low serum (2% FBS) media. Total RNA was extracted from cells at different times after differentiation. RT-PCR demonstrated the presence of $Ca_v1.1$ and α_2/δ_1 subunits message after 15 days of differentiation. However, the relative amount of α_2/δ_1 subunit message was consistently larger than for the $Ca_v1.1$ subunit from day 15 to day 26. In addition, several isoforms of the α_2/δ_1 subunit were detected during the same time span. To determine the lineage of BM-MSC differentiation, we examined genes specific for muscle. At day 15 cells expressed myogenin and the cardiac isoforms of troponins T and I, and GATA4. Because cells showed a robust expression of α_2/δ_1 subunit mRNA, we used the α_2/δ_1 subunit monoclonal antibody 20A to sort cells using flow cytometry. Sorted cells cultured for an additional 30 days contracted with electrical stimulation. Cultures from sorted cells showed a similar relationship between $Ca_v1.1$ and α_2/δ_1 subunit as found in unsorted cultures. Cardiac TnT was also detected in these cells. These results show that cardiac and skeletal muscle genes are co-expressed in the population of BM-MSC at early stages of differentiation into muscle cells and suggest that further differentiation into cardiac or skeletal muscle depends on other local factors, such as neighboring cells or extracellular cues.