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 $Ca_v\beta$ 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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