3523-Pos

Interplay between Calmodulin and the Ca^{2+} Channel Beta Subunit in Ca^{2+}-Dependent Inactivation of the L-Type Channel $Ca_v1.2$

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$Ca_v1.2$ is the most prominent L-type voltage dependent Ca^{2+} channel in heart and brain and has physiological importance in the cardiovascular and central nervous system due to its direct relation to cardiac muscle contractility, learning and memory. $Ca_v\beta$ subunits supports voltage gating of $Ca_v1.2$ calcium channel. Chronically increased $Ca_v1.2$ activity contributes to the etiology of heart disease and Alzheimer's disease. Ca^{2+} influx is tightly regulated through negative feedback via voltage-dependent inactivation (VDI) and Ca^{2+}-dependent inactivation (CDI) of $Ca_v1.2$. Our long term interest is to determine the molecular mechanisms that control VDI and CDI. At present we seek to understand the molecular details of how β2 subunit and the ubiquitous Ca^{2+}-binding protein calmodulin, regulate the function of voltage-gated Ca^{2+} channels. In the present study, we employed biochemical and fluorescence anisotropy techniques to identify the exact binding site for β2 subunit on the α1 subunit $Ca_v1.2$. Our findings indicate that the residues 1588-1675 of α1 subunit serves as a common binding region for β2 subunit and Calmodulin. Furthermore, we investigated the affinities of β2 subunit for different peptides within 1534-1697 fragments of $Ca_v1.2$ and observed an affinity in the nM range with peptides 1614-1635 and 1644-1670. This work may help us to form the basis for future work on the dysregulation of Cav1.2 under pathological conditions such as heart failure and ultimately also Alzheimer's disease.

3524-Pos

Role of AKAP150 in the Regulation of the Synaptic L-Type Chalcium Channel Cav1.2

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The A-kinase anchor protein 150 (AKAP150) is a major scaffolding protein for PKA localization in brain. It plays a critical role for PKA-mediated $Ca_v1.2$ regulation. The L-type calcium channel $Ca_v1.2$ contributes to Ca^{2+} influx into cardiac and smooth muscles, neurons, and endocrine cells. In brain, $Ca_v1.2$ is majorly localized in postsynaptic sites and important for synaptic function. Protein kinase A (PKA) upregulates $Ca_v1.2$ activity in response to adrenergic stimulation. The primary phosphorylation site by PKA is located on serine 1928 of the central, pore-forming $\alpha_1$1.2 subunit. We have shown previously that $Ca_v1.2$ forms a complex with the β_2-adrenergic receptor and PKA. We also found that AKAP150 associates with β_2AR in vivo and in vitro. To investigate the significance of AKAP 150 anchored PKA activity on $Ca_v1.2$ regulation, we analyzed the functional and physical association of β_2AR and $Ca_v1.2$ among wild type, AKAP150 KO, and AKAP D36 (lacking the PKA biding site) mice.

3525-Pos

The Second Loop of Orai Channels Fine-Tunes Ca^{2+} Feedback Regulation

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Interaction of STIM1, targeted to the endoplasmic reticulum and the Orai1 channel located in the plasma-membrane induce endogenous Ca^{2+} release activated Ca^{2+} (CRAC) currents. A key regulator and hallmark of CRAC currents is the Ca^{2+} dependent inactivation (CDI). Within the Orai family, Orai3 exhibits a striking CDI in comparison to Orai1. An attribute of Orai1 is its slower phase of reactivation. Several domains within the N-and C-terminus of Orai, Stim1 and the Ca^{2+} binding protein Calmodulin regulate CDI. Here we show, that a chimera of Orai3 with the second loop of Orai1 decreased CDI in comparison to wild-type Orai3 without inducing a reactivation phase. Among the Orai proteins, this 40 amino acid long stretch differs in a set of non-conserved residues. Side-directed mutagenesis in both Orai1 and Orai3 altered both CDI and reactivation, suggesting that several amino-acids within the loop contribute to the divergent feedback characteristics. These results suggest that the second loop of Orai channels is an essential site to fine-tune CDI in combination with further domains and proteins. Supported by FWF P21118, Hertha Firnberg grant T442 and DOCfFORTE of ÖAW.

3526-Pos

Positively Charged as Well as Hydrophobic Amino Acids in Orais' Conserved N-Terminal Domain Contribute to Orai Function

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The two proteins STIM1 and Orai1 represent the key components fully reconstituting the Ca2+ release-activated Ca2+ (CRAC) current. While the C-termini of both proteins play an essential role in their store-operated coupling, the role of Orai1 N-terminus in the STIM1/Orai1 signaling machinery remains so far elusive. Orai1 N-terminus contains proline- and arginine-rich regions at the very beginning which are lacking in Orai2 and Orai3. At the end of the N-terminal cytoplasmic stretch close to the first transmembrane segment all Orai proteins include a highly conserved amino acid domain that contains positively charged as well as hydrophobic amino acids. This stretch has also been suggested to interact with STIM1.

In attempt to elucidate the role of these conserved amino acids we mutated positively charged to either neutral or negatively charged amino acids. Single neutral mutations had no effect on store-operated currents, however, substantially reduced fast inactivation of Orai1 and Orai3, while double mutations strongly reduced current densities. Single negatively charged amino acids increased store-operated currents in correlation with abolished fast inactivation, while double negative mutations also decreased current density. Furthermore mutation of some hydrophobic amino acids within this conserved region resulted in 1.5-fold increased store-operated currents, while inactivation remained unaffected. In summary, we identified respective amino acids within Orais'conserved N-terminal region that play a modulatory role in Orai function. (supported by the Habilitation Scholarship JKU Linz, Austria and FWF P21118)

3527-Pos

Regulatory Role of N-terminal Orai Domains in Current Activation

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STIM1 and Orai1 represent the key components of the CRAC (Ca2+ release-activated Ca2+) channel signalling machinery. The Orai family comprises three members designated Orai1, Orai2 and Orai3. While the C-termini of all three Orai proteins are involved in the coupling of STIM1 to Orai, the exact role of their N-termini in the communication of STIM1 and Orai is still unclear. All Orai N-termini display a highly conserved cluster of positively charged and hydrophobic amino acids located close to the first transmembrane region. In contrast, only the Orai1 N-terminus but not that of Orai2 and Orai3 contains an arginine- and proline-rich region.

In this study we focused on the role of the N-termini of Orai 1/3 proteins for current activation. It has been shown that an Orai1 N-terminal deletion mutant lacking the first 73 amino acids is still sufficient for current activation. Accordingly, an Orai1 N-terminal deletion mutant Δ1-47 lacking the arginine- and proline-rich region displayed a similar STIM1-dependent store-operated activation but showed an altered reactivation profile compared to wild-type Orai1. Further, Orai1 and Orai3 mutants with corresponding N-terminal deletions exhibited distinct STIM1-dependent activation in response to store-depletion. Moreover, activation of Orai3 via STIM1 or 2-APB apparently involved separate N-terminal regions.

In summary, conserved regions within the N-termini of Orai 1/3 proteins play a distinct role in STIM1-mediated channel activation, and separate domains contribute to STIM1 or 2-APB induced Orai3 activation. (supported by a ÖAW scholarship and FWF P21118)

3528-Pos

Conformational Rearrangement within STIM1 C-terminus Crucial for Coupling to Orai1

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Ca^{2+} influx in non-excitable cells is mainly carried by store-operated channels (SOCs), where Orai1 (CRACM1) and STIM1 represent the two molecular key players in this process. STIM1 functions as an endoplasmic reticulum located Ca^{2+} sensor and transmits the signal of store depletion to the plasma membrane by coupling to Orai1, which in turn causes channel activation. STIM1 C-terminus itself can act as a surrogate of full length STIM1 and is sufficient for the activation of Orai1 currents. 2-aminoethoxydiphenylborate (2-APB) has been shown to induce enhanced association of STIM1 C-terminus with Orai1 suggesting a conformational change within the former that drives this association. Indeed we were able to monitor 2-APB induced STIM1 C-terminal conformational rearrangement by fluorescence microscopy. In the absence of 2-APB the change in conformation was only seen for STIM1 C-terminus coupled to Orai1 but not for that part remaining in the cytosol suggesting this conformational change crucial for Orai1 binding. Moreover, we were able to mimic this conformational rearrangement by introducing selective point mutations into