Platform BE: Voltage-gated Ca Channels

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A Spider Toxin and its Recombinant Isoform Block T-Type and N-Type Calcium Channels with High Affinity

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T-type calcium channels are widely distributed in diverse tissues and dysfunctions of these channels contribute to a variety of disorders and diseases. Notably, few specific ligands are available for physiological identification of T-type calcium channels. Here we identify ω-agatoxin IIA (ω-Aga-IIA), a polypeptide toxin purified from venom of American Funnel Web spider, *Agelenopsis aperta,* as a high affinity low voltage-activated (T-type) calcium channel antagonist. In whole cell recordings of the human α_{1I} channel stably expressed in HEK cells, ω-Aga-IIA partially inhibited T-type current with an EC_{50} of 1.05 ± 0.62 nM. ω-Aga-IIA also partially blocked α_{1B} calcium channels with a higher efficacy than its effect on α_{1I} channel, with a comparable EC_{50} of 0.17 ± 0.056 nM. ω-Aga-IIA partially inhibited T-type and N-type calcium current at saturating concentrations without shifting the *I-V* curve. We also developed a heterologous expression system (*E. coli*) and a modified on-column protein refolding method for production of a ω-Aga-IIA isoform, ω-Agatoxin IIC (ω-Aga-IIC). Recombinant ω-Aga-IIC exhibited similar RP-HPLC elution profiles as ω-Aga-IIA and blocked α_{1I}/α_{1B} channels with high potency (EC_{50} of 1.01 ± 0.38 and 0.16 ± 0.049, respectively). The high affinities of ω-Aga-IIA and ω-Aga-IIC for α_{1I} and α_{1B} calcium channels indicates the presence of an evolutionarily conserved binding site on high- and low voltage-activated calcium channels. With the successfully production and refolding of recombinant ω-Aga-IIC, a valuable tool has become available for further studies of calcium channel pharmacology and function.

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Caveolin-3 Inhibits $Ca_v3.2$ (α1H) Currents and Regulates Hypertrophic Signaling in Ventricular Myocytes

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Voltage gated T-type Ca^{2+} channel $Ca_v3.2$ subunit, responsible for T-type Ca^{2+} current (I_{Ca_vT}) is expressed in different tissues including the heart and participates in Ca^{2+} influx, hormonal secretion, pacemaker activity and arrhythmia. The $Ca_v3.2$ channels are reported to be up regulated and contribute to the altered Ca^{2+} signaling and pathogenesis of cardiac hypertrophy via the activation of calcineurin/nuclear factor of activated T cells (NFAT) pathway. Caveolae containing scaffolding protein caveolin-3 (Cav-3) localize many ion channels and signaling proteins, and provide temporal and spatial regulation of intracellular Ca^{2+} in cardiomyocytes. However, the mechanism of altered Ca^{2+} signaling in cardiac hypertrophy is not clearly defined. We investigated the role of caveolae and $Ca_v3.2$ channels in the regulation of Ca^{2+} signaling during angiotensin-II induced cardiac hypertrophy in ventricular myocytes. Immunogold labeling and electron microscopy analysis demonstrated the co-localization of $Ca_v3.2$ channel and Cav-3 relative to caveolae in the ventricular myocytes. GST pull-down analysis confirmed that the N-terminus region of Cav-3 interacts with $Ca_v3.2$ channels. Impact of Cav-3 association with $Ca_V3.2$ was analyzed by whole cell patch clamp technique. Co-expression of Cav-3 specifically inhibited $I_{Cav3.2}$ in heterologously expressed HEK293 cells. In the neonatal ventricular myocytes, overexpression of Cav-3 inhibited $I_{Ca,T}$ and specifically inhibited the adenovirus ($AdCa_v3.2$) mediated peak $Ca_v3.2$ currents. In addition, overexpression of Cav-3 prevented the angiotensin-II induced hypertrophic responses in neonatal mouse cardiomyocytes. Over expression of Cav-3 prevented the angiotensin-II induced translocation of NFAT4 to the nucleus and also inhibited the phosphorylation of extracellular signal-regulated kinase, ERK. Overall our results demonstrate that over expression of Cav-3 suppresses pathological hypertrophic responses in cardiomyocytes via the inhibition of the $I_{Cav3.2}$. We conclude that Cav-3 may play a crucial role in protective signaling mechanisms in the ventricular myocytes during hypertrophic cardiomyopathy.

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Molecular Mechanism of Calcium Channel Regulation in the Fight-Or-Flight Response

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During the fight-or-flight response, the sympathetic nervous system stimulates L-type calcium currents in the heart conducted by Cav1.2 channels through activation of β-adrenergic receptors, adenylyl cyclase, and phosphorylation by cAMP-dependent protein kinase (PKA), thereby increasing cardiac contractility and beat rate. The channel α_1 subunit C-terminus contains binding sites for multiple regulatory proteins including the PKA/A kinase anchoring protein 15 (AKAP15) complex. The C-terminus is proteolytically cleaved but remains associated non-covalently with the truncated channel and acts as a potent autoinhibitor of channel activity. Relief of this autoinhibition provides an attractive mechanism for cellular regulatory signals to produce the large increases in calcium current observed physiologically. We reconstituted regulation of Cav1.2 channels in non-muscle tsA-201 cells by forming an autoregulatory signaling complex composed of the Cav1.2Δ1800 channel, the noncovalently-associated distal C-terminal domain, the auxiliary $\alpha_2\delta_1$ and β_{2b} subunits, and AKAP15. During whole-cell recordings of channel activity we observed a 3.6-fold range of Cav1.2 activity from a minimum in the presence of protein kinase inhibitors to a maximum with activation of adenylyl cyclase with forskolin. Equivalent modulation was not observed for the full-length (untruncated) channel or the truncated channel without the distal C-terminus. Basal channel activity in unstimulated cells was regulated by phosphorylation of two novel sites at Ser1700 in a PKA consensus sequence and Thr1704 in a casein kinase 2 consensus sequence, both strategically located at the interface between the distal and proximal C-terminal regulatory domains. Further stimulation of channel activity via PKA signaling required only phosphorylation of Ser1700. Phosphorylation at Ser1928 did not significantly alter channel activity. These results define the signaling complex required for Cav1.2 channel regulation and elucidate the sites of phosphorylation that regulate channel activity.

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The Calcium Channel Single Channel Conductance Heirarchy is N>L>T at Physiological External Calcium: Implications for Presynaptic Transmitter Release Site Gating

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A number of studies support the conclusion that single Ca_V channels Ca^{2+} nanodomains gate molecular signaling pathways. Thus, at presynaptic terminals single $Ca_V2.2$ channels trigger fusion of synaptic vesicle (SVs) by saturating a nearby calcium sensor.[2] It is generally accepted that Ca_V1, Ca_V2, and Ca_V3 families (L, N and T, respectively) exhibit a decreasing order of single channel conductance.[1] Since nanodomain dimensions are proportional to single channel current amplitude (*i*), high-conductance L type channels would be expected to be favored over the intermediate conductance N-type. Since the L>N>T hierarchy was determined with high $Ba^{2+}{}_{EXT}$, we tested the idea that this sequence may differ at physiological $Ca^{2+}{}_{EXT}$.

We recorded *i* values for all three Ca_V families across a broad range of $Ca^{2+}{}_{EXT}$, spanning the physiological range. We focused on i_{-65mV} to avoid non-linear current-to-voltage relationship complications and for direct relevance to the gating of synaptic transmission.[3] A $Ca_V2.2>Ca_V1>Ca_V3.2$ hierarchy was determined for i_{-65mV} at 1-2 mM $Ca^{2+}{}_{EXT}$. Mathematical modeling predicts that the $Ca_V2.2$ Ca^{2+} nanodomain is ~25% more extensive than that generated by Ca_V1. We also calculated single channel 'SV fusion' domains, defined as the radii where the channel would saturate ≥50% of 5-binding site calcium sensors. With a sensor binding affinity of 10μM a single $Ca_V2.2$ can activate a calcium-fusion sensor located on the proximal face of the synaptic vesicle.[4] These findings may explain why Ca_V2 family channels are preferred for transmitter release site gating.

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Mechanism of Auxiliary Beta-Subunit-Mediated Membrane Targeting of $Ca_v1.2$ Channels

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Ca^{2+} influx via Ca_V1 and 2 channels drives essential physiological processes ranging from synaptic transmission to muscle contraction. Membrane-targeting of Ca_V1 and 2 channels is requisite for their physiological function. Association of a pore-forming α_1 with a cytosolic β is necessary for trafficking Ca_V1 and 2 channels to the cell surface, but the mechanisms underlying this phenomenon are poorly understood. One prevalent idea is that β binds to the intracellular I-II loop of α_1 and masks an endoplasmic reticulum (ER) retention signal, while other work suggests a critical, but undefined role for the α_1 C-terminus. We hypothesized that major determinants of $Ca_V1.2$ channel ER retention and β-dependent export reside within the five intracellular loops and termini of the pore-forming α_{1C} subunit. We generated 31 chimeric