as ''quantal'' Ca2+ release. Such quantal behavior of IP3R is thought to be due to the feedback regulation of the channel by luminal Ca2+. A high level of luminal Ca2+ enhances the sensitivity of IP3R to IP3, while a reduced luminal Ca2+ level desensitizes IP3R. Despite its importance, the molecular basis underling the regulation of IP3R by luminal Ca2+ is unknown. Ryanodine receptors (RyRs), another family of intracellular Ca2+ release channels, also exhibit quantal Ca2+ release in response to agonists, and are regulated by luminal Ca2+. We have recently demonstrated that mutations in the TM10 helix (the pore inner helix) of the RyR2 channel markedly alter the sensitivity of the channel to activation by luminal Ca2+. Given the high degree of sequence homology in the channel pore-forming region between RyR and IP3R, we hypothesize that the TM6 helix in IP3R, corresponding to TM10 in RyR, is also important for luminal Ca2+ regulation of IP3R. To test this hypothesis, we have generated a number of mutations in the TM6 of IP3R and established stable, inducible HEK293 cell lines expressing these mutants. By monitoring the ER luminal Ca2+ level using a fluorescent ER Ca2+ sensor protein, D1ER, we found that mutations in TM6 either increase or decrease the rate of IP3-induced Ca2+ release in permeablized mutant cells. These mutations also affect the sensitivity of ATP-triggered Ca2+ release in intact cells. Further studies at the single channel level should provide new insights into the role of the pore-forming region in the luminal Ca2+ regulation of IP3R.

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A C-terminal Fragment of Chromogranin B Amplifies Inostiol (1,4,5)-Trisphohsphate Receptor Mediated Signaling

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Chromogranin B (CGB) is a low affinity, high capacity calcium binding protein belonging to the granin family. It is located in the lumen of the endoplasmic reticulum (ER) and is also found in secretory granules. Addition of CGB amplifies calcium release from ER stores and increases the activity of the inostiol (1,4,5)-trisphohsphate receptor (InsP3R). We previously demonstrated that CGB is non-uniformly distributed within neurons, and its spatial localization is cell type specific. We also showed that stimulation of the InsP3R in neurons leads to initiation of intracellular calcium release where the concentration of CGB is highest. When we expressed the N-terminal region of CGB, which binds to the third intralumenal loop of the InsP3R, the functional interaction between CGB and the InsP3R was disrupted and the initiation site of calcium release was altered. We now report that a 20 amino acid fragment of the C-terminal region plays a critical role in regulating calcium transients from the InsP3R. Addition of the C-terminal region of CGB increased the activity of single InsP3R currents in lipid bilayers. When intracellular calcium transients were monitored in 3T3 cells lacking CGB, InsP3R dependent calcium release was markedly amplified after expression of full length CGB or expression of the C-terminal region. In contrast, expression of the N-terminal region was unable to amplify the intracellular calcium transients. In SHSY5Y cells with endogenous CGB, expression of the C-terminal region induced a prolonged response to extracelluar agonists compared to native cells whereas expression of the N-terminal region depressed calcium signaling and altered the signal initiation site. These effects of CGB on calcium transients in neuronal cells indicate its importance in physiological processes and will guide investigation into pathophysiological processes.

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Comparison of IP_3R and RyR Expression and Ca^{2+} Release Characteristics in Isolated Cardiac Nuclei

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In cardiac muscle, the role of the inositol trisphosphate receptor (IP_3R) and its regulation is not fully understood. A contribution to nuclear Ca^{2+} signalling has been proposed. This study compares expression and Ca^{2+} release characteristics of the IP_3R and the ryanodine receptor (RyR) in purified functional cardiac nuclei. It also examines whether the IP_3R may exist as a multi-protein complex in these preparations. Quantitative immunoblotting of IP_3R and RyR protein levels in isolated nuclei demonstrated greater expression of the IP_3R; nucleolin was used as an internal control for quantification. Ca^{2+} release in response to IP_3 and caffeine from single isolated nuclei was used to compare IP_3R and RyR activity. Changes in nuclear [Ca^{2+}] were measured as fluorescence signals from nuclei loaded with 10μM Fluo 5N-AM. IP_3 or caffeine was applied by hydrostatic pressure ejection and signals expressed as ratios (F/F_0) of fluorescence counts relative to baseline. Ca^{2+} release in response to IP_3 (10μM) was significantly greater than that released in response to caffeine (10mM) (0.12 ± 0.02 v's 0.017 ± 0.002 $[Ca^{2+}]_{Nuc}$ (F/F_0) for IP_3 and caffeine respectively, n=6). When tetracaine (100μM) was applied to the nuclei, IP_3-mediated Ca^{2+} release was unaffected but the response to caffeine was abolished, suggesting RyR activation does not contribute to IP_3-mediated nuclear Ca^{2+} release. The potential for other nuclear proteins interacting with the nuclear IP_3R was also investigated. Immunoblot analysis demonstrated expression of both FKBP12 and calcineurin in cardiac nuclei. These proteins are known to interact with the IP_3R in other tissue types. Co-immunoprecipitation experiments using an anti-IP_3R (type II) antibody suggest IP_3R/calcineurin/FKBP12 interaction specifically at the nucleus. These results highlight the existence of a nuclear multi-protein IP_3R complex, providing further scope for regulation of cardiac nuclear Ca^{2+} release.

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Type 2 Inositol 1,4,5-trisphosphate Receptor Phosphorylation and Modulation by Ca^{2+}/Calmodulin-dependent Protein Kinase II

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$InsP_3$-mediated intracellular Ca transients can activate Ca^{2+}-calmodulin-dependent protein kinase II (CaMKII), a multifunctional Serine/Threonine protein kinase involved in many signaling pathways. Recent results show that $InsP_3Rs$ in the heart ($InsP_3R2$) are primarily targeted to the nuclear envelope in ventricular cardiac myocytes. Here it forms a macromolecular complex with CaMKIIδ (Bare et al, 2005, *JBC*) . Upon stimulation of $InsP_3$ production, Ca^{2+} released through the $InsP_3R2$ activates CaMKIIδ, allowing it to act on downstream targets, such as histone deacetylases 4 & 5 (HDAC4 & HDAC5) (Zhang et al, 2007, *JBC*). Additionally, CaMKII activity feedback modulates $InsP_3R2$ function by direct phosphorylation and results in a decrease in the channel's open probability. The results of this study show that in planar lipid bilayers the channel activity of $InsP_3Rs$ can be inhibited by CaMKII-mediated phosphorylation, and that effect can be reversed by addition of protein phosphatases. Furthermore, the N-terminal 1078 amino acids of the rat $InsP_3R2$ have been shown to interact with, as well as be phosphorylated by CaMKII in *in vitro* kinase assays. A smaller fragment spanning amino acids 1-708 of the $InsP_3R2$ has been shown to be phosphorylated in a CaMKII-dependent manner. Conversely, C-terminal regions were not phosphorylated by CaMKII *in vitro*. We have also shown that the N-terminal region of the rat $InsP_3R1$ spanning amino acids 1-1081 can be phosphorylated by CaMKII. Our results from mass spectrometry and *in vitro* kinase assays indicate that the putative CaMKII regulatory phosphorylation site lies within amino acids 150-340 of the $InsP_3R2$.
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Regulation Of Inositol 1,4,5-Trisphosphate Receptor Isoforms By O-Linked Glycosylation

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The inositiol 1,4,5 trisphospate receptor (InsP3R), an intracellular calcium channel, is a family of three isoforms. All three isoforms display a significant level of sequence identity yet they differ in expression level, localization and many functional aspects. We previously showed that InsP3R type 1 is modified by O-linked β-N-acetylglucosamine glycosylation (O-GlcNAcylated). Through this dynamic and inducible modification a single monosaccharide is covalently attached to serine and threonine residues of the protein backbone, providing protein regulation similar to O-phosphorylation. We also reported that increased O-GlcNAcylation of the InsP3R type 1 reduced the percent of cells that responded to addition of extracellular agonists and those that did respond had a decreased InsP3 dependent calcium release from the endoplasmic reticulum (ER). We now report that the InsP3R type 3 is also O-GlcNAcylated. Interestingly, the functional impact of O-GlcNAcylation on InsP3R type 3 channel is opposite to the effect measured with the InsP3R type 1. Human cholangiocytoma cells (MzChA-1) contain >90% InsP3R type 3. When these cells were incubated in hyperglycemic media there was an increase in the percent cells responding to InsP3 generating stimuli and there was an increase in the InsP3 dependent calcium release from the ER. A difference in functional response between InsP3R isoforms was reported previously for phosphorylation by cyclic AMP dependent protein kinase (PKA). In contrast, the InsP3R type 2 showed no detectable O-GlcNAc glycosylation and no significant functional changes even though the enzymes necessary for both the addition and removal of the monosccharide are present in all cell types tested. The dynamic and inducible nature of O-GlcNAcylation and the isoform specificity suggests that this form of modification of the InsP3R and subsequent changes in intracellular