Calcium Fluxes, Sparks & Waves II

1528-Pos

Polyhistidine Peptide Inhibitor of the Aβ Calcium Channel Potently Blocks the Aβ-Induced Calcium Response in Cells

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Based on the consistent demonstrations that the Aβ peptide of Alzheimer's disease forms calcium permeant channels in artificial membranes, we have proposed that the intracellular calcium increase observed in cells exposed to Aβ is initiated by calcium fluxes through Aβ channels. We have found that a small four histidine peptide, NAHis04, potently inhibits the Aβ-induced calcium channel currents in artificial lipids membrane. Here we report that NaHis04 also potently blocks the intracellular calcium increase which is observed in cells exposed to Aβ. PC12 cells loaded with Fura 2AM show a rapid increase in fluorescence with rapidly return to base line after Aβ is added to the medium. This fluorescence change occurs even when the medium contains nitrendipine, a voltage-gated calcium channel blocker, but fails to occur when application of Aβ is preceded by addition of NAHis04. Steep dose response curves of percentage of responding cells and cell viability show that NAHis04 inhibits in the μm range in an apparently cooperative manner. We have developed numerous models of Aβ pores in which the first part of the Aβ sequence forms a large beta barrel ending at His13. We have modeled how up to four NAHis04 peptides may block these types of pores by binding to side chains of Aβ residues Glu 11, His 13, and His 14.

1529-Pos

Elucidation of Ca^{2+} Influx through Alzheimer's Aβ Channels in the Aβ-Induced Cellular Ca^{2+} Response

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The Alzheimer's disease Aβ peptide interaction with the plasma membrane of cells results in a response characterized by the elevation of the intracellular (cytoplasmic) Ca^{2+} concentration which may critically perturb Ca^{2+} homeostasis. Calcium influx into the cytosol can occur across the plasma membrane via receptor-mediated, voltage-gated, store-operated calcium channels, or from internal stores. Based on the ion channel formation by Aβ peptides on artificial membranes *w*e have proposed that perturbation in Ca2+ homeostasis induced by Aβ could be caused by external calcium entering through ion channels formed by Aβ in the plasma membrane. The activity of these channels would permit the entrance of extracellular calcium ions into the cell, subsequently triggering the release of calcium from internal stores. To elucidate the contribution of calcium influx through the Aβ ion channels to the Aβ-induced calcium response we used specific blockers of plasma membrane channels and specific inhibitors of the mechanisms that permit the release of calcium from the ER, the largest intracellular store. With all those mechanisms blocked and inhibited, we visualized a fast raising, short-lasting calcium entry immediately after PC12 cells were exposed to Aβ. This calcium signal was identified as calcium flowing through the Aβ channels by using specific Aβ channel blockers. The Aβ channel blockers did not affect other mechanisms that contribute to the cytosolic calcium increase, and their use prevented the calcium entry through the Aβ channels and consequent development of the whole cellular Aβ-induced calcium response.

1530-Pos

Functional Analysis of GPCR and Calcium Channel Targets Using Quest Fluo-8

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Calcium flux assays are preferred methods in drug discovery for screening G protein coupled receptors (GPCRs) and calcium channels. Quest Fluo-8 AM, Fluo-3 AM and Fluo-4 AM are evaluated for several GPCR and ion channel targets. They share the same assay principle. All the three fluorogenic calcium indicators are in the form of non-fluorescent AM esters. Once inside cells, the lipophilic AM blocking groups are cleaved by non-specific cellular esterases, resulting in negatively charged fluorescent dyes that stay inside cells, and their fluorescence intensities are greatly enhanced upon binding to calcium. When cells stimulated with bioactive compounds, the receptor signals release of intracellular calcium, which greatly increase their fluorescence signals. In conclusion, Fluo-3 AM, Fluo-4 AM and Quest Fluo-8 AM are robust tools for evaluating GPCR and calcium channel targets and screening their agonists and antagonists with fluorescence microplate readers, fluorescence microscopes or flow cytometers.

1531-Pos

Persistent Calcium Sparklet Activity of L-Type Calcium Channels: Link Between PKC and c-Src

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Ca^{2+} sparklets are a fluorescence signal associated with Ca^{2+} entry through L-type calcium ($Ca_v1.2$) channels, which are primary Ca^{2+} entry pathway in many excitable cells. Ca^{2+} sparklets are quantal in nature (q = 34 nM $\Delta[Ca^{2+}]_i$) and their activity is categorized as low ($0<nP_s<0.2$) or persistent ($nP_s>0.2$), where n = no. of quantal levels and P_s = active sparklet probability. Both types of Ca^{2+} sparklets are present in vascular smooth muscle cells (VSMCs) but only low activity Ca^{2+} sparklets are present in heterologously expressed $Ca_v1.2$ channels unless PKCα is activated. The latter effect presumably requires $Ca_v1.2$ phosphorylation, perhaps at the canonical PKA phosphorylation site, S^{1901}, yet the exact phosphorylation site remains unclear. Using TIRF microscopy in conjunction with whole cell voltage clamp, we detected persistent Ca^{2+} sparklet activity in HEK 293 cells co-expressing $S^{1901}A$ $Ca_v1.2c$ and PKCα, indicating that PKCα does not phosphorylate S^{1901}. Also, persistent Ca^{2+} sparklets were detectable in cells expressing WT $Ca_v1.2c$ in the absence of PKCα if c-Src was co-expressed. Furthermore, Ca^{2+} sparklet activity was reduced in cells expressing WT $Ca_v1.2c$ and kinase dead c-Src (7/9 cells). To test if phosphorylation of a previously identified $Ca_v1.2c$ tyrosine phosphorylation site (Y^{2122}) by c-Src mediated persistent Ca^{2+} sparklet activity, we co-expressed $Y^{2122}F$ $Ca_v1.2c$ and c-Src in HEK cells. Persistent Ca^{2+} sparklet activity was present under these conditions; however, Ca^{2+} sparklet activity was reduced in cells co-expressing $Y^{2139}FCa_v1.2c$ and c-Src. These data suggest that c-Src may phosphorylate $Ca_v1.2c$ at Y^{2139} under basal conditions to produce persistent Ca^{2+} sparklet activity. Future experiments on HEK cells expressing $Y^{2139}FCa_v1.2c$ and PKCα will allow us to determine if persistent Ca^{2+} sparklet activity in VSMCs is evoked by a common mechanism involving PKCα and c-Src.

1532-Pos

Optical Stimulation of Ca^{2+} Transients in Smooth Muscle Cells

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The modulation of intracellular Ca^{2+} plays a huge role in controlling important cell functions such as cell division, signaling, contraction and cell death. To date, intracellular Ca^{2+} dynamics have mainly been investigated using electrophysiological measurement techniques such as patch clamp experiments often used in combination with fluorescence imaging techniques. To activate intracellular channels, drugs are often used. However, because of diffusion and mixing constraints there is little precision in the time course of channel activation and deactivation. In addition, the invasive nature of conventional whole cell patch clamp techniques disturbs the intracellular environment and may so alter channel behaviour.

We report a non-invasive technique that induces controlled Ca^{2+} responses in isolated smooth muscle cells using a holosteric incoherent light source as a stimulus. Using a conventional epi-fluorescence microscope configuration, cells labeled with a Ca^{2+} fluorescent indicator (Fluo-3AM) were stimulated using the low intensity light ($<1.5mW \cong \lambda=488nm$) and the resultant Ca^{2+} transients were visualized using a highly sensitive CCD camera. We will describe the cell stimulation protocol used and present data demonstrating the efficacy of this low cost and minimally invasive technique. We will also describe the investigation into the origin of the light induced Ca^{2+} responses.

1533-Pos

Agonist-Evoked Calcium Wave Progression Requires Calcium and Ip_3 in Smooth Muscle

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Smooth muscle responds to IP_3-generating agonists by producing Ca^{2+} waves to spread information within and between cells. The mechanism of wave progression has been investigated in voltage-clamped single smooth muscle cells. Agonist-evoked waves initiated as a uniform rise in $[Ca^{2+}]_c$ over a substantial length (~30 μm) of the cell. During regenerative propagation, the wave-front was approximately 1/3 the length (~9 μm) of the $[Ca^{2+}]_c$ change at the initiation site. The wave-front progressed at a constant velocity though amplitude varied through the cell. Differences in sensitivity to IP_3 may explain the variation in amplitude; local release of IP_3 evoked $[Ca^{2+}]_c$ increases of varying amplitude in different regions of the cell. The wave-front does not progress by Ca^{2+}-dependent positive feedback alone. In support, colliding $[Ca^{2+}]_c$