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The transient receptor potential (TRP) channel TRPC3 is a non-voltage gated Ca^{2+}-permeable cation channel that is expressed in skeletal muscle. It is assumed that TRPC3 is important for the cellular Ca^{2+} homeostasis and that the channel is involved in Ca^{2+} dependent signal transduction. To study the role of TRPC3 in skeletal muscle we investigated gene expression and cellular localization of the TRPC3 protein. We further tested whether expression and localization of TRPC3 are altered in murine muscular dystrophy (mdx), a muscle disease characterized by abnormal cellular Ca^{2+} regulation. Using RT-PCR and Western blot techniques, we did not find differences in TRPC3 gene expression in limb muscles and diaphragm between mdx and control mice. Immunofluorescent staining of isolated interosseus fibers with an anti-TRPC3 antibody revealed a cross striation pattern near the sarcolemma and a faint cytoplasmic fluorescence. Double labelling experiments showed co-localization of TRPC3 with vinculin and dystrophin, but not with the ryanodine receptor or the dihydropyridine receptor. The latter results were confirmed for both genotypes, however, mdx fibers showed a more prominent cytoplasmic TRPC3 staining. The strong cytoplasmic TRPC3 signal diminished, while the sarcolemmal staining increased, after incubation of mdx fibers with Gd^{3+} (50 μM), nifedipine (50 μM), epidermal growth factor, 2-aminoethoxydiphenly borate (2-APB) or a Ca^{2+}-free solution. In control fibers, the effects of the ion channel blockers were lesser. Only Gd^{3+} and nifedipine stimulated the translocation of TRPC3 to the sarcolemma. Our data suggest a costameric localization of TRPC3 in skeletal muscle and a Ca^{2+}-dependent trafficking of the channel from cytoplasmic pools to the sarcolemma. In dystrophin-deficient mdx fibers TRPC3 seems to be displaced to the cytoplasm, an effect that can be reverted by inhibition of Ca^{2+} influx.

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Synaptic Transmission

3058-Pos Monomeric Helical α-Synuclein Forms Highly Conductive Ion Channels

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Monomeric wild type α-synuclein (αS) and two mutants associated with familial Parkinson's disease, E46K and A53T, form ion channels with well defined conductance states in planar bilayer membranes containing 25–50% anionic lipid (PG) and 50% phosphatidyl-ethanolamine (PE) in the presence of a *trans*-negative potential. In contrast, another familial mutant, A30P, known to have a lower membrane affinity, did not form ion channels. Membrane permeabilization by oligomeric αS, compared to that by the monomer, differed by a low probability of discrete channel formation, no requirement for curvature-inducing lipid, and a pronounced decrease in the dependence of channel activity on anionic lipid and trans-membrane potential.

Circular dichroism (CD) analysis revealed a significant increase in helical content of the oligomeric form of αS upon binding to liposomes with optimum for channel formation lipid composition. This implies partial conversion of oligomer from β-strand to α-helix conformation. Fluorescence correlation spectroscopy was employed to measure the lateral mobility of αS after binding of monomeric aS to planar bilayer membranes under conditions favorable for channel formation. It was found that the lateral mobility of αS is similar to that of 19 kDa channel-forming domain of colicin E1, which is known to function as a monomer in the membrane-bound state, and is slightly larger then that of lipids. Together with the CD data, this implied the absence of oligomerization of αS upon membrane-binding.

It was inferred that discrete ion channels with well defined conductance states were formed by one or several molecules of monomeric αS in an α-helical conformation and that such channels have a role in the normal function and pathophysiology of the protein

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3059-Pos Kv Channels Ion Independent Effect on Frequency and Characteristics of Fusion Events

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Kv2.1 channel is commonly expresses in the soma and dendrites of neurons, where it could influence the release of neuropeptides and neurotrophins and in neuroendocrine cells, where it could influence hormone release. The traditional role of this channel is to inhibit neurotransmitter release by influencing the membrane potential and thereby inhibiting voltage-gated calcium channels activation. Recently (Singer-Lahat *et al.*, 2007), we suggested an unexpected role for Kv2.1 of facilitating neuropeptide release from rat PC12 cells expressing recombinant fluorescent atrial natriuretic factor polypeptide. The enhancement was independent of the channel ion conducting function and occurred through a direct interaction with syntaxin 1A, a member of the SNARE proteins which are component of the vesicle release machinery. These data implied a complex role for this channel in the regulation of exocytosis, and raised the need for understanding the mechanism underlying this enhancement effect.

Here, we further investigated the enhancement effect on release from bovine chromaffin cells using amperometry, which allows direct measurements of single secretion events. We show that over expression of Kv2.1 wild-type or a non-conducting mutant channel enhances release (~ two fold) of catecholamines triggered by external application of high potassium solution, compared with control cells. Detailed single spike analysis reveals that the enhancement can be attributed mostly to a higher fusion events

frequency, indicating a possible involvement of Kv2.1 in vesicle docking and/or priming. In addition, significant changes in single spike parameters are obtained, suggesting a role for Kv2.1 in regulating the vesicle fusion efficiency.

This study substantiates the non-conducting function of Kv2.1 channel in regulating vesicle exocytosis and provides new mechanistic insights into the machinery that participating in the regulation of secretion.

3060-Pos Overexpression Of Kv2.1 Channel In Drg Neurons Enhances Exocytosis Independent Of Its Ion-conducting Function

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It is commonly accepted that voltage-gated K^+ (Kv) channels inhibit release indirectly by hyperpolarizing membrane potential. Recently we showed that a direct interaction of Kv2.1 channel with the key component of the fusion machinery, syntaxin 1A, causes facilitation of exocytosis in PC12 cells (Singer-Lahat et al., 2007). Here we extended the study to dorsal root ganglion (DRG) neurons. Exocytosis was measured as the change in membrane capacitance (ΔCm) in response to depolarization from -70 to 0 mV. These experiments were done by whole cell voltage clamp configuration with Cs ion in the electrode solution to block K^+ currents. In agreement with our results in PC12 cells, overexpression of Kv2.1 enhanced peptide release, compared to the release from control DRG neurons without affecting Ca^{2+} currents. Moreover, the enhancement was diminished when a mutant Kv2.1 channels with deleted C terminal syntaxin-binding domain, C1a, was overexpressed in DRG neurons. Thus, the Kv2.1 channel has a dual functioning, mediated by association with the secretory apparatus and by its pore.

References

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3061-Pos A Maximum-Entropy Algorithm for Deconvolution of Excitatory Postsynaptic Currents with a High Temporal Resolution

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Chemical synaptic transmission, involving the exocytotic release of neurotransmitter from synaptic vesicles, results in excitatory postsynaptic currents (EPSCs) that can be measured in a postsynaptic neuron by tight-seal, whole-cell recording. Such records are often complex, with overlapping signals that display a large range of amplitudes thought to reflect exocytosis both from individual and various ensembles of vesicles. Statistical analysis of the kinetics and amplitudes of such complex EPSCs is essential in the development of mechanistic models of transmitter release. In the auditory system, for example, the synaptic propagation of information with high temporal fidelity requires that the dynamics of exocytosis be determined at a similar precision.

We developed a high-resolution algorithm to detect exocytotic events in complex EPSC records. Using a novel maximum-likelihood algorithm with sub-sample precision to align EPSCs, we characterized their kinetics in a parameter-free way. Combining this approach with maximum-entropy deconvolution, we identified independent release events at a temporal resolution of less than 250 μs. This approach indicates that the large exocytotic events in hair cells of the amphibian papilla in the bullfrog's inner ear have no detectable substructure, suggesting that transmitter release from multiple vesicles is highly synchronous.

Maximum-entropy deconvolution of the long records required for statistical analysis challenges the capabilities of contemporary workstations. We therefore used numerical techniques developed for the analysis of large astronomical datasets to write software that performs the deconvolution on a timescale of the order of the number of samples. The algorithm is available as a software package for use by other researchers in the field.

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3062-Pos Acute action of Thyroid Hormones in mice neuromuscular junction

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Muscular weakness is a hallmark of thyroid dysfunction, whether excess or insufficient thyroid hormones (THs) are produced. Since THs have rapid, nongenomic effects on spontaneous transmitter release in amphibian skeletal muscle, we hypothesized that the muscular weakness in these pathological conditions could result in part from rapid effects of THs on neuromuscular performance. Using male Swiss-Webster mice, we have explored direct effects of THs on transmitter release, and the effect of Methimazole (MMI), on muscular performance and transmitter release. Triiodothyronine (T_3), (0.25–250 nM) directly added to a mouse diaphragm preparation increases spontaneous neurotransmitter release in 2–5 min and is dose-dependent. Mice administered MMI (0.1%) in the drinking water, were tested for weight changes, Thyrotropin (TSH) and T_3 levels, and front limb strength, at 24 and 48 h after treatment. We

observed a significant weight loss during the two days of MMI exposure, depending on initial weight. Plasma TSH levels at 24 and 48 h after MMI were significantly reduced ($p<0.05$) as compared to controls, and T_3 levels were increased ($p<0.001$). Upper limb strength was evaluated using the hanging wire task at 24 and 48 h after MMI exposure. All treated animals showed reduced muscle strength compared to controls. Miniature endplate current frequency was also reduced in MMI treated animals, and diaphragm preparations showed reduced fluorescent alpha-bungarotoxin labeling compared to controls. A preliminary analysis of the muscle proteome in control and MMI treated animals suggests the involvement of alpha-actin in the THs effects. Together, these results strongly suggest THs may acutely regulate mammalian neuromuscular function. Similar rapid THs effects may contribute to the muscular weakness of thyroid dysfunction before and during initial treatment with MMI.

Peptide & Toxin Ion Channels

3063-Pos High Membrane Cholesterol Levels Characterize Cells Identified By Their Aβ Binding Affinity

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Abundant evidence establishes that the Alzheimer's disease (AD) Aβ peptide is a cell-selective cytotoxic agent. We have recently demonstrated that neuronal cells, when analyzed on the basis of their capacity to bind Aβ, can be separated into two main subpopulations: one that distinguishably bind Aβ, and one that does not bind Aβ. The selective cell membrane binding of Aβ is established by distinctive characteristics of subpopulations of cells which are maintained even after multiple cell divisions. Because the level of cholesterol in the cell membrane has been considered an influential factor associated with Aβ toxicity, we investigated the level of membrane cholesterol in subpopulations of cells with differential Aβ membrane binding. We also studied the effect of changing the levels of membrane cholesterol on the membrane affinity for Aβ membrane. Using flow-cytometry and cell sorting we analyzed Aβ binding and the membrane content of cholesterol in PC12 cells and ex-vivo hippocampal neurons. Fluorescent Aβ and filipin were used to detect membrane bound Aβ and membrane cholesterol, respectively. Membrane cholesterol was either enriched or depleted by growing cells on cholesterol-enriched media or by interfering with the biosynthesis of cholesterol. We found that populations of cells that exhibited Aβ binding affinities also show higher membrane cholesterol levels, compared to cells that did not bind Aβ. The same direct relation was also observed when membranes were artificially enrich or depleted of cholesterol. This membrane characteristic was confirmed after cell sorting based on Aβ binding affinities, and was found to be maintained after several days in cultures and multiple cell divisions. We conclude that the level of cholesterol in the membrane is one of distinctive the membrane factors influencing the membrane binding affinity for Aβ.

3064-Pos G_{M1} and PS Enrichment of Cell Membranes Assists Aβ Membrane Binding and Ion Channel Formation

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Aβ binding to cell membranes and Aβ aggregation to structures that form membrane ion channels has been considered crucial steps in the cytotoxicity of Aβ. Studies suggest that the seeding process to Aβ aggregation occurs in lipid raft mediated by clusters of monosialoganglioside G_{M1}, and that phosphatidylserine (PS) is a surface membrane receptor for Aβ. Aβ membrane binding is followed by intracellular calcium increases produced by the insertion and consequent formation of Aβ ion channels. This study examine whether the formation of aggregate structures corresponding to the cytotoxic Aβ ion channels correlates with G_{M1} and PS enrichment of cell membranes. Fluorescence microscopy and spectrophotometer evaluation showed that the GM1 and PS content of the cell membrane can be gradually increased by exogenous timely exposure of cells to G_{M1} and to PS. Membrane G_{M1} was recognized with fluorescence-labeled cholera toxin, and PS with annexin V-FITC. The binding of Aβ was tracked by Aβ42-AMCA. The formation and identification of Aβ channels was tracked by measuring the increase in intracellular calcium and by blocking the channels with a specific Aβ channel blocker. The increase in the intracellular calcium concentration and the reduction of viability observed after cells are exposed to Aβ is significantly enhanced in cells enriched with G_{M1} and PS. Both, Aβ-induced effects are totally prevented when cells are cultured in the presence of a specific Aβ channel blocker. PS enrichment is notoriously more effective than G_{M1} enrichment in favoring Aβ membrane binding. Our results strongly suggest the role of PS as a Aβ membrane receptor, and that the Aβ aggregation in the cell membrane lipid rafts mediated by clusters of G_{M1} additionally promote the formation of structures with the capacity to form of Aβ channels.

3065-Pos Characterization of Amyloid-Beta Membrane Association and Permeabilization: Dependence on membrane charge, curvature, and phase

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Much evidence has demonstrated involvement of the peptide amyloid-β (Aβ) in instigating neuronal degeneration in Alzheimer's Disease. Whereas it was previously thought that Aβ becomes cytotoxic only as insoluble fibrillar aggregates, recent studies suggest that soluble intermediate sized oligomeric species cause cell death through membrane permeabilization. Here we characterize the steps leading to the formation of ion conducting channels,