2206-Pos Board B192

Positively Charged Amino Acids in the C-Terminal Domain of SNAP-25 Affect Fusion Pore Structure and Dynamics

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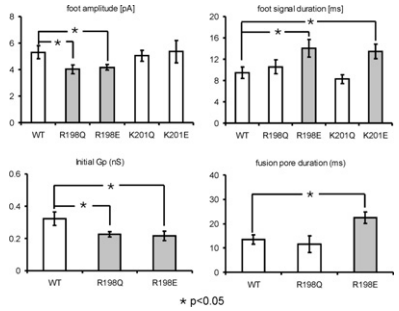
SNAP25 is a t-SNARE protein mediating exocytosis in neuronal cell types. Previous results with a construct lacking the nine C-terminal residues (SNAP25Δ9) showed changed fusion pore properties, suggesting a model for fusion pore mechanics that couple C-terminal zipping of the SNARE complex to the opening of a proteolipid fusion pore (Fang 2008 PNAS,105:15388). The deleted fragment contains the positively charged residues R198andK201, which are located in layers 7-8 of the SNARE complex at its C-terminal end. To study the role of these two residues in the structure and dynamics of the fusion pore, carbon fiber amperometry and cell-attached capacitance measurement of individual fusion and release events were performed using bovine chromaffin cells overexpressing wild type SNAP-25 or the point mutation constructs R198Q/E, K201Q/E to substitute the positively charged amino acid with a neutral or positively charged one. The R198E/K201E mutants increased fusion pore duration similar to SNAP25Δ9. Furthermore, the R198E/R198Q also showed a smaller fusion pore conductance. These results support the conclusion that fusion pore properties depend on the precise arrangements at the C-terminal end of the SNARE complex. Supported by NIH grant GM085808.



2207-Pos Board B193

Activity-Dependent Fusion Pore Dilation Mediated by a Dynamin I-Syndapin Pathway

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Chormaffin cells of adrenal medulla serve a primary role in setting a proper homeostatic status under basal sympathetic tone as well as a physiological response to sympathetic stress. In order to function under these diverse conditions, chromaffin cells exhibit a differential release of catecholamine and co-packaged peptide transmitter molecules. Under basal sympathetic firing, catecholamine is selectively secreted through a restricted fusion pore of ''Kiss and Run'' exocytic mode. On the other hand, under acute stress, elevated sympathetic activity increases cytosolic calcium, leading to dilation of fusion pore and finally full granule collapse into plasma membrane. Both catecholamine and peptide transmitters are expelled in this condition. Thus, activity-dependent differential transmitter release is regulated by fusion pore dilation. Previous studies have shown that dynamin I plays a critical role in controlling fusion pore dilation in chromaffin cells. Here, we employ electrochemical, electrophysiological and fluorescence based approaches to investigate the molecular mechanism responsible for dynamin I-dependent fusion pore dilation. We show that syndapins (synaptic dynamin-associated proteins) are a primary molecular component of dynamin I-dependent fusion pore regulation. Disruption of dynamin I-syndapin interaction decreases normal activity-mediated catecholamine release. Our results suggest that fusion pore dilation is regulated by a dynamin I-syndapin-dependent signaling mechanism.

2208-Pos Board B194

Oxidation and Palmitoylation of SNAP-25B In Vitro

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SNAP-25 plays a critical role in neuronal exocytosis. The SNAP-25 linker region contains a cysteine-rich domain, which is the site of various post-translational modifications such as palmitoylation and possibly oxidation. Palmitoylation anchors SNAP-25 to the membrane, while the level of oxidation may regulate the extent of transmitter release. Using a biotinylation/chemiluminescence assay, within a reconstituted system, and mass spectrometry (LTQ Orbitrap) we characterize the modification of the four cysteine residues in the SNAP-25B linker. We show that the palmitoyl transferase DHHC17 increases the extent of SNAP-25B palmitoylation *in vitro*. We verified that oxidation of SNAP-25B leads to disulfide bond formation within the cysteine-rich domain, the predominant form containing two disulfide bonds. As expected, oxidation of cysteines prevented palmitoylation. Through additional experimentation we hope to elucidate the effects of palmitoylation and oxidative stress on SNARE complex formation and vesicle fusion.

2209-Pos Board B195

Glucose Regulates the Kinetics of Insulin Granule Fusion

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A key step in glucose homeostasis is the glucose-induced secretion of insulin from pancreatic beta cells. The control of amount of insulin secretion has largely been thought of as dependent on glucose regulation of the size and shape of the calcium signal that in turn regulates the numbers of fused granules. However, past work indicates that differences in the kinetics of insulin granule fusion exist and that these may be important determinants of the amount of insulin release (Ma et al., 2004; Michael et al., 2006). Here we have tested the hypothesis insulin granule fusion kinetics are regulated in a glucose-dependent manner.

Isolated mouse islets were bathed in a solution containing the fluorescent dye, sulforhodamine B (SRB). These islets were imaged with 2-photon microscopy and stimulated with glucose. Fusion of individual granules was observed as the sudden appearance of spots of SRB fluorescence (diameter = 300nm ± 12.9nm (mean+/-SEM, N=5 islets); consistent with insulin granule fusion (Ma et al., 2004). 15mM glucose triggered granule fusion with a latency of 118s ± 26 (mean+/-SEM, N=5) compared to 627s ± 117 (mean+/-SEM, N=5, Student's t test $p<0.01$) with 6 mM glucose. Half-maximal cumulative granule fusion occurred at 456s ± 71 (mean+/-SEM) with 15 mM glucose stimulation compared with 1008s ± 57 (mean+/-SEM, $p<0.01$). Plots of SRB fluorescence intensity over time for each granule showed an initial peak fluorescence after granule fusion and then a decay of differing time-courses. Measured 10 seconds after fusion, 42.8% of granules with 15 mM glucose and 56.5% of granules with 6mM glucose stimulation still had >30% of peak fluorescence.

References

We conclude these differences suggest differences in granule fusion behavior that are regulated by glucose.

Ma et al. (2004) PNAS 101, 9266-9271

Michael et al. (2006) Diabetes, 600-607

2210-Pos Board B196

Multiple Sites of Contact Exist between Synaptotagmin 1 and SNAREs and the Complex is Structurally Heterogeneous

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Synaptotagmin 1 (syt 1) is a vesicle anchored membrane protein consisting of two C2 domains that acts as the Ca2+ sensor in neuronal exocytosis. Neuronal fusion is mediated by the SNAREs and the interaction of syt 1 with the SNARE complex is thought to be critical to this process. Using site-direct spin labeling and continuous wave EPR, three sites of interaction on a soluble fragment of syt 1 were identified to the soluble core SNARE complex that occur in a Ca2+ independent manner. These include: the polybasic region of the C2B domain, the sites opposite to Ca2+ binding loop of C2B, and a region near loop 2 of C2A. The distances between the C2A and C2B domains of syt 1 were measured using pulse EPR in solution and in the presence of the soluble core SNARE complex under conditions where syt1 is completely bound to SNAREs. The distances, which have broad distributions, are virtually unchanged in the presence of SNAREs indicating that the two C2 domains assume relative orientations that are heterogeneous when bound to the SNAREs. Moreover, the two C2 domains assume a roughly antiparallel orientation. When reconstituted into bilayers composed of POPC:POPS (3:1), the SNAREs associate with the C2 domains of syt1, but only in the absence of Ca2+. Under this set of conditions, ternary interactions between syt1/SNAREs and membranes are not observed. A model for the molecular function of syt1 based upon these data will be presented. (supported by NIGMS, GM 072694).

2211-Pos Board B197

Titration of Synaptotagmin I Expression Differentially Regulates Release of Neuropeptide Y and Norepinephrine

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Christopher D. Wedell, Lauren R. French, **Amy B. Harkins**.

Synaptotagmin (syt) I is a primary regulator of Ca^{2+}-dependent vesicle secretion. Pheochromocytoma (PC12) cells are used as an immortalized cell model system for neurons to study regulated vesicle release. In this study, we stably transfected multiple PC12 cell lines with a single plasmid that contains a syt I-targeting short hairpin RNA to knockdown expression of syt I. Stable cells were selected, expanded, and tested for stable incorporation of the plasmid with PCR and for specific targeting of syt I with immunoblot analysis. As previously reported (Cahill et al., 2007), individual