3534-Pos
SNARE Complex Assembly in Retinal Bipolar Neuron Exocytosis
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Three kinetic components of exocytosis have been described in retinal bipolar neurons. They are thought to reflect the fusion of a docked pool of ribbon-tethered synaptic vesicles termed the rapid pool, the releasable pool of ribbon-tethered vesicles, and a cytoplasmic reserve pool. In neurons, assembly of SNARE proteins facilitates exocytosis. We asked whether these pools could be distinguished on the basis of SNARE complex formation. Syntaxin3B is a t-SNARE in ribbon synapses. We generated a fluorescent peptide based on the syntaxin3B SNARE binding motif from goldfish. The peptide was dialyzed into isolated synaptic terminals of goldfish retinal bipolar cells via a whole-terminal recording electrode. A scrambled peptide served as control. Exocytosis was monitored with membrane capacitance measurements. Beginning one minute after break-in, a 1s stimulation, sufficient to deplete the releasable pool, was given every 60 seconds. The first exocytotic response was not significantly altered by the syntaxin3B peptide. However, by the fourth pulse, the exocytotic response in terminals dialyzed with the syntaxin3B peptide was reduced by 89% relative to the first, whereas that with the control peptide was reduced by only 45% ($p < 0.04$). This effect was not due to a reduction in calcium influx. Next, we implemented a pulse train protocol that captures the three components of release. Control terminals showed both depletion and replenishment of the pools. Terminals dialyzed with the syntaxin3B peptide showed: 1) immediate loss of the exocytotic component attributed to the reserve pool 2) decreased refilling of the rapid and releasable pools. The results demonstrate that reserve pool vesicles are unlike those in the rapid and releasable pools. It is likely that pool refilling is inhibited by the syntaxin3B peptide because reserve vesicles establish new SNARE complexes when they join a fusion-competent vesicle pool.

3535-Pos
Role of the Synaptobrevin C-terminus in Fusion Pore Formation
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Neurotransmitter release is mediated by the SNARE proteins synaptobrevin II (sybII) also called VAMP2, syntaxin and SNAP-25 generating a force transfer to the membranes and inducing fusion pore formation. However, the molecular mechanism by which this force leads to fusion pore formation remains elusive. To determine a possible role of the sybII TM domain in fusion pore formation, sybII constructs in which one or two polar or non-polar residues were added at the C-terminus of the protein were expressed in double knock-out (DKO) embryonic mouse chromaffin cells deficient in sybII and cellubrevin1. Exocytosis was stimulated by flash photolysis of caged-calcium (NP-EGTA), and the capability of the mutated constructs to support exocytosis was monitored by whole-cell patch clamp capacitance measurements while the associated transmitter release was monitored by carbon fiber amperometry. We found that the ability of sybII to support exocytosis is inhibited by addition of one or two residues to the sybII C-terminus depending on their energy of transfer from water to the membrane interface2, following a Boltzmann distribution. These results suggest that C-terminal zipping of the SNARE complex pulls the C-terminus of sybII deeper into the vesicle membrane, with this movement disrupting the vesicular membrane continuity and leading to fusion pore formation. In contrast to current models, fusion thus begins with molecular rearrangements at the intravesicular membrane leaflet and not between the apposed endoplasmic leaflets.
1 M. Borisovska, Y. Zhao, Y. Tsytsyura et al., Embo J 24 (12), 2114 (2005).
2 W. C. Wimley and S. H. White, Nat Struct Biol 3 (10), 842 (1996).
Supported by NIH grants R01NS38200, R01GM85808, T32GM8267, the Nanobiotechnology Center (NSF) and DFG grants SFB523-B16 and SFB530-C10.

3536-Pos
Effects of Rab3 and Rab27 on Exocytotic Activity and Docking in Mouse Chromaffin Cells
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The small GTPases Rab3A and Rab27A are endogenously expressed and specifically localized to dense-core vesicles in PC12 cells and are implicated in the late steps of dense-core vesicle exocytosis. What remains unknown is the individual and collective impact of these proteins on the size of the releasable pools and the rate at which vesicles are primed into these pools. Chromaffin cells isolated from Rab3A-/-, Rab27-/-, and double knock-out mice were used to examine the density of docked vesicles, granule mobility at the membrane, and exocytotic activity. Docked granules were visualized using electron micrographs and quantified based on their placement with respect to the plasma membrane. Mobility of granules was examined using total internal reflection fluorescence (TIRF) microscopy with virus-infected chromaffin cells expressing NPY-cherry. Changes in membrane capacitance were recorded from chromaffin cells in adrenal slices using patch clamp electrophysiology, providing data reflecting fusion kinetics and the refilling of releasable pools. The present study has identified the contributions of Rab3 and Rab27 to release readiness of dense-core vesicles in primary mouse chromaffin cells.

3537-Pos
Link between Structure and Cooperativity in the C2a Domains of Synaptotagmin
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Synaptotagmin has recently been shown to be a major Ca+2 sensor in exocytosis; however, the role of Ca+2 binding in the process is poorly described beyond the location of the binding sites. To address this, we have studied the most highly conserved sequences in the C2 family by mutagenesis, X-ray crystallography and biophysical characterization. We have shown that a single, conservative point mutation in this highly region of the C2A domain of Synaptotagmin 1 is capable of altering the cooperativity between the C2 domain and Ca+2. We conclude that this locus is a unique characteristic of the C2 domain and is involved in discriminating between the activities of C2A vs. C2B in the synaptotagmin molecule. We show that static loop motion in the Ca+2 binding pocket can be an important regulatory feature of the synaptotagmin molecule.

3538-Pos
Quantitative Analysis of Domain Formation after Snare Mediated Fusion of Synaptic Vesicles
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In synapses, synaptic vesicles are recycled shortly after fusion with the plasma membrane by endocytosis. If membrane components diffused freely away from the fusion site before this endocytosis, a complete reassembly of these synaptic vesicle membrane components (proteins and lipids) would be necessary for synaptic vesicle recycling. Therefore, this recycling would be highly facilitated in the case that complete mixing of synaptic vesicles with the plasma membrane did not occur, but rather if the synaptic vesicle components remained clustered in a patch on the plasma membrane. To test this hypothesis, we applied Förster resonance energy transfer (FRET) to study the fusion of purified synaptic vesicles with artificial membranes containing the SNAREs syntaxin 1 and SNAP-25 and a lipid composition that mimicked the plasma membrane. We compared the extent of mixing for fluorescently labeled phospholipids (with a varying degree of acyl-chain saturation) upon fusion. We found that phospholipids with saturated acyl-chains mixed with vesicular membrane to a higher extent. Full mixing was not achieved for phospholipids with unsaturated acyl-chains. This indicates that stable membrane domains of different compositions might be present after fusion.

3539-Pos
Primed Vesicles of the Readily Releasable Pool Appear to be in a Hemifused State
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The fusion of secretory vesicles with the plasma membrane occurs in three biochemically distinct steps: initial tethering and docking of the vesicle with the membrane, priming of the fusion machinery, and merging of the lipid bilayers. All three processes revolve around the regulated assembly of low-energy complexes formed by the coil-coiling of three members of the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) protein family. According to the hypothesis of distal to proximal (N- to C-terminal) ''zipping'' of SNAREs during exocytosis, priming of vesicles was suggested to correspond to only partial N-terminal SNARE complex assembly. Here we describe evidence that SNAREs might prime vesicles by hemifusing them with the plasma membrane.