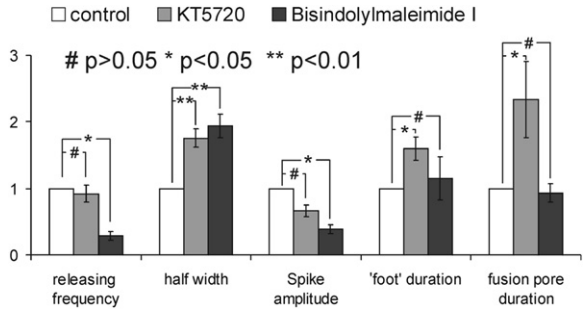
release, carbon fiber amperometry and cell-attached capacitance measurements were performed in bovine chromaffin cells. In cells treated with the PKC inhibitor, bisindolylmaleimide I (100nM, 10Ki), release frequency was significantly reduced to 5.0 ± 1.2events/min (from control 17.1 ± 2.0events/min). Amperometric spikes also showed increased half width of 22.3 ± 2.0ms (from 11.5 ± 0.9ms control) and smaller amperometric spike amplitude of 21.9 ± 3.7pA (from 56.2 ± 9.4pA control). In the cells treated with PKA inhibitor, KT 5720 (500nM, 10Ki), the duration of foot signals of amperometric events was prolonged to 14.4 ± 1.6ms (from 9.0 ± 1.0 ms control), consistent with prolonged fusion pore duration in cell-attached capacitance measurements of 27.7 ± 6.8ms (from 11.8 ± 1.4ms control). In contrast to PKC inhibitor, KT 5720 did not affect the release frequency.These results indicate that PKC affects the rate of fusion pore formation and release after full fusion but not early fusion pore expansion, while PKA specifically affects the expansion of the early fusion pore as well as release after full fusion.



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Effects of Calcium and PIP2 on the Membrane Binding of Synaptotagmin I

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Synaptotagmin I (Syt I) appears to act as the Ca^{2+} sensor in neuronal exocytosis and it is known to interact both with membranes and with SNAREs, which form the conserved core protein machinery for the fusion process. The interactions of Syt I with membranes were examined here with a combination of vesicle sedimentation and site-directed spin labeling (SDSL). Several interesting features of the interaction are revealed. First, Syt I binds to PC/PS bilayers in a Ca^{2+}-independent manner though one of its cytosolic C2 domains, C2B. The interaction is mediated by the polybasic region of C2B domain, which associates in the electrostatic double-layer, but does not penetrate into the bilayer interior. Second, the affinity of C2B is increased approximately 20 fold in the presence of Ca^{2+} and now interacts through its Ca^{2+}-binding loops. Remarkably, in the presence of Ca^{2+}, C2A, C2B and a tandem fragment containing both C2A and C2B have approximately the same affinity, indicating the free energy of C2 domain interactions in Syt 1 are not additive. This may be due to demixing of the PS in the bilayer or the effects of curvature strain that are induced by the C2 domains. Finally, $PI(4,5)P_2$ is a lipid that is critical to membrane fusion. Our preliminary data indicate that the addition of 1 mol% $PI(4,5)P_2$ has little effect on the Ca^{2+}-dependent binding of C2A; however, the membrane binding of both C2B and the tandem C2A-C2B domains is enhanced by $PI(4,5)P_2$. As seen for other polybasic segments, the C2 domains appear to sequester or alter the lateral distribution of $PI(4,5)P_2$ in the bilayer.

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A Novel Approach For Wireless Communication Of *In Vivo* Data From Freely Moving Research Animals

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In vivo electrochemistry has become a fascinating research tool allowing neuroscientists to study the release of oxidizable neurotransmitters, such as dopamine and norepinephrine in the brain of freely moving animals (Garris et al., 1997, J. Neurochem., 68(1): 152–161). The main limitation of this technique is the wired connection from the working electrode at the animal's head to the data acquisition apparatus, thus restricting the animal's freedom of motion. To overcome this limitation, we are designing an electronic device with the capability of performing fast-scan cyclic voltammetry measurements and wirelessly transmitting the recorded data. The device consists of two parts: the base station, which is connected to a PC, and the remote unit, which the rat carries on its back. The base station can wirelessly transmit the potential waveform applied to the working electrode, using the Advanced Audio Distribution Profile (A2DP) protocol. At the remote unit, a capacitance compensation circuit partially removes the capacitive background current present in voltammetric measurements due to charging of the Debye double layer. This increases the device's dynamic range, allowing for the detection of lower neurotransmitter levels. Although the forward telemetry (PC to remote unit) is functional, we have not yet characterized the reverse telemetry (remote unit to PC) in A2DP format. After finalizing the design, the device will be tested *in vivo* and subsequently employed in behavioral experiments, allowing researchers to obtain data from freely behaving rodents.

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Intracellular Ca^{2+} In Physiological Range Affects The Forward Rate Of Priming Of Large Dense Core Vesicles, But Not The Backward Rate

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Secretory vesicles which undergo Ca^{2+}-dependent exocytosis pass several consecutive molecular states before release. While docking describes the anchoring of the vesicles to the plasma membrane, priming is necessary to render the vesicles release-competent. Many regulatory proteins and second messengers mediate the transition between these different molecular states.

By combining total internal reflection fluorescence microscopy (TIRFM) and analysis of the caging diameter (CD) we show that different molecular prefusion states of large dense core vesicles (LDCVs) can be distinguished by their different mobility (Nofal et al., J. Neurosci. 2007, 27:1386–95). Furthermore, we established simultaneous TIRFM measurements with whole-cell patch-clamp recordings which enables us to set a stable composition of the intracellular conditions, e.g. intracellular Ca^{2+} (Becherer et al., PLoS ONE 2007, 6:e505).

We investigated the Ca^{2+} dependence of both priming and unpriming reactions by varying the intracellular Ca^{2+} concentration within the physiological range from 50–800 nM.

CD analysis reveals that both lateral and axial mobility of LDCVs under resting conditions (100 nM $[Ca^{2+}]_i$) are elevated, whereas mobilities are reduced with raising $[Ca^{2+}]_i$ from 200 nM to 800 nM. Further increases of Ca^{2+} levels above 800 nM again lead to an increase in mobility. Interestingly, the dwell time of LDCVs appear to be independent of $[Ca^{2+}]_i$ in this range, arguing against the Ca^{2+}-dependence of docking. Quantitative analysis of individual parameters, such as dwelltime in a specific molecular state and frequency of interstate changes, demonstrate that the forward rate of priming is increased with raising $[Ca^{2+}]_i$ while the backward rate remains unaffected.

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Role Of SPIN90 (SH3 Protein Interacting With Nck, 90kda) In The Formation Of Endocytic Vesicle And Its Movement In Receptor-mediated Endocytosis

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Endocytosis is a key mechanism for mediating diverse cellular functions, like uptake of nutrients, recycling of synaptic vesicles and intracellular signaling. The formation and targeting of vesicles to their acceptor compartment from the plasma membrane are tightly controlled for regulating tissue homeostasis. To gain insight into the effect of SPIN90 in the formation of vesicles, we measured the interaction between syndapin and dynamin in SPIN90 overexpressed and deficient fibroblasts. It is reported that syndapin is the phophorylation-regulated dynamin I partner in vivo and its interaction is crucial for SVE. SPIN90-SH3 domain binds with dynamin I-PRD in synapses and PRD domain of SPIN90 interacts to syndapin-SH3 in fibroblasts are already reported. Here, we show that the syndapin-dynamin interaction is maintained in SPIN90-N terminal (SH3 and PRD domain containing part) overexpressed cells comparing to that in mock overexpressed cells. In addition, SPIN90 C terminus (642–722aa) interacts with Rab5a small GTPase which has a role for early endosome movement and fusion were found. For verifying this, immuno-fluorescence and live cell imaging technique were used. We examined that SPIN90 is co-localized with Rab5 in fibroblast, and the movement of gfp-Rab5 positive endosome is delayed when the SPIN90-CC (Rab5 binding) part is overexpressed. From these results, we proposed that SPIN90 has a role in the formation and movement of early endosome.

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TIRF-FRET As An Approach To Quantitative Analysis Of Dynamic Molecular Interactions On Secretory Granules In Live Cells

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