Supplementary information – Controlling synaptotagmin activity by electrostatic screening

Supplementary figure

Supplementary figure 1 Purification of CGs from bovine adrenal glands. (a) Overview over the protocol for CG purification. After differential centrifugation, CGs were separated from contaminating organelles on a continuous sucrose density gradient (from 0.3 M to 2.0 M). Due to their high protein content, CGs exhibit a buoyant density that is higher than of most other organelles. (b) Profile of synaptobrevin (marker for CG membranes) and succinate dehydrogenase complex subunit A (SDHA, marker for mitochondria) across the gradient fractions. Aliquots of equal volume from the each fraction were subjected to SDS-PAGE and immunoblotting. Mitochondria (SDHA) contaminants were mainly present between fraction 7 and 11, whereas CGs (synaptobrevin) were present between fraction 11 and 16. Purified CGs were collected from the pellet (fraction 16, red). (c) Distribution of VAMP-4 (marker for immature CGs) and synaptotagmin-1 (present on both immature and mature CGs) across the gradient. Immature CGs were mainly detected in fraction 12 and 13. (d) Shown are full-length blots of representative marker proteins.
Supplementary figure 2 Characterization of SNARE-dependent fusion of CGs with LUVs containing Q-SNARE complexes. (a) Quantification of fusion experiments shown in Figure 1C. All values are normalized to fusion observed under standard conditions. No addition represents basal fusion without any treatment or Ca$^{2+}$. Data were presented as the percentage of basal fusion reactions after 20 min of reaction time. (b) The light chain of tetanus toxin (TeNT) quantitatively cleaves synaptobrevin in CG membranes. CGs were incubated for 30 min at 37°C with 200 nM of purified light chain or a mutant light chain that is inactivated by a point mutation in the Zn$^{2+}$-coordination site. Synaptophysin served as further control to ensure specificity of toxin action. (c) Lysophosphatidylcholine (LPC) inhibits fusion in a dose-dependent manner. (d,e) SNARE-dependent content mixing using liposomes preloaded with calcein. Dequenching was inhibited by preincubation of the LUVs with soluble Syb$_{1.96}$ and by preincubation of CGs with soluble SyxH3/SN25.
**Supplementary figure 3** Influence of Ca\(^{2+}\) on SNARE-mediated fusion between CGs and LUVs: further characterization and quantification. (a) Dose-dependent inhibition by Ca\(^{2+}\) using standard conditions in the absence of ATP. This inhibition resembles that observed previously for purified synaptic vesicles. (b,c) CG fusion in the presence of ATP (5 mM). 300 µM Ca\(^{2+}\) was added resulting in 84 µM of free Ca\(^{2+}\) concentration in the presence of 5 mM ATP and enhanced fusion. 300 µM MgCl\(_2\) had no effect. Soluble Syb\(_{1-96}\) completely blocked fusion regardless of whether Ca\(^{2+}\) was present or not. (d) Ca\(^{2+}\) dose-response curve of CG fusion in the absence and the presence of ATP. Free Ca\(^{2+}\) concentration in the presence of ATP was calibrated using the simulation (see Ca\(^{2+}\) calibration in the Online Methods). Ca\(^{2+}\) was able to accelerate CG fusion only in the presence of ATP (red). (e) SNARE assembly was also enhanced by Ca\(^{2+}\) in the presence of ATP. Data was presented as the percentage of total SNARE assembly induced by 1 µM unlabeled Syb\(_{1-96}\).
Supplementary figure 4 (a) Sequential addition of CaCl$_2$ and ATP caused a step-increase in the fusion reaction upon the 2$^{nd}$ addition, regardless of the order in which CaCl$_2$ and ATP were added. (b) Ca$^{2+}$/ATP-dependent enhancement of fusion is also observable when full length syntaxin-1A and SNAP-25A are used as acceptors in the LUVs. Again, CaCl$_2$ increased CG fusion only in the presence of PI(4,5)P$_2$ in the target membrane (red solid line). Preincubation of Syb$_{1-96}$ completely abolished Ca$^{2+}$-induced CG fusion in the presence of PI(4,5)P$_2$ (green line). (c) PI(4,5)P$_2$ (1%) in LUVs was replaced by PI3P (1%). (d) LUVs contained no phosphoinositides but the PS concentration was increased to 40% (57% PC, 3% labeled PE). 5 mM ATP was added in all of fusion reactions.
Supplementary figure 5 Ca\textsuperscript{2+}-dependent enhancement of SV fusion does not require ATP hydrolysis but is dependent on PI(4,5)P\textsubscript{2} in the target membrane. (a,b) In the presence of 5 mM ATP, Ca\textsuperscript{2+} (84 µM) increased SV fusion only if the target membrane contains PI(4,5)P\textsubscript{2}. Soluble synaptobrevin (Syb\textsubscript{1-96}), an inhibitor of SNARE assembly, blocked Ca\textsuperscript{2+}-dependent SV fusion indicating SNARE-dependent fusion. Dose-response curve of Ca\textsuperscript{2+}-induced SV fusion in the presence of 0.5% PI(4,5)P\textsubscript{2} shows EC\textsubscript{50} of 35.4 ± 3 µM. Fusion was normalized as the percentage of basal fusion without Ca\textsuperscript{2+} after 20 min reaction time. (c) Ca\textsuperscript{2+}-dependent enhancement of synaptic vesicle fusion in the presence of ATP also does not require ATP hydrolysis.