Supporting Information

Yao and Sakaba 10.1073/pnas.1100608109

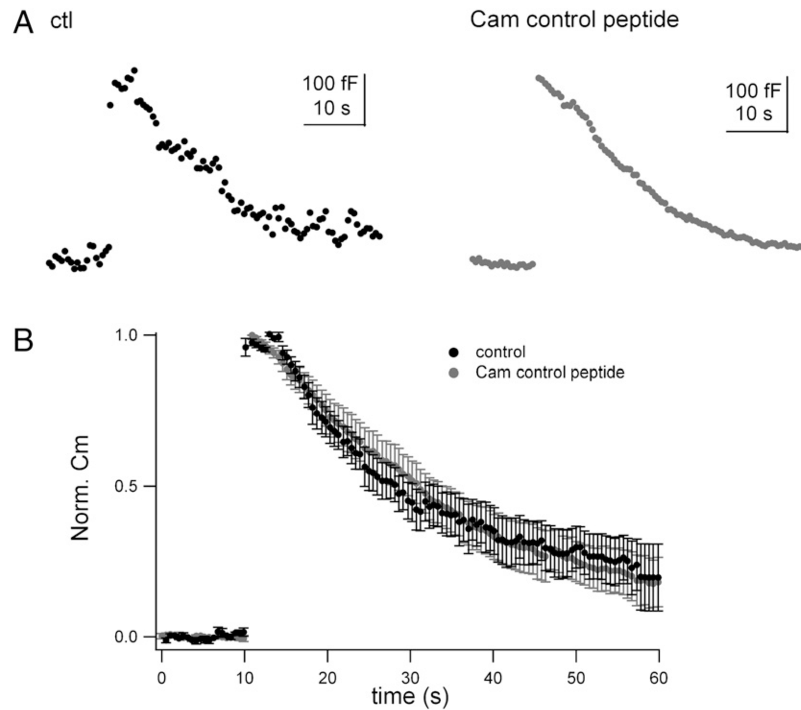

Fig. S1. A control peptide of calmodulin inhibitory peptide did not block endocytosis during a 20-ms pulse under 0.05–0.1 mM EGTA. A set of experiments similar to that of Fig. 1*A*, also including 0.05–0.1 mM EGTA in the pipette solution both for the control experiment (no peptide) and calmodulin control peptide (20 μM). A 20-ms depolarizing pulse to 0 mV was applied to the terminal. Individual capacitance traces under control condition (*A*, *Left*) and with the control peptide (*Right*) are shown. (*B*) Normalized capacitance traces with or without control peptide. Each capacitance trace was normalized to the peak value before averaging across cells, and there was no significant statistical difference between the two groups (n = 9 for control peptide).

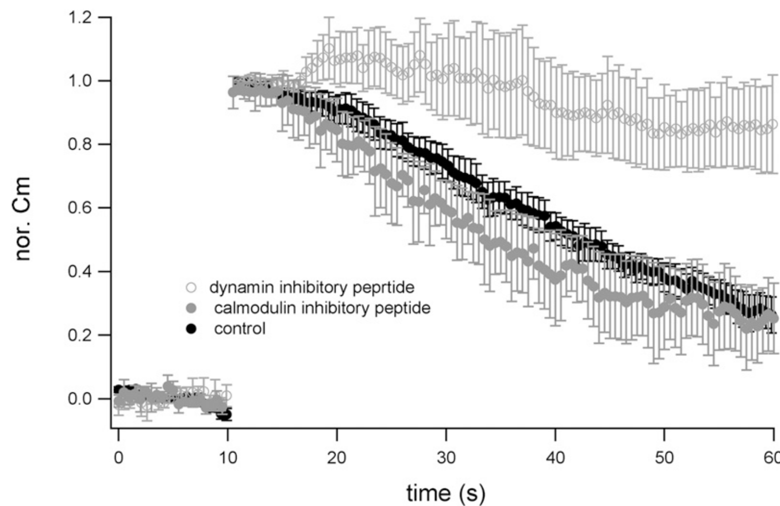

Fig. S2. Calmodulin inhibitory peptide did not block endocytosis following a 5-ms depolarizing pulse. The presynaptic patch pipette contained 0.5 mM EGTA, and the figure shows the normalized time course of endocytosis. When endocytosis following a 5-ms depolarizing pulse of 0 mV was monitored, calmodulin inhibitor did not block endocytosis. In contrast, dynamin inhibitory peptide (1 mM) blocked endocytosis. The concentration of calmodulin inhibitory peptide was either 20 or 100 μM in the presynaptic patch pipette (four cells each). The peptide concentration was raised in some cases to ensure that the concentration was sufficiently high in the presynaptic terminal. The results were similar, irrespective of the concentration and were pooled. Summary data are shown in Table S2. For a single exponential fit, capacitance jumps divided by endocytotic rates equal time constants.

Table S1. Summary of the endocytosis data obtained from a 50-ms pulse protocol (0.5 mM EGTA in the patch pipette)

Condition	*n*	Cm jump (fF)	Endocytosis rate (fF/s)
Control	12	379 ± 42	11.2 ± 1.8
CaM inhibitory peptide	10	344 ± 42	8.7 ± 1.3
Calmodulin binding domain (CBD)	6	375 ± 29	8.6 ± 2.1
KT5720	5	306 ± 10	5.1 ± 1.5*

Individual traces were fitted by an exponential, and average capacitance jumps and rates of endocytosis are shown. For a single exponential fit, capacitance jumps divided by endocytotic rates equal time constants. Statistical significance: $*P < 0.05$.

Table S2. Summary of the endocytosis data obtained from a 5-ms pulse protocol (0.5 mM EGTA in the patch pipette)

Condition	*n*	Cm jump (fF)	Endocytosis rate (fF/s)
Control	6	212 ± 29	6.4 ± 1.0
CaM inhibitory peptide	8	180 ± 21	6.8 ± 1.0
Dyn inhhibitory peptide	5	157 ± 24	0.9 ± 0.3**

The data are analyzed from Fig S2. Statistical significance: $**P < 0.01$.

Table S3. Summary of the endocytosis data obtained from a 500-ms pulse protocol (0.5 mM EGTA in the patch pipette)

Condition	*n*	Cm jump (fF)	Fast endo (fF/s)	Slow endo (fF/s)
Control	9	1,153 ± 116	145 ± 23	36.0 ± 7.9
CaM inhibitory peptide	6	1,350 ± 118	125 ± 31	10.0 ± 0.9*
CBD	5	1,144 ± 125	85 ± 13*	13.7 ± 2.4*

Individual traces were fitted by a double exponential, and average capacitance jumps and rates of endocytosis (fast and slow components) are shown. Endocytotic rates were estimated by dividing the amplitude of each component by the time constant. For KT5720 and MDL 12330A, decays were so slow that the data could not be fitted by an exponential. For a single exponential fit, capacitance jumps divided by endocytotic rates equal time constants, but for a double-exponential fit, the rates depend on the exact amplitude and time constant of each component. In Table S4, the amplitudes and time constants are shown. Statistical significance: $*P < 0.05$.

Table S4. Time constants and amplitudes of the double exponential fit to the time course of endocytosis (500 ms in duration, 0.5 mM EGTA in the presynaptic patch pipette)

Condition	Fast (fF)	τ_{fast} (s)	Slow (fF)	τ_{slow} (s)
Control	368 ± 72	2.8 ± 0.5	813 ± 101	25.8 ± 2.9
CaM inhibitory peptide	442 ± 71	5.4 ± 1.6	125 ± 31	79.5 ± 13.2
CBD	344 ± 50	4.3 ± 0.7	732 ± 162	57.6 ± 10.5

Table S5. Time course of transmitter release during a step pulse (50 ms in duration, 0.5 mM EGTA in the presynaptic patch pipette)

Condition	*n*	Fast time constant (ms)	Slow time constant (ms)
Control	7	1.6 ± 0.2 (57 ± 5%)	36.7 ± 12.2
CaM inhibitory peptide	5	1.4 ± 0.3 (55 ± 3%)	17.1 ± 1.9
KT5720	5	1.5 ± 0.3 (54 ± 4%)	24.2 ± 5.5

Data from Fig. 4 were analyzed. Time constants of transmitter release were obtained by a double-exponential fit to the cumulative release during a depolarizing pulse (1, 2). The values were comparable to those in previous studies. It seems that the time course of release was not changed by the reagents. It is likely that calmodulin inhibitory peptide mainly affected the replenishment of fast-releasing synaptic vesicles.

1. Forsythe ID (1994) Direct patch recording from identified presynaptic terminals mediating glutamatergic EPSCs in the rat CNS, in vitro. *J Physiol* 479:381–387.
2. Wu XS, et al. (2009) Ca(2+) and calmodulin initiate all forms of endocytosis during depolarization at a nerve terminal. *Nat Neurosci* 12:1003–1010.