Supporting Information

Extracellular osmotic stress reduces the vesicle size while keeping a constant neurotransmitter concentration

Hoda Fathali, [†] *Johan Duneval,* [†] *Soodabeh Majdi*[‡]*, and Ann-Sofie Cans*[†]

[†] Department of Chemistry and Chemical Engineering, Chalmers University of Technology, 412 96 Gothenburg, Sweden

[‡]Department of Chemistry and Molecular Biology, University of Gothenburg, 41296 Gothenburg, Sweden

Table of content

Table S1: The kinetics of exocytosis at chromaffin cells in isotonic and hypertonic (730 mOsm/kg) buffers is summarized by three main amperometric spike parameters; the peak half width (t_{half}) measured as the width of the peak at half maximum amplitude corresponds to the duration of catecholamine release from pore expansion, the peak rise time (t_{rise}) determined from the timing for the current transient to rise from 25 to 75% of the peak current maximal amplitude and is considered to indicate catecholamine release from the initial fusion pore through the time for pore expansion, and the maximum peak current amplitude (I_{max}) that relates to the maximal flux of neurotransmitter during exocytosis release. N is the number of cells used in the isotonic and hypertonic experiments. The reported spike parameter values are the group average of averages from each single cell recording. Statistical significance was tested using t-test for unpaired data.

	t_{half} (ms)	t_{rise} (ms)	I_{max} (pA)	N
Isotonic	8.43± 0.52	1.77± 0.086	118.8± 13.33	22
Hypertonic	7.57± 0.73	2.00± 0.23	61.82± 6.51	20
p-value	0.3390	0.3438	0.0006 ***	

Table S2: Kinetics of prespike foot features from amperometric recording of exocytosis release at chromaffin cells exposed to an isotonic and a hypertonic environment. Here four main prespike foot parameters are presented; "Foot molecules" presents the number of catecholamine molecules released through the initial fusion pore before pore dilation or closing, "Foot (I_{max})" presents the maximum flow of catecholamines through the initial fusion pore before dilation or closing, "Foot Duration" is the time the initial fusion pore is stable before dilation or closing, and "Foot Frequency" is percentage of total number of amperometric spikes recorded that display pre-spike foot features. N is number of the cells used in the isotonic and hypertonic experiments. The reported values are the group average of averages from each single cell recording. Statistical significance was tested using t-test for unpaired data.

	Foot Molecules	Foot I_{max} (pA)	Foot Duration (ms)	Foot Frequency %	N
Isotonic	346038± 31283	10.4± 0.71	9.62± 0.49	37.7	22
Hypertonic	495829± 101889	7.1± 0.55	19.34±4.6	35.6	20
P-value	0.1515	0.0008 ***	0.0353 *	0.6483	

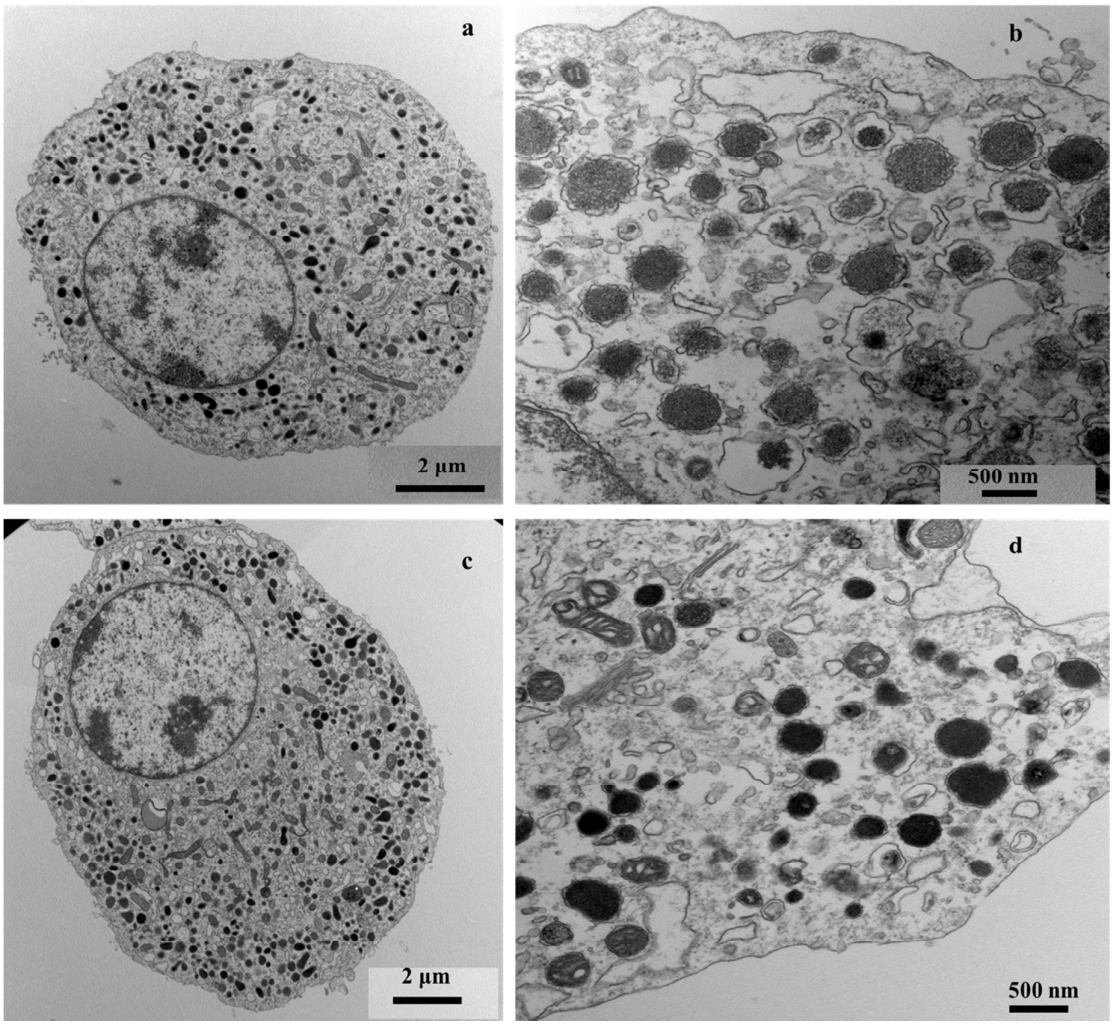

Figure S1: Representative TEM images of chromaffin cells exposed to a) isotonic buffer b) isotonic buffer c) hypertonic buffer and d) hypertonic buffer. Chromaffin cells were chemically fixed and prepared for TEM imaging after 10 min incubation in either isotonic or hypertonic buffer.

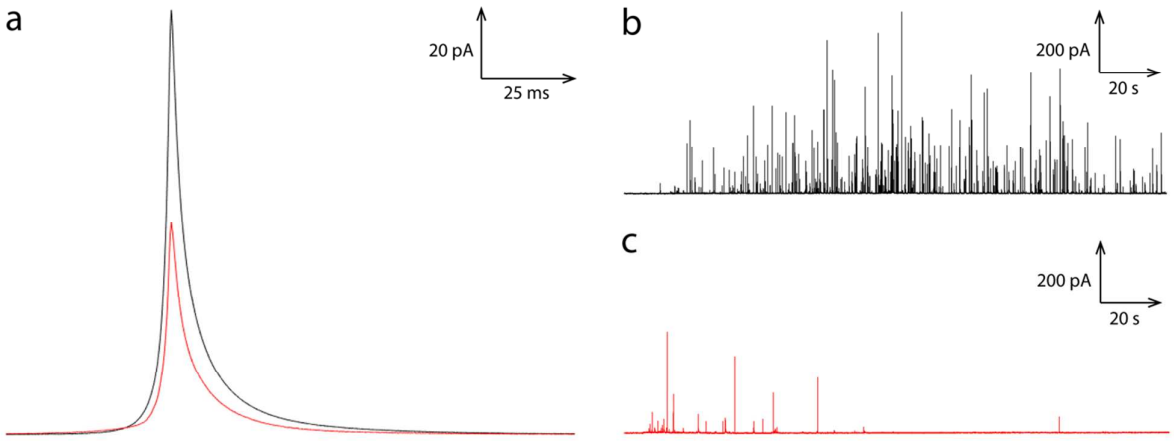

Figure S2: Amperometric recording of exocytosis from a single chromaffin cell after stimulation with Ba^{2+}-solution. a) Enlargement of an average amperometric spike from exocytosis measurement at chromaffin cells in isotonic (black) and hypertonic (red) environments. b) A representative amperometric trace of exocytosis recorded from a chromaffin cell in isotonic buffer. c) A representative amperometric trace of exocytosis recorded from a chromaffin cell in hypertonic buffer.

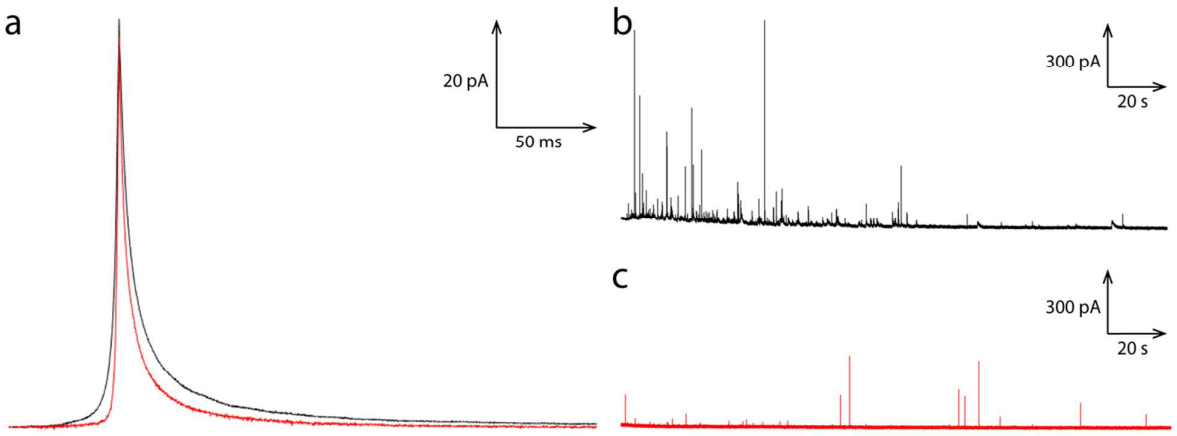

Figure S3: Amperometric recording of catecholamine release from vesicle rupture in situ of a chromaffin cell using intracellular electrochemical cytometry. a) Enlargement of an average amperometric spike from intracellular cytometry measurement in chromaffin cells placed in isotonic (black) and hypertonic (red) buffers. b) A representative amperometric trace of vesicle rupture recorded by intracellular cytometry in a cell subjected to isotonic buffer. c) A representative amperometric trace of vesicle rupture recorded by intracellular cytometry in a cell exposed to hypertonic buffer.