

Luminal Protein within Secretory Granules Affects Fusion Pore Expansion

Annita Ngatchou Weiss,^{1,*} Arun Anantharam,² Mary A. Bittner,¹ Daniel Axelrod,^{1,3} and Ronald W. Holz^{1,*}

¹Department of Pharmacology, University of Michigan Medical School, Ann Arbor Michigan; ²Department of Biological Sciences, Wayne State University, Detroit, Michigan; and ³Department of Physics and LSA Biophysics, University of Michigan, Ann Arbor, Michigan

Supporting Material

SUPPLEMENTAL FIGURES

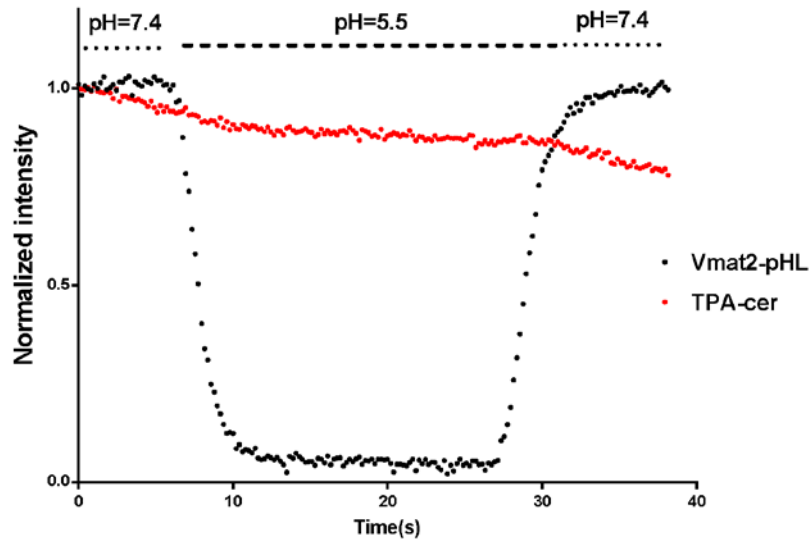


Fig. S1. Absence of pH dependency of cerulean fluorescence in chromaffin cells.

Chromaffin cells were transfected with a plasmid encoding tPA-cer or VMAT2-phluorin (pHL). Four days later, the cells were fixed with paraformaldehyde (4%), permeabilized with ice-cold methanol and imaged in epifluorescence. Individual cells were perfused with physiological salt solution at pH 7.4 (buffered with 15 mM HEPES) and with physiological salt solution at pH 5.5 (buffered with 15 mM MES) as indicated. There were 10 cells/group.

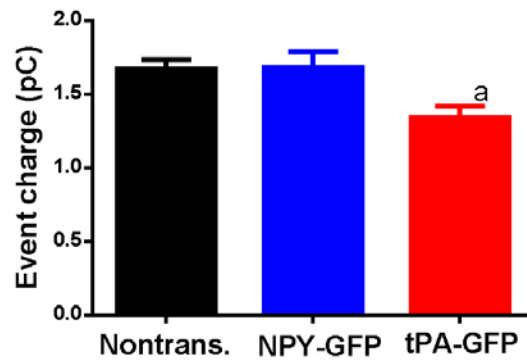


Fig. S2. Amperometric charge per event from non-transfected, NPY-GFP- and tPA-GFP-expressing cells. The average of the medians in non-transfected cells (15 cells), NPY-GFP-expressing cells (13 cells) and tPA-GFP-expressing cells (14 cells). ^a $p < 0.05$ vs. non-transfected and NPY-GFP-expressing cells.

Video 1. Fusion of tPA-cer-containing secretory granules, P/S and $P+2S$ changes. The circles identify 3 granules undergoing fusion and the corresponding changes in P/S and $P+2S$. The discharge of tPA-cer occurs over many seconds and is associated with long-lived increases in P/S and $P+2S$. The blue circle shows an event in which the duration of the P/S and $P+2S$ increases is correlated with the time course of the tPA-cer discharge. The white and red circles show fusion events in which the increases in P/S and $P+2S$ remain even after the discharge of all detectable tPA-cer. The movie is 10X real time.

Video 2. Fusion of NPY-cer-containing secretory granules, P/S and $P+2S$ changes. Circles identify 3 granules undergoing fusion and the corresponding changes in P/S and $P+2S$. NPY-cer discharge and increases in P/S had durations of no greater than several hundred milliseconds. There was little change in $P+2S$ in these examples. The movie is real time.