SUPPLEMENTARY MATERIAL

for

"Pancreatic Beta Cells Secrete Insulin in Fast- And Slow-release Forms"

Darren J. Michael[1], Robert A. Ritzel[2], Leena Haataja[3] and Robert H. Chow[1*]

Adenovirus for Expression of Cytoplasmic GFP

Construction (1) and use (2) of adenovirus containing the gene for cytoplasmic GFP have been described.

Multiphoton Imaging Setup

An upright microscope (Nikon TE2000, Melville, NY) was configured for multiphoton imaging. Light (800 nm) from a Ti-sapphire laser (Mai-Tai, Spectra-Physics, Mountain View, CA) was sent through an external conditioning unit (TrimScope, LaVision BioTech, Goettingen, Germany) that expanded the original beam into multiple beamlets spaced evenly along a line. These beamlets were swept across the field of view to excite emission from the zinc fluorescent indicator Newport Green. Light emitted from the sample was focused onto an electron multiplication charge-coupled device camera (Ixon, DV885, Andor, Belfast, Ireland). A computer synchronized image acquisition with scanning of the beamlets. Typical acquisition time for a single frame was less than 300 milliseconds. During time lapse imaging, individual frames were collected continuously at ~3 Hz, a rate that was similar to the repetition rate we used for time lapse TIRF microscopy. For these experiments, cells were grown on glass coverslips and mounted in a constant temperature chamber (33°C, FCS2, Bioptechs, Butler, PA) on the day of the experiment. The microscope objective (60x, NA 1.25, oil immersion, Nikon USA) was warmed (objective heater, Bioptechs) to reduce temperature gradients. The fine positioning of the microscope objective was controlled by a piezoelectric positioner (PiFoc, Physik Instrumente, Tustin, CA). Throughout the experiment, cells were perfused (~0.6 mL per minute) with extracellular solution. To stimulate secretion, a small bolus (~0.3 mL) of high potassium solution containing Newport Green was injected into the perfusion system. Before each stimulus, the sample was first stained by introducing a similar bolus of Newport Green in standard extracellular solution. Each of four samples

of human pancreatic beta cells was stimulated twice, using a different field of view during each stimulus. Between the stimuli, tissue was allowed to recover for approximately 10 minutes. Only cells within small clusters were analyzed. Background fluorescence was subtracted from images to aid identification of regions of interest.

Multiphoton Imaging of Secretion at Planes above the Coverslip

Primary cultures of pancreatic beta cells were stimulated to secrete in the presence of Newport Green. During the stimulation, fluorescence intensity increased in discrete spots near the plasma membrane. As with the experiments using near-critical angle illumination described in the manuscript (Fig. 2), these newly appearing fluorescent spots next to cells imaged by multiphoton illumination had widely varying lifetimes (Fig. S1). Examples of short- and long-lived vesicle-sized spots are shown in Figure S1.

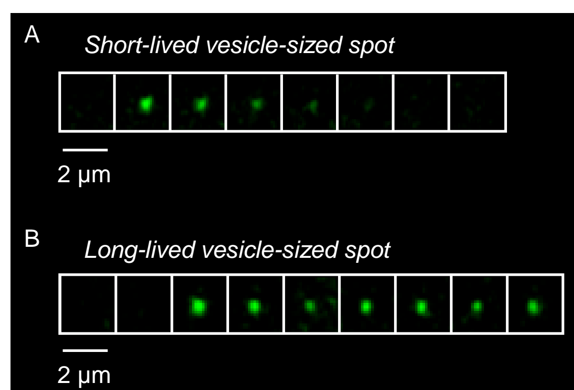

Figure S1. Native cargo, released between cells, also leaves insulin vesicles at widely varying rates. (A) Short-lived vesicle-sized spot (every second frame; ~500 ms between the panels shown in the montage) (B) Long-lived vesicle-sized spot (every sixth frame; approximately 2 s between the panels shown in the montage).

Most vesicles do not secrete slow-release insulin.

It is also possible to visualize membrane-proximal vesicles in endocrine cells using transient expression of cytoplasmic GFP. In cells expressing cytoplasmic GFP, secretory vesicles appear in TIRF micrographs as dark “holes” where the cytoplasmic fluorescence is excluded (3).

Primary cultured rat pancreatic beta cells expressing cytoplasmic GFP (Fig. S2A) were bathed in Rhodzin, and then stimulated. Stimulation caused sudden changes in the

appearance of some membrane-proximal holes. Of the holes that showed a change in appearance, most (>80%) vanished rapidly, suggesting collapse of the vesicle membrane into the plasma membrane. Less frequently (<20%), the holes enlarged suddenly, but remained visible, suggesting swelling or sudden movement of the vesicle towards the plasma membrane.

Stimulation in the presence of bath-applied Rhodzin led to the sudden appearance of red fluorescent spots with diameters and lifetimes similar to those reported in the manuscript (Figs. 1,4). Fluorescent Rhodzin-stained spots appeared at only a fraction of sites where exocytosis was reported by either disappearance or dilation of a hole (n=26 of 100, 14 cells; Figs. S2B,C,D). Two examples where sites did overlap are shown. In one example a short-lived vesicle-sized spot appeared where a hole collapsed (Fig. 5C). In the other example two long-lived vesicle-sized spots appeared sequentially in neighboring holes that remained intact (Fig. S2D).

Thus, the results in cells expressing cytoplasmic GFP corroborate the results in cells expressing fluorescently tagged C-peptide—not all vesicles undergoing exocytosis release a slowly dissolving dense core that can be detected by fluorescent zinc indicator. There must be extensive variability in the contents of insulin vesicles, with the dense cores ranging from very slowly dissolving to rapidly dissolving or possibly even absent.

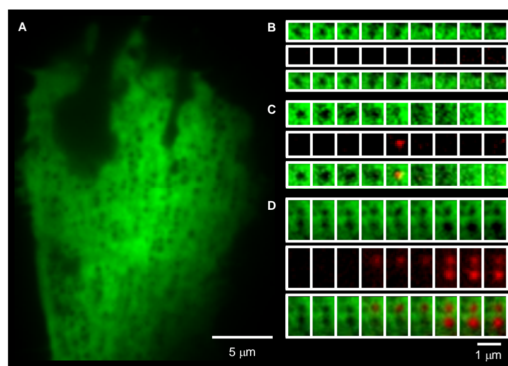

Figure S2. Most vesicles do not secrete slow-release insulin. (A) A primary cultured rat pancreatic beta cell expressing cytoplasmic GFP—membrane proximal insulin vesicles appear as holes within the boundary of the cell. (B) Many holes suddenly disappeared without an accompanying change in fluorescence intensity for Rhodzin. (C/D) Punctate changes in fluorescence intensity for Rhodzin sometimes appeared at sites of exocytosis marked by sudden changes in appearance for holes. The lifetimes of these spots varied widely. Examples of short-lived (C) and long-lived (D) Rhodzin spots are shown. The montages in B, C and D show every third frame acquired at 0.25 seconds per frame, yielding 0.75 seconds per frame shown.

References

1. Wrede CE, Dickson LM, Lingohr MK, Briaud I, Rhodes CJ: Protein kinase B/Akt prevents fatty acid-induced apoptosis in pancreatic beta-cells (INS-1). *J Biol Chem* 277:49676-49684, 2002
2. Michael DJ, Geng X, Cawley NX, Loh YP, Rhodes CJ, Drain P, Chow RH: Fluorescent Cargo Proteins In Pancreatic Beta Cells: Design Determines Secretion Kinetics at Exocytosis. *Biophys J* 87:L3-L5, 2004
3. Taraska JW, Perrais D, Ohara-Imaizumi M, Nagamatsu S, Almers W: Secretory granules are recaptured largely intact after stimulated exocytosis in cultured endocrine cells. *Proceedings of the National Academy of Sciences of the United States of America* 100:2070-2075, 2003