The role of F-actin in vesicular secretion

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Acute pancreatitis is an inflammatory process that leads to auto-digestion of pancreatic tissue by premature activation of digestive enzymes within pancreatic acinar cells. One of the prominent cellular events in acute pancreatitis is formation of vacuoles and rapid disruption of the actin cytoskeleton surrounding the lumen of pancreatic ducts (Nemoto *et al.*, 2004). The mechanisms behind reorganization of F-acin during vesicular secretion are still unclear, however it is generally accepted that secretory granules are coated with F-actin in response to secretagogue stimulation. F-actin coating of secretory vesicles may be a key process which regulates vesicle content release in exocytosis. Therefore, this study investigates the role of F-actin in vesicular secretion in pancreatic acinar cells.

Experiments were performed using clusters of acini, isolated from the pancreas of 2-5 week-old male CD-1 mice by digestion with collagenase. The effects of inhibiting F-actin polymerization were examined with 10 μM of Latrunculin B treatment prior to cell stimulation. Latrunculin is an actin perturbing drug which reduces F-actin and may have effects on fusion pore dynamics (Larina *et al.*, 2007). Firstly, the effects of increasing treatment time (0 to 35 min) of Latrunculin B on the F-actin coating of secretory vesicles were examined in confocal microscopy. After various treatment times, the cells were incubated with fluorescein for 5 minutes then stimulated with acetylcholine for 2 min. For the visualization of F-acin, acini were fixed with 4% paraformaldehyde and stained with Alexa 633-phalloidin. Latrunculin B inhibited F-actin coating of secretory granules at all time points. The longer exposure to the drug strongly decreased the F-actin. 5 minutes of exposure to the drug did not significantly change the structure and the size of the secretory vesicles. After 15 minutes of Latrunculin B treatment, the average size of the secretory granules increased three fold in comparison to the size of granules in control cells. The measured size of control vesicles was 1.05 ± 0.19 μm (mean diameter ± SEM, n=100) while the size of vesicles in Latrunculin B treated cells was 3.14 ± 0.09 μm. Latrunculin B did not affect the the total number of exocytic events suggesting that F-actin plays a role in secretory vesicle maintenance. Therefore we hypothesize that the increased size of the secretory vesicles of Latrunculin B treated cells may be due to the vesicle swelling during exocytosis. To examine the appearance of zymogen granules in real time, two-photon excitation imaging of pancreatic acinar cells was performed. Time course Latrunculin B treatment and acetylcholine stimulation was processed in the acini for real time experimentation in equivalent conditions to those described above as an extracellular tracer. Vesicle fusion was visualized using sulforhodamine B over 10 minutes after stimulation under two-photon microscopy. Real time analysis revealed that secretory vesicles appeared as same size as the vesicles in control cells then swell with sequential exocytosis after stimulation resulted in Latrunculin B treatment for 15 minutes. Corresponding to the fixed cells imaging result, 5 minutes treatment of Latrunculin B did not cause the secretory vesicle extension.

In conclusion, the results of this study suggest that loss of F-actin during secretion weakens the secretory vesicle membrane and destabilizes granules. This supports the idea that impaired F-actin polymerization leads to retention of enzymes in the cytosol which may contribute to auto-digestion of the vacuolar membrane in acute pancreatitis. We are currently working on the mechanisms of granule swelling after Latrunculin treatment and its potential relationship to acute pancreatitis.

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