

ORIGINAL RESEARCH

Early to Late Endosome Trafficking Controls Secretion and Zymogen Activation in Rodent and Human Pancreatic Acinar Cells



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SUMMARY

In addition to zymogen granule secretion, acinar cells express an anterograde endosomal secretory pathway coordinated in the early endosome (EE). Altered trafficking from EEs to late endosomes/lysosomes or apical membrane during acute pancreatitis controls secretion and zymogen activation.

BACKGROUND & AIMS: Pancreatic acinar cells have an expanded apical endosomal system, the physiologic and pathophysiologic significance of which is still emerging. Phosphatidylinositol-3,5-bisphosphate [PI(3,5)P₂] is an essential phospholipid generated by phosphatidylinositol 3-phosphate 5-kinase (PIKfyve), which phosphorylates phosphatidylinositol-3-phosphate (PI3P). PI(3,5)P₂ is necessary for maturation of early endosomes (EE) to late endosomes (LE). Inhibition of EE to LE trafficking enhances anterograde endosomal trafficking and secretion at the plasma membrane by default through a recycling endosome (RE) intermediate. We assessed the effects of modulating PIKfyve activity on apical trafficking and pancreatitis responses in pancreatic acinar cells.

METHODS: Inhibition of EE to LE trafficking was achieved using pharmacologic inhibitors of PIKfyve, expression of dominant negative PIKfyve K1877E, or constitutively active Rab5-GTP Q79L. Anterograde endosomal trafficking was manipulated by expression of constitutively active and dominant negative Rab11a mutants. The effects of these agents on secretion, endolysosomal exocytosis of lysosome associated membrane protein (LAMP1), and trypsinogen activation in response to supramaximal cholecystokinin (CCK-8), bile acids, and cigarette toxin was determined.

RESULTS: PIKfyve inhibition increased basal and stimulated secretion. Adenoviral overexpression of PIKfyve decreased secretion leading to cellular death. Expression of Rab5-GTP Q79L or Rab11a-GTP Q70L enhanced secretion. Conversely, dominant-negative Rab11a-GDP S25N reduced secretion. High-dose CCK inhibited endolysosomal exocytosis that was reversed by PIKfyve inhibition. PIKfyve inhibition blocked intracellular trypsin accumulation and cellular damage

responses to supramaximal CCK-8, tobacco toxin, and bile salts in both rodent and human acini.

CONCLUSIONS: These data demonstrate that EE-LE trafficking acutely controls acinar secretion and the intracellular activation of zymogens, leading to the pathogenicity of acute pancreatitis. (*Cell Mol Gastroenterol Hepatol* 2015;1:695-709; <http://dx.doi.org/10.1016/j.jcmgh.2015.08.002>)

Keywords: Endosome; Pancreatitis; PIKfyve; Trypsin.

Acute pancreatitis is an inflammatory disease of the exocrine pancreas initiated in part by the premature activation of proteolytic zymogens by lysosomal hydrolases in an unidentified intracellular compartment.¹ The coincident activation of nuclear factor- κ B promotes inflammation and cellular damage in this disease.² Ductal obstruction, retrograde perfusion of bile salts, chronic ethanol exposure, hypercalcemia, hyperlipidemia, or infection can lead to acute experimental pancreatitis. These treatments are relevant to the etiologies of clinical disease. In addition to zymogen activation, a common feature of acute pancreatitis is the pronounced inhibition of digestive enzyme secretion from the acinar cell.³ Acinar preparations stimulated with

Abbreviations used in this paper: AP, adaptor protein; BFA, brefeldin A; CCK, cholecystokinin; CLP, constitutive-like pathway; DMEM, Dulbecco's minimal essential medium; DMSO, dimethyl sulfoxide; EE, early endosome; GDP, guanosine diphosphate; GFP, green fluorescent protein; GTP, guanosine triphosphate; HA, hemagglutinin; LAMP1, lysosome-associated membrane protein; LDH, lactate dehydrogenase; LE, late endosome; LY294002, 2-morpholin-4-yl-8-phenylchromen-4-one; MRP, minor-regulated pathway; PI, phosphatidylinositol; PIKfyve, phosphatidylinositol 3-phosphate 5-kinase; PI(3)P, phosphatidylinositol 3-phosphate; PI(3,5)P₂, phosphatidylinositol-3,5-bisphosphate; RE, recycling endosome; TGN, *trans*-Golgi network; VAMP8, vesicle-associated membrane protein 8; Vps34, phosphatidylinositol 3-kinase; WT, wild type; YM201636, 6-amino-N-[3-(4-morpholin-4-yl)pyrido[2,3-furo[2,4-b]pyrimidin-2-yl]phenyl]pyridine-3-carboxamide; ZG, zymogen granule.

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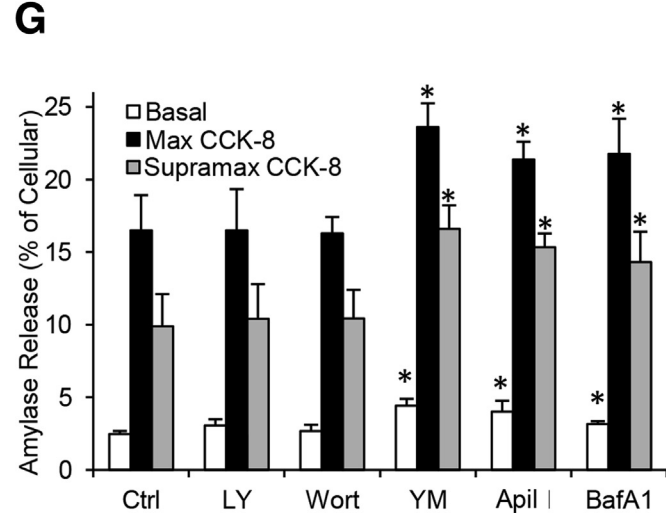
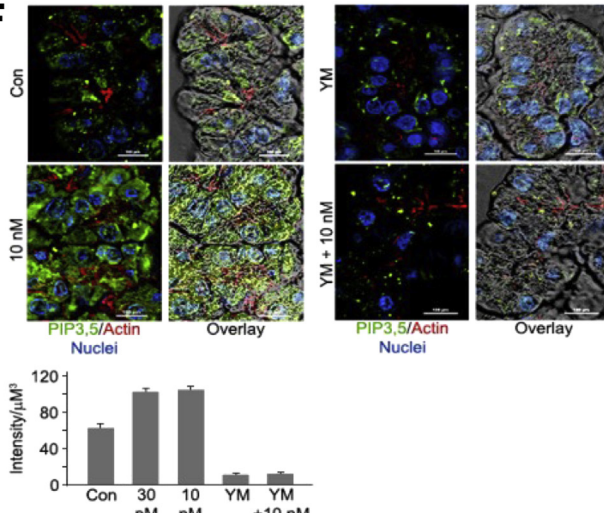
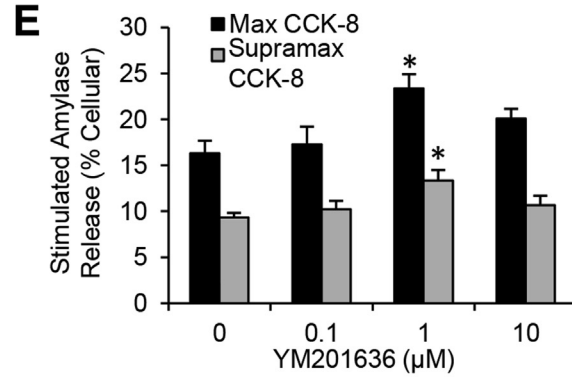
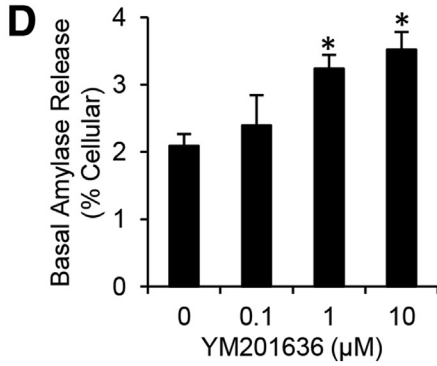
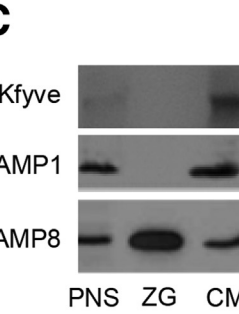
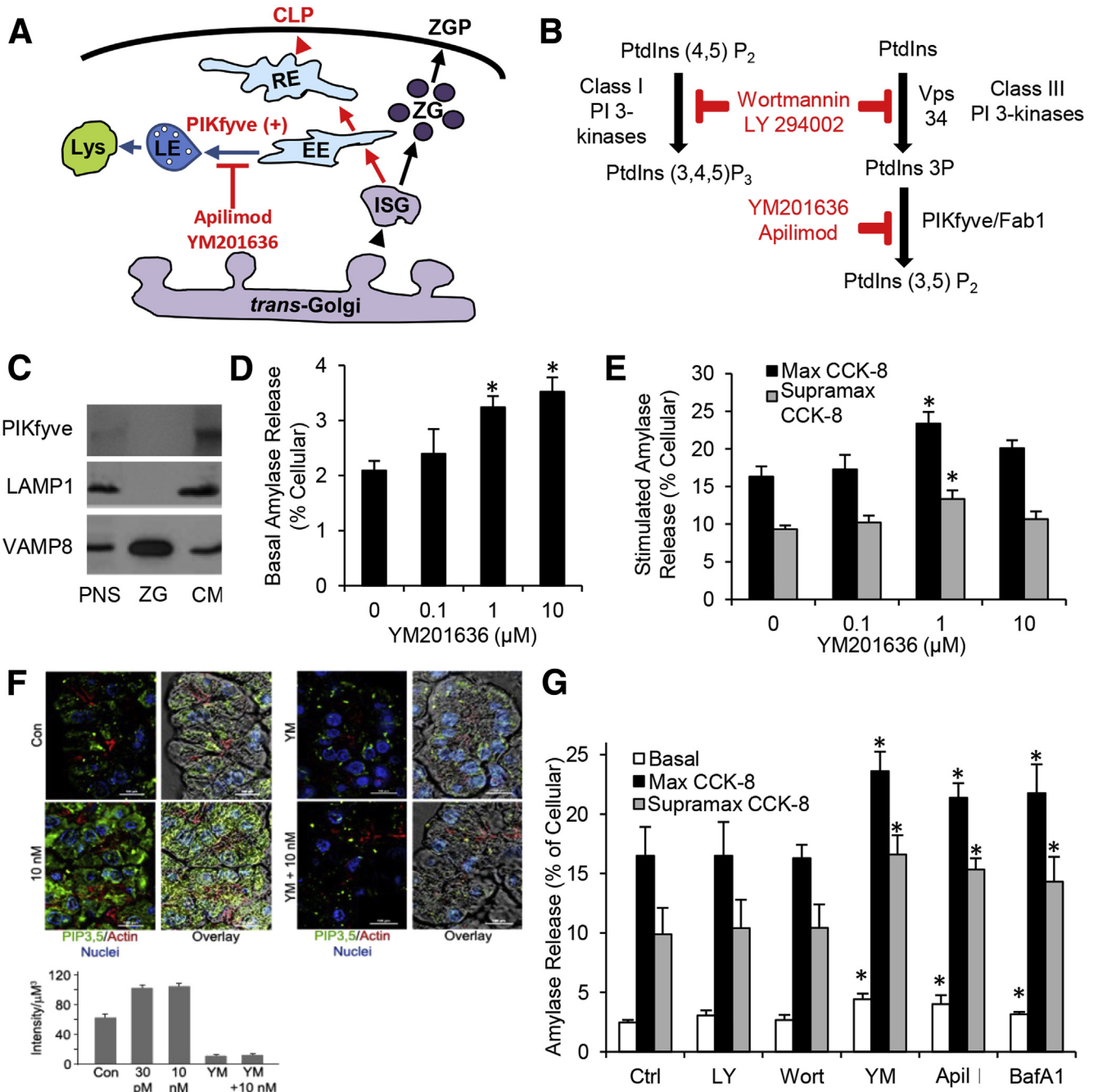
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high-dose cholecystikinin (CCK), bile salts, or cigarette smoke toxin are useful to induce early events of pancreatitis in the absence of an inflammatory response. Stimulation of acinar cells with the high-affinity CCK agonist JMV-180 or bombesin show no high-dose secretory inhibition or accumulation of activated zymogens, suggesting that secretory inhibition may play a key role in accumulating activated zymogens in the acinar cell and causing injury during pancreatitis.³

Phosphatidylinositols (PI) regulate membrane trafficking events based on their reversible phosphorylation of the

inositol ring.⁴ Class III PI3-kinase Vps34 (phosphatidylinositol 3-kinase) phosphorylates PI to generate PI3 phosphate [PI(3)P], which is essential for formation of the early endosomal (EE) compartment.⁵ The PI3-kinase inhibitors wortmannin and LY294002 (2-morpholin-4-yl-8-phenylchromen-4-one) were shown to fully block intracellular trypsinogen activation in isolated acini and in vivo.^{6,7} However, high concentrations of these inhibitors also block class I PI3-kinases, which generate phosphatidylinositol (3,4,5)-trisphosphate [PI(3,4,5)P₃] from phosphatidylinositol 4,5-bisphosphate [PI(4,5)P₂] (see Figure 1). Genetic



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