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Molecular Characterisation of Pancreatic Zymogen Granule Ion Channel and Regulator Proteins Involved in Exocytosis

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In pancreatic acinar cells Ca^{2+}-dependent secretagogues promote the fusion of zymogen granules (ZG) with the apical plasma membrane (PM) and exocytosis of digestive enzymes. In addition to exocytotic fusion complexes between SNARE proteins in the ZG membrane (ZGM) and the apical PM, enzyme secretion elicited by Ca^{2+}-dependent secretagogues requires cytosolic Cl^- and K^+ and is inhibited by blockers of Cl^-- and K^+-channels. We have identified a Cl^--conductance activated by ATP, and a K^+-conductance (with properties similar to ATP-sensitive K^+-channels), regulated by the granule matrix protein Zg-16p in the ZGM. Both conductances are inversely regulated by a 65-kD *mdr1* gene product. We have also identified a novel Ca^{2+}-activated anion conductance in ZGM, the Ca^{2+}-sensitivity of which increases 50-fold when Cl^- is replaced by I^-. This conductance is blocked by micromolar H_2-DIDS or DTT, reminiscent of a family of epithelial Ca^{2+}-activated Cl^--channels (CaCC). Expression of a CaCC in exocrine pancreas has been confirmed by RT-PCR analysis, and by immunoblotting and immunogold labeling of ZG membranes. These data suggest that ion channels in the ZGM are essential elements in pancreatic exocytosis.

Exocytosis in many cell types is strictly controlled by Ca^{2+}. In pancreatic acinar cells the action of Ca^{2+}-dependent secretagogues leads to the fusion of zymogen granules (ZG) with the apical plasma membrane (PM) and to exocytosis of digestive enzymes into the lumen. Although exocytotic fusion complexes between protein families (SNARE proteins, syncollin) in the ZG and apical plasma membranes have been identified (1-3), these proteins are not sufficient to reconstitute the Ca^{2+}-dependent exocytotic process in vitro, let alone in intact cells (4). Therefore additional mechanisms have to be invoked to fully account for exocytosis.

Key Words: *Secretion; Acinar Cells; Anion Channels; mdr1 P-Glycoprotein; Sulfonylurea Receptor*

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Enzyme secretion evoked by Ca^{2+}-dependent secretagogues in permeabilized pancreatic acini, is critically dependent on the presence of Cl^- and K^+ in the cytosol and is abolished by application of Cl^- and K^+ channel blockers (5). This suggests that regulated Cl^- and K^+ channels present in the membrane of ZG promote enzyme secretion elicited by secretagogues. We have demonstrated the presence of regulated Cl^-- (6, 7) and K^+ conductances (8) in osmotic lysis experiments with isolated rat pancreatic ZG used as a quantitative assay to measure macroscopic ion fluxes. The Cl^- conductance is activated by ATP and nonhydrolyzable ATP analogs, such as AMP-PCP, and is blocked by the Cl^- channel blocker DIDS (6, 7). The K^+ conductance is blocked by typical K^+-channel blockers, e.g., quinidine and Ba^{++}. Additional characteristics of this conductance, including inhibition by ATP, nonhydrolyzable ATP analogues and glibenclamide, and activation by diazoxide, are similar to those of the "classical" ATP-sensitive K^+-channels (8).

In our attempts to identify the underlying channel proteins, we have characterised two ZG membrane (ZGM) associated proteins that regulate Cl^-- and K^+ conductances: 1) Using *mdr1a* knock-out mice we have provided evidence that ZG Cl^- and K^+ conductances are regulated by two small molecular weight *mdr1a* and *mdr1b* gene products in ZGM that are distinct from the known *mdr1* proteins with a molecular weight of ~180 kDa (9, 10). This ~65 kDa *mdr1* gene product is most likely the receptor for ^{3}H-glibenclamide in pancreatic ZG and other secretory granules (11, 12). 2) We have purified and identified the high affinity receptor (K_d ~8 nM) for a benzoyldihydrocinnamic acid derivative of dihydropyridine (BZDC-DHP), which specifically blocks the K^+-selective conductance in ZG, as "ZG-16p", a recently cloned ZG protein of unknown function with homology to lectins (13). We hypothesise that "ZG-16p", as part of the submembranous granule matrix, is involved in the regulation of the ZG ATP-sensitive K^+ conductance.

We have also identified a novel Ca^{2+}-activated anion conductance in ZG. Its Ca^{2+}-sensitivity increases 10-50-fold (from 100-500 μM to 5-10 μM) when Cl^- is replaced by I^- or NO_3^-. Moreover, H_2-DIDS (100 μM) or DTT

(25 μM) block the Ca^{2+}-activated anion conductance. The functional properties of this anion conductance are reminiscent of a family of epithelial Ca^{2+}-activated Cl^--channels (CaCC) first identified (14) and cloned in bovine trachea (15). To confirm CaCC expression in rat pancreas, we used rat pancreatic mRNA in a reverse transcription-PCR reaction. The PCR primers were based on the sequence of the mouse mCaCC-1 isoform. A PCR product of 539 bp was generated in a standard PCR reaction, and subcloned. The sequence consisted entirely of open reading frame (179 amino acids) and exhibited 81%, 77% and 57% amino acid similarity, respectively, to mCaCC-1, -2 and -3 (mgob-5), the three extant mouse isoforms known (16-18). Using antibodies raised against the bovine tracheal CaCC isoform and immunofluorescence light microscopy, basolateral labelling was found in acinar cells. Moreover, ZG also expressed CaCC in their membranes as shown by immunoblotting and immunogold labelling. This finding suggests that a CaCC could account for the Ca^{2+}-activated anion conductance of isolated ZG. We propose that a CaCC expressed in plasma membranes and ZG of pancreatic acinar cells is involved in hormone-stimulated secretion of salt and digestive enzymes.

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