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Control of the Priming and Triggering Phases of Exocytosis in the Pancreatic Acinar Cell

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The primary function of the pancreatic acinar cell is to secrete a complex mixture of digestive enzymes. These enzymes are synthesised as inactive zymogens, and are stored within the apical pole acinar cell in membrane-bound storage vesicles called zymogen granules. On stimulation, the zymogen granules migrate to and then fuse with the apical plasma membrane, a process known as regulated exocytosis. The fusion of the granules with the plasma membrane results in the secretion of the granules' contents into the acinar lumen. Until relatively recently very little was known about the mechanism of exocytosis in the acinar cell. However, with the advent of the use of bacterial toxin-permeabilized acini (1, 2), and the development of an in vitro zymogen granule/plasma membrane fusion assay (3), details are now beginning to emerge about the exocytotic mechanism.

Recently it was demonstrated that exocytosis in the acinar cell is composed of at least two biochemically distinct steps (4). The first step requires requires ATP, but not Ca^{2+}, and primes the exocytotic machinery, whereas the second step requires Ca^{2+}, but not ATP, and triggers granule exocytosis. The priming and triggering steps are reflected in the biphasic kinetics of stimulated zymogen secretion. On stimulation there is an initial rapid phase of secretion that lasts approximately five min, which is followed by a second, much slower, sustained phase of zymogen release that can continue up to one hour. The initial phase of secretion represents the Ca^{2+} triggered exocytosis of a pool of primed granules, whilst the slower kinetics of the sustained phase reflects the recruitment of granules to the apical plasma membrane and the ATP-dependent re-priming of the exocytotic apparatus. Work from my laboratory indicates that the priming and triggering of exocytosis are independently controlled via distinct intracellular signalling pathways.

Secretagogues such as cholecystokinin octapeptide (CCK8) and acetylcholine (Ach) trigger exocytosis (zymogen secretion) by eliciting oscillations in intracellular Ca^{2+} concentration (5). Exactly how elevating the ambient Ca^{2+} concentration triggers exocytosis of the zymogen granules is unclear. Examination of the mechanism of exocytosis in neuroendocrine cells has indicated that Ca^{2+} most likely triggers exocytosis by producing conformational changes in individual components of the SNARE complex (6). The SNARE complex mediates the docking and fusion of the synaptic vesicle with the plasma membrane. It consists of three integral membrane proteins (vamp 2, syntaxin 1 and SNAP 25) and two cytosolic proteins (NSF and α/β snap). The conformational changes in the SNARE complex proteins are mediated through a Ca^{2+} sensor, possibly synaptotagmin (6). Vamp 2, syntaxin 2 and syntaxin 3 are known to be required for zymogen granule/plasma membrane fusion (7), but how Ca^{2+} modifies these proteins to trigger exocytosis is unknown. Further research is required to fully understand the mechanism evoked by Ca^{2+} to trigger exocytotic membrane fusion not only in pancreatic acinar cell but all secretory cells.

The ATP-dependent priming of exocytosis is not unique to the acinar cell and was originally observed in neuroendocrine cells. Research examining exocytosis in chromaffin cells has shown that, at least in neuroendocrine cells, the ATP-dependent priming of exocytosis is the result of a number of ATP-dependent events; these include: the sequential phosphorylation of phosphoinositol to generate phosphoinositol bisphosphate (PIP_2), the phosphorylation of an unknown protein or proteins and the NSF-catalysed, disassembly of the SNARE complex (6). Secretagogue stimulation of acini does evoke the generation of PIP_2 and activates PKC, however no pancreatic equivalent of the SNARE complex has been identified. Whether the same series of events that prime exocytosis in neuroendocrine cells also prime exocytosis in acinar cells is currently unknown.

Over the course of the past two to three years my laboratory has examined the control of the ATP-dependent priming of exocytosis. These studies have shown that the sustained, slower, phase of secretagogue stimulated secretion, which includes the ATP-dependent

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priming of exocytosis, is absolutely dependent on a sustained increase in diacylglycerol (DAG) produced by the PC-PLC catalysed hydrolysis of PC. All evidence indicates that DAG activates protein kinase C to up-regulate both the repriming of exocytosis and the recruitment of zymogen granules to the plasma membrane. DAG may also be modulating other DAG-binding proteins, like Munc-13, which are involved in regulating the exocytotic machinery in neuronal cells (6). Our other studies have shown that the low-affinity CCK receptor inhibits stimulated amylase secretion by generating an intracellular signal that blocks the ATP-dependent priming of the exocytotic machinery (8). The exact identity of the inhibitory signal is currently unknown, however, we do know that the signal is hydrophobic and remains membrane associated. The only intracellular signal generated by the low-affinity CCK receptor that has these characteristics is DAG. Furthermore, we found that artificially elevating intracellular DAG concentration by blocking DAG hydrolysis markedly inhibited the sustained phase of CCK8-stimulated secretion. This finding, plus other published work (9), strongly supports the idea that DAG, probably via activation of PKC, mediates the inhibitory action of the low-affinity CCK receptor on the ATP-dependent priming of exocytosis.

In conclusion, our studies have shown that exocytosis in the acinar cell is composed of at least two biochemically distinct steps that are independently controlled by different intracellular signalling pathways. Secretagogues evoke increases in intracellular calcium that initiate secretion by directly triggering exocytosis of a pool of primed granules. In addition, secretagogues stimulate a sustained increase DAG, which is absolutely required to maintain the secretory response by up-regulating the repriming of the exocyotic apparatus and possibly the recruitment of granules to the apical plasma membrane. Paradoxically, we also found that DAG can inhibit the sustained phase of secretion, possibly by down-regulating the repriming of exocytosis. Thus DAG, and by implication PKC, appear to be critical factors in the regulation of the sustained phase of exocytosis including the ATP-dependent priming of the exocytotic apparatus. Further research is required to determine how DAG/PKC differentially regulates the ATP-dependent priming of regulated exocytosis.

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