Ca²⁺ signaling in pancreatic acinar cells: physiology and pathophysiology

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The pancreatic acinar cell is a classical model for studies of secretion and signal transduction mechanisms. Because of the extensive endoplasmic reticulum and the large granular compartment, it has been possible - by direct measurements - to obtain considerable insights into intracellular Ca²⁺ handling under both normal and pathological conditions. Recent studies have also revealed important characteristics of stimulus-secretion coupling mechanisms in isolated human pancreatic acinar cells. The acinar cells are potentially dangerous because of the high intra-granular concentration of proteases, which become inappropriately activated in the human disease acute pancreatitis. This disease is due to toxic Ca²⁺ signals generated by excessive liberation of Ca²⁺ from both the endoplasmic reticulum and the secretory granules.

Introduction

The crucial role of changes in the cytosolic Ca²⁺ concentration ($[Ca^{2+}]_i$) for the control of muscle contraction and relaxation was well understood already in the 1960's (1). However, a proper appreciation of the role of Ca²⁺ in the regulation of secretion from exocrine glands only began to emerge in the 1970's (Figure 1). Selinger and colleagues (2) from Israel and Alonso and colleagues (3) from Argentina demonstrated ATP-dependent Ca²⁺ uptake into microsomal vesicular preparations from salivary gland cells and thereby established that there was a source of intracellular non-mitochondrial Ca²⁺ from which Ca²⁺ could in principle be released for signaling purposes. Shortly thereafter, Nielsen and I (4) - working at that time in Copenhagen, Denmark - demonstrated that the physiological stimulants of salivary secretion, acetylcholine (ACh) and adrenaline, evoked release of Ca²⁺ from internal stores in the salivary gland cells and we proposed that the crucial store was in the endoplasmic reticulum (ER). Together with Matthews and Williams (5) in Cambridge, England, I

obtained similar results in studies of the exocrine pancreas, and Case and Clausen (6) - working at that time in Denmark - also produced the same type of evidence.

After several years of confusion about whether pancreatic enzyme secretion is Ca²⁺-dependent or -independent, it became clear that there is an initial phase of stimulant-evoked enzyme secretion that is completely independent of extracellular Ca²⁺, whereas the following phase of sustained secretion depends acutely on the presence of external Ca²⁺ (7). The initial phase of stimulant-evoked enzyme secretion follows very closely after the intracellular Ca²⁺ release (5) and data obtained later, utilizing patch clamp membrane capacitance measurements (8), showed that the exocytotic release has a time course very similar to the time course - during a single Ca²⁺ spike - of the change in $[Ca^{2+}]_i$ near the apical membrane through which secretion occurs (Figure 2). These findings provide the basis for our current concept of stimulus-secretion coupling in exocrine glands.

It is not the purpose of this article to provide a comprehensive review of what is now known about the mechan-

isms underlying physiological and pathological Ca^{2+} signaling in the pancreatic acinar cells, because several such review articles have been published recently (9-11). The purpose of this article is to focus attention on a couple of critical questions, which have received recent attention.

Why is the sustained phase of enzyme secretion acutely dependent on external Ca^{2+}?

Considering the existence of large intracellular Ca^{2+} stores in pancreatic acinar cells and the evidence that Ca^{2+} is released from such stores upon stimulation with physiological stimulants (for example, ACh), it is not immediately obvious why secretion should depend on external Ca^{2+}. One could easily imagine that Ca^{2+} could be released upon stimulation and then taken up again into the stores when stimulation ceases. However, the pancreatic acinar cell - like all other cells - has Ca^{2+} pumps in the plasma membrane (plasma membrane Ca^{2+}-activated ATPase - PMCA) and these pumps increase the rate of Ca^{2+} extrusion as soon as $[Ca^{2+}]_i$ increases from the basal resting level (12). In fact, the rate of PMCA-mediated Ca^{2+} extrusion is sufficient to pump out all the Ca^{2+} released into the cytosol within a few minutes, when the ER is emptied during maximal stimulation (11,12).

Figure 3 shows a simplified diagram in which the major shifts in Ca^{2+} handling during the transitions from rest to activity and back again to rest are depicted. The initial sharp rise in $[Ca^{2+}]_i$ (monitored by changes in the Ca^{2+}-activated Cl^- current) is due to opening of Ca^{2+} release channels (predominantly inositol trisphosphate receptors (IP_3Rs) in the ER, but also involving ryanodine receptors and non-ER acidic Ca^{2+} stores) (for a recent review, see Ref. 11). The subsequent decline in $[Ca^{2+}]_i$ is principally due to PMCA-mediated Ca^{2+} extrusion, since the Ca^{2+} pumps in the ER (sarco(endo)plasmic reticulum Ca^{2+}-activated ATPase - SERCA) have little impact as long as the Ca^{2+} release channels are open. During the sustained phase of stimulation, when $[Ca^{2+}]_i$ is stable at a moderately elevated level, there is again equilibrium - as in the resting state - with Ca^{2+} influx from the external solution into the ER matching the release from the ER and extrusion across the plasma membrane, but the Ca^{2+} fluxes are much higher in the stimulated state than under resting conditions (compare scenarios [1] and [2] in Figure 3). When stimulation ceases, the Ca^{2+} release channels close and now SERCA has a significant impact, causing net Ca^{2+} reuptake into the stores (scenario [3] in Figure 3). The interesting point here is that, whereas $[Ca^{2+}]_i$ returns to the resting level very quickly upon cessation of stimulation, it takes a long time before the ER is replenished with Ca^{2+} (Figure 3). This reuptake of Ca^{2+} into the ER can be blocked by the specific SERCA pump inhibitor thapsigargin and is completely dependent on the presence of external Ca^{2+} (13,14). This is in complete accord with the finding that all the Ca^{2+} released from the intracellular stores is pumped out of the
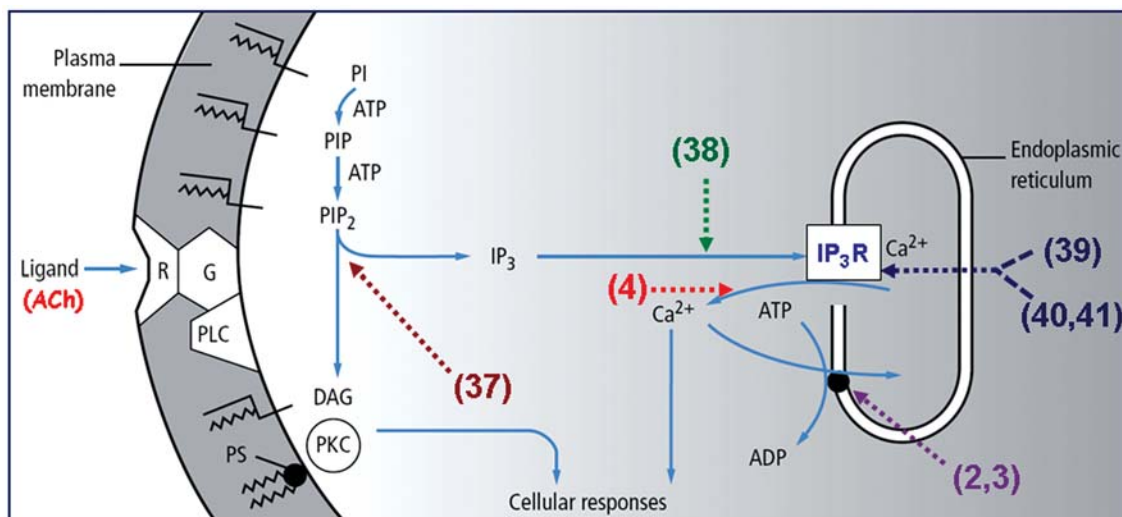

Figure 1. Basic aspects of Ca^{2+} signal generation in electrically non-excitable tissues by means of Ca^{2+} release from intracellular stores. The figure is based on a standard textbook diagram (adapted from Ref. 36). The numbers (2-4 and 37-41) in parentheses refer to the publication of the original discoveries and correspond to numbers in the reference list. ACh = acetylcholine; R = receptor; G = GTP-binding protein; PLC = phospholipase C; PKC = protein kinase C; PS =phosphatidyl serine; PI = phosphatidyl inositol; PIP = phosphatidyl inositol phosphate; DAG = diacylglycerol; IP_3R = inositol trisphosphate receptor; ATP = adenosine triphosphate; ADP = adenosine diphosphate.

cell by PMCAs and therefore has to re-enter the cell in order to refill the ER (11,13). Finally, after several minutes of active Ca²⁺ reuptake into the ER we come back to the pre-stimulation resting level (scenario [4] in Figure 3).

How does Ca²⁺ enter acinar cells during the sustained phase of stimulation?

After many years of uncertainty and debate about the molecular mechanism responsible for Ca²⁺ entry secondary to depletion of intracellular stores, a consensus has now emerged. There is a sensor, STIM1, in the ER membrane that monitors [Ca²⁺] in the ER lumen ([Ca²⁺]$_{Lu}$). When Ca²⁺ is released from the ER, in response to stimulation, [Ca²⁺]$_{Lu}$ falls and this triggers translocation of STIM1 to the so-called puncta in the ER membrane. These puncta are localized in parts of the ER very close to the plasma membrane. During ER Ca²⁺ depletion, STIM1 is localized in the puncta together with the Ca²⁺ entry channel CRACM1 (also known as Orai1) (15), which is the classical well-

characterized and very Ca²⁺-selective, Ca²⁺ release-activated Ca²⁺ channel (16). We have recently demonstrated STIM1 translocation, triggered by ER Ca²⁺ depletion, to puncta very close to the plasma membrane and co-localized with Orai1 in pancreatic cells (pancreatic cell line, PANC1). Furthermore, we have shown that this translocation does not require ATP (17).

What is physiological stimulation?

In experiments of the type shown in Figures 2B and 3, sustained stimulation with μM concentrations of ACh was used. Is this physiological? Almost certainly not! Prolonged stimulation of this intensity will destroy the acinar cells (18). Unfortunately, we do not have any direct evidence relating to the actual ACh concentrations attained during physiological nerve stimulation near the muscarinic receptor sites on the baso-lateral acinar membranes. In order to answer the question about the type of stimulation that could be regarded as physiological, it may therefore be
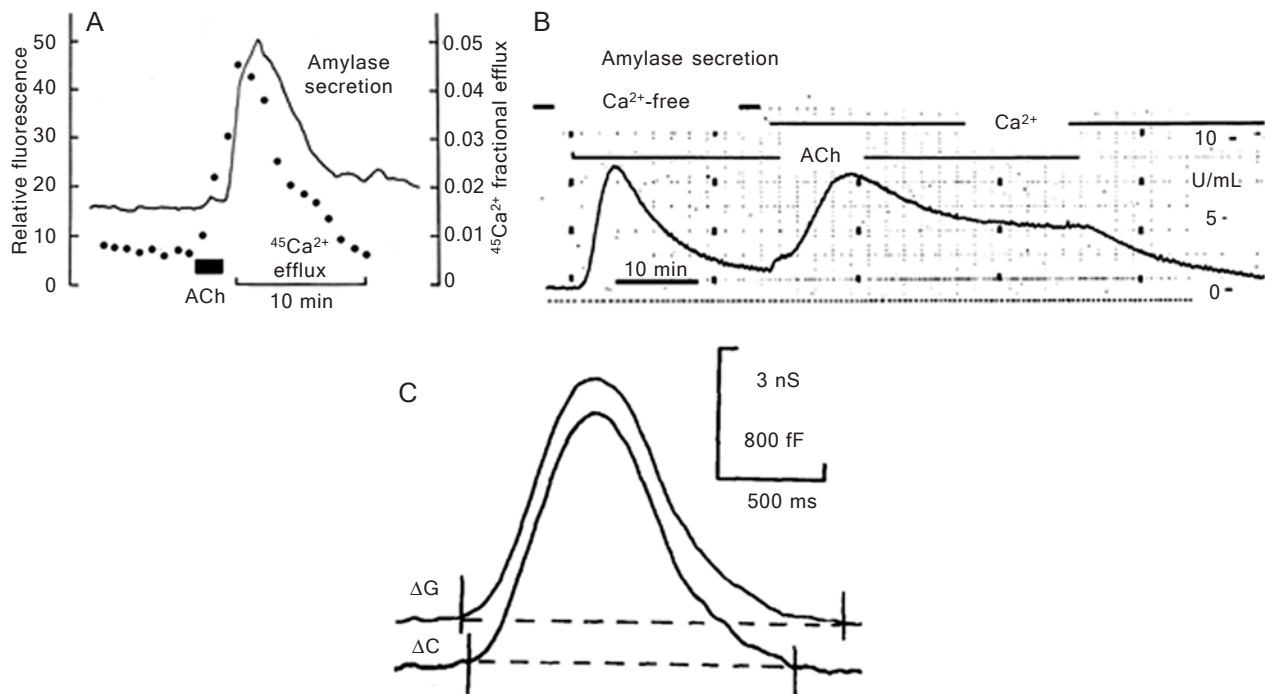

Figure 2. Basic aspects of pancreatic acinar stimulus-secretion coupling. *A*, Simultaneous measurements in isolated pancreatic fragments of intracellular Ca²⁺ release (assessed by fractional efflux of pre-loaded radioactive calcium) and amylase secretion assessed by an on-line fluorescence-based technique (adapted from Ref. 5). *B*, Ca²⁺-dependence of acetylcholine (ACh; 1 μM)-induced amylase secretion from isolated pancreatic fragments. Initially, the fragments were superfused by a Ca²⁺-free solution, then (during continued ACh stimulation) Ca²⁺ was added to the inflowing solution. Finally, ACh was removed (adapted from Ref. 7). *C*, Simultaneous conductance and capacitance measurements from a single isolated pancreatic acinar cell. The changes shown represent a single spike taken from a train of such events elicited by intracellular IP₃ infusion. The conductance trace (ΔG) refers to the Ca²⁺-activated Cl⁻ conductance and therefore shows the time course of the [Ca²⁺]$_i$ change. The capacitance trace (ΔC) monitors insertion and retrieval of granule membrane (adapted from Ref. 8).
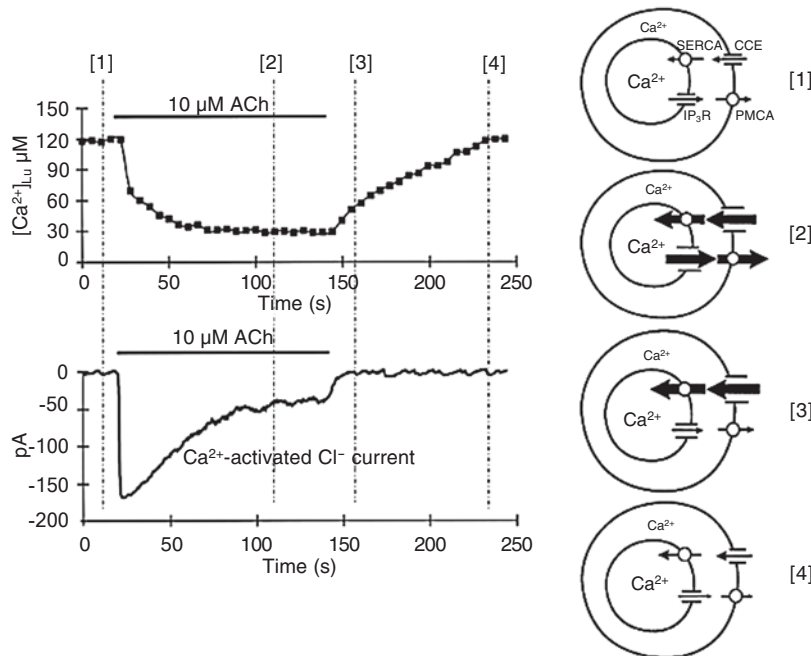
Figure 3. Simultaneous recording of $[Ca^{2+}]$ in the endoplasmic reticulum (ER) lumen ($[Ca^{2+}]_{Lu}$) and the Ca^{2+}-activated Cl^- current (monitoring changes in the cytosolic Ca^{2+} concentration) before, during and after acetylcholine (ACh; 10 µM) stimulation. To the right are shown cartoons indicating in a simplified manner (and only qualitatively) the rate of Ca^{2+} transport through 4 crucial transport proteins, namely the capacitative Ca^{2+} entry channels (CCE) - also known as store-operated Ca^{2+} channels, the Ca^{2+} pumps in the ER (SERCA), the IP_3 receptors (IP_3R), and the Ca^{2+} pumps in the plasma membrane (PMCA). The numbers refer to the times indicated in the main part of the figure. For further explanation, see text (adapted from Ref. 13).

better to consider stimulation with the circulating hormone cholecystokinin (CCK). From animal experiments, as well as experiments on man, we have good data about the CCK levels in plasma after a normal meal. They are in the range 1-10 pM (19). When pancreatic acinar cells are exposed to such concentrations of CCK, there is no sustained elevation of $[Ca^{2+}]_i$, but rather repetitive short-lasting $[Ca^{2+}]_i$ spikes. These are mostly local, in the apical granule-containing region, but do occasionally spread out to the whole cell (20,21). In order to obtain a sustained global elevation of $[Ca^{2+}]_i$, of the type shown in Figure 3, it is necessary to use nM concentrations of CCK, completely outside the physiological range of this hormone (18,19).

These considerations indicate that physiological stimuli, in general, evoke repetitive, short-lasting and local $[Ca^{2+}]_i$ elevations and that sustained (and inevitably global) $[Ca^{2+}]_i$ elevations are pathological phenomena. However, the relevance of the CCK data described above to human biology and medicine has been disputed, because of a study indicating that human pancreatic acinar cells lack functional CCK receptors (22,23). We therefore re-investigated this question, taking advantage of a long-established and well-functioning collaboration with the Division of Surgery and Oncology at the Royal Liverpool Hospital, which enabled us to transfer normal human pancreatic tissue - obtained from patients (with full informed consent) undergoing surgery for treatment of pancreatic cancer - very quickly to our laboratory for isolation of acinar cells and cell clusters (24). As shown in Figure 4, these isolated human acinar cells were polarized, exactly like mouse and rat

cells, and produced repetitive short-lasting $[Ca^{2+}]_i$ spikes in response to stimulation with physiological (pM) concentrations of CCK. These spikes were mixtures of local (in the apical pole) and global $[Ca^{2+}]_i$ transients and - similar to what had originally been found in the mouse acinar cells (20,21), even the global $[Ca^{2+}]_i$ elevations always started in the apical (granular) pole (Figure 4A). These repetitive $[Ca^{2+}]_i$ spikes were able to generate appropriate secretory and metabolic responses, as assessed by several different methods (Figure 4).

We can therefore conclude that physiological stimuli evoke repetitive $[Ca^{2+}]_i$ spikes, that these spikes often only represent local $[Ca^{2+}]_i$ elevations in the apical granular pole and that global $[Ca^{2+}]_i$ elevations have the character of $[Ca^{2+}]_i$ waves that always begin in the apical pole and then spread out towards the base of the cell.

Why do sustained global $[Ca^{2+}]_i$ elevations cause necrosis?

If sustained stimulation of the type shown in Figure 3 - with almost complete emptying of the ER - is allowed to continue for more than 15-20 min, activation of intracellular trypsin occurs (18,25). This initiates the very dangerous autodigestion process, which causes the often fatal human disease acute pancreatitis (10). Although hyperstimulation (stimulation of the pancreas with unphysiologically high concentrations of ACh or CCK) does experimentally evoke changes in mouse pancreatic acinar cells that are very similar to those observed in human pathology, it is
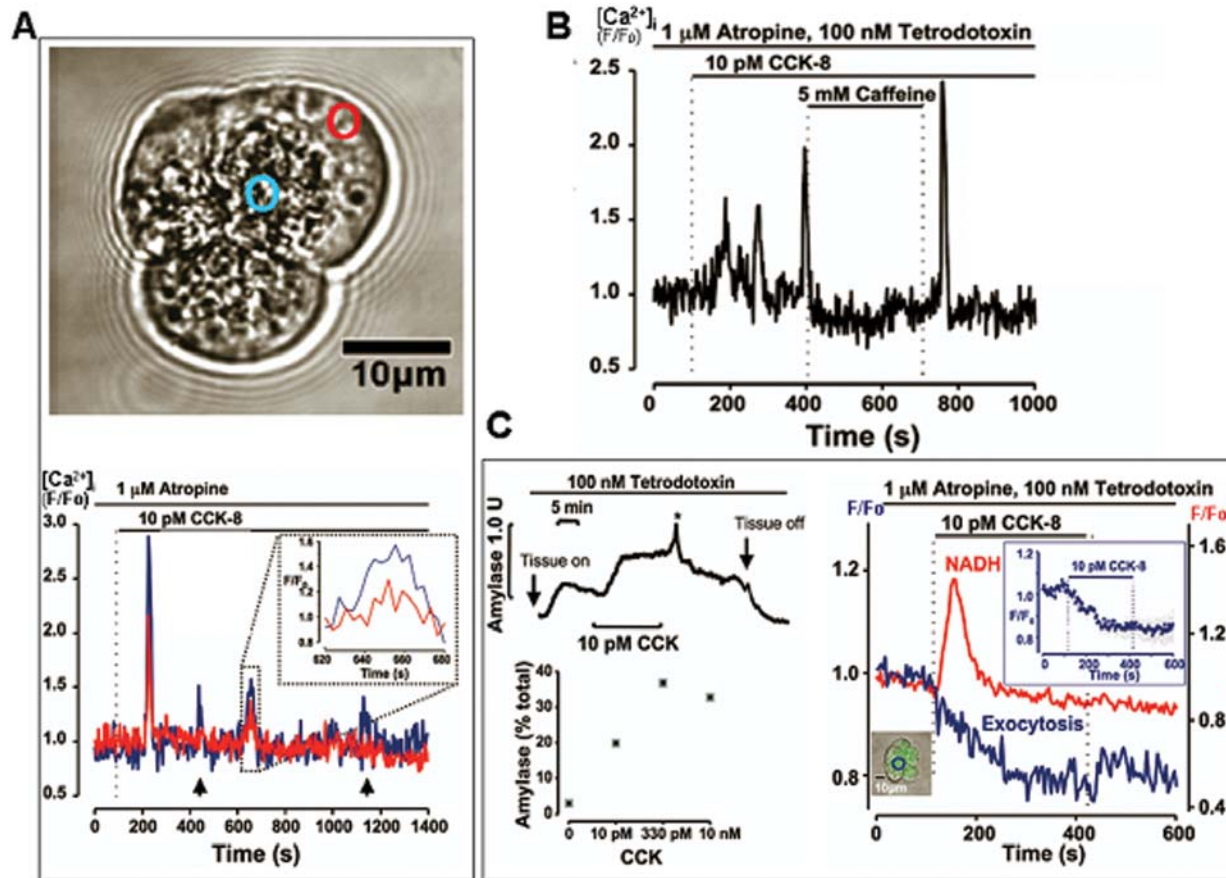
Figure 4. Ca²⁺ signaling and secretion in isolated human pancreatic acinar cells. *A*, Transmitted light image of an isolated cluster of three acinar cells. Below are seen traces of [Ca²⁺]ᵢ during stimulation with 10 pM cholecystokinin (CCK), obtained from the basal (red) and the apical (blue-granular) areas of another isolated pancreatic acinar cell. *B*, Ca²⁺ spikes generated in response to stimulation with CCK (10 pM) in the presence of both atropine and tetrodotoxin, indicating that these spikes cannot have been produced by the theoretically possible release of acetylcholine from stimulated nerve endings adhering to the cells. The spikes are suppressed, reversibly, by caffeine - a known inhibitor of inositol phosphate receptors (IP₃Rs). *C*, Amylase secretion measured directly by a specific enzymatic fluorescence-based technique (left) or by decrease in intracellular quinacrine fluorescence (right). Quinacrine accumulates in acid ATP-containing granules and disappearance of fluorescence is therefore a sign of exocytosis. There is simultaneous recording of NADH autofluorescence, which monitors mitochondrial activity. The traces to the right thus demonstrate CCK-elicited activation of the Krebs cycle as well as secretion (adapted from Ref. 24).

clear that hyperstimulation of this type is not responsible for acute pancreatitis. Pancreatitis is typically caused by alcohol abuse or complications arising from biliary disease (gall stones) (10). It turns out that non-oxidative alcohol metabolites, namely fatty acid ethyl esters, and bile acids are capable of eliciting sustained global [Ca²⁺]ᵢ elevations and necrosis (26-28). This necrosis is Ca²⁺-dependent, since it can be prevented by incubating the acinar cells with membrane-permeant Ca²⁺ chelators (27). Although there are no doubt many factors that play a role in the disease development (29), the crucial point is that acute pancreatitis is one of the clearest examples known of [Ca²⁺]ᵢ toxicity. Basically, acute pancreatitis is caused by

the wrong kind of Ca²⁺ signal, namely a global sustained elevation of [Ca²⁺]ᵢ, rather than the physiologically important repetitive local Ca²⁺ spikes in the secretory pole that control normal secretion.

Since intracellular trypsin activation is regarded as the most important process initiating acute pancreatitis, it is essential to understand in which intracellular compartment this occurs. Recent work from my laboratory demonstrated that trypsin activation, elicited by a prolonged elevated [Ca²⁺]ᵢ, occurs in post-exocytotic endocytic vacuoles (30). Taken together with the well-established fact that hyperstimulation only produces very little secretion and that one of the characteristics of acute pancreatitis is a secretion

defect (31), these data lead us to conclude that prolonged global $[Ca^{2+}]_i$ elevation fails to induce sustained secretion. At this point in time, the nature of this secretion defect is not entirely clear. Hyperstimulation clearly elicits insertion of granule membrane into the apical plasma membrane, since endocytosis of a fluorescent extracellular fluid phase marker was observed in our experiments (30). This indicates that hyperstimulation induces formation of a fusion pore large enough to allow molecules of the size of, for example, Texas Red Dextran to be taken up. Since there is little real protein secretion in acute pancreatitis, it would appear that the fusion pore is insufficiently large to allow the passage of large protein molecules. It is now becoming clear that fusion pore expansion is an important part of the secretion process (32) and it is a plausible hypothesis, which still needs to be critically tested, that it is the fusion pore expansion that is defective during conditions leading to acute pancreatitis.

Is the ER the only intracellular store from which Ca^{2+} can be released?

The mechanism by which trypsin activation occurs in the granules/vacuoles is not clear. It is not immediately obvious why Ca^{2+} depletion of the ER should trigger transformation of trypsinogen to trypsin inside the granules. In this context, it is important to realize that, although stimulant-elicited Ca^{2+} release from the ER is essential for Ca^{2+} signal generation and therefore secretion (11), it is not the only store from which Ca^{2+} can be released. Many years ago, we demonstrated in studies of isolated granules from pancreatic acinar cells that IP_3 can liberate Ca^{2+} stored in these organelles (33) and more recently we have shown, in experiments on permeabilized acinar cells, that high concentrations of Ca^{2+}-releasing messengers, including IP_3, release Ca^{2+} not only from the ER, but also from an acid pool specifically localized in the granular area (34). This pool is most likely dominated by the granules. It is important for pathophysiological considerations that bile acids, which are known to be capable of inducing acute pancre-

atitis, release Ca^{2+} from the acid pool in the granular area at concentrations that are pathophysiologically relevant (35).

Taken together, these data suggest that release of Ca^{2+} from the secretory granules may be an important part of the mechanism by which trypsin activation occurs inside the granules. This does not mean that the release from the ER is of no importance. It is clear that sustained elevation of $[Ca^{2+}]_i$ is essential for both trypsin activation and vacuole formation and that this, to a large extent, depends on the ER Ca^{2+} depletion, which is linked to activation of Ca^{2+} entry channels in the plasma membrane as explained above. Most likely, trypsin activation depends on both Ca^{2+} release from the ER, triggering sustained Ca^{2+} entry that maintains the plateau of elevated $[Ca^{2+}]_i$, as well as Ca^{2+} depletion from the granules which, in a way that is still obscure, changes the configuration of the granule matrix (10).

Conclusion

Our understanding of the complex set of processes responsible for Ca^{2+} signaling in pancreatic acinar cells has improved enormously in recent years. With the help of direct measurements of $[Ca^{2+}]$ in various intracellular compartments, we now have good data describing the basic movements of Ca^{2+} in response to stimulation under both normal physiological and pathological conditions. This basic knowledge concerning Ca^{2+} movements between various well-defined compartments has also helped our understanding of the mechanism by which the important human disease acute pancreatitis is initiated. However, several crucial points require further clarification. Most importantly, we do not at present understand how a sustained elevated $[Ca^{2+}]_i$, in combination with Ca^{2+} depletion of the ER as well as the secretory granules, triggers the fatal trypsin activation that ultimately kills the acinar cell with devastating results. Progress in this respect would be important for the development of better therapeutic approaches.

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