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Quantal Calcium Release and Calcium Entry in the Pancreatic Acinar Cell

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In the past decade, there have been remarkable advances in our understanding of the calcium messenger system that mediates the effects of various agonists. The purpose of the present article is to describe two areas of current interest in the calcium signaling field—quantal calcium release and calcium entry into the cell—using the pancreatic acinar cell as a model. Proposed mechanisms describing these phenomena and the role they play in the kinetics of calcium movements in the cell are discussed.

OVERVIEW

The major function of the pancreatic acinar cell is to synthesize, store, and release digestive enzymes [1]. The regulated secretion of digestive enzymes occurs in response to a number of hormones and neurotransmitters [2,3]. The intracellular messenger systems mediating secretion are of two separate types. Agents such as vasoactive intestinal polypeptide and secretin cause secretion by activating adenylate cyclase and increasing cyclic AMP. In contrast, cholinergic agents, cholecystokinin, bombesin peptides, and substance P peptides activate phosphoinositide breakdown and calcium transports. These processes, in turn, mediate enzyme secretion.

Historically, the pancreatic acinar cell has furnished an important model in providing the initial observations about the phosphoinositide/calcium pathway. The initial observation of phosphatidyinositol turnover was made, using pigeon pancreatic slices, by the Hokins in 1953 [4]. Inositol 1,4,5-trisphosphate was first demonstrated to mobilize intracellular calcium stores by Streb et al. in 1983, using permeabilized pancreatic acinar cells [5]. It is now generally accepted that agonists such as cholecystokinin, cholinergic agents, bombesin peptides, and substance P peptides cause a phospholipase C-mediated hydrolysis of the phosphorylated phosphatidylinositol derivative, phosphatidylinositol 4,5-bisphosphate, to inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol [3,6–8]. IP_3, in turn, mobilizes calcium from an internal store [5,9], while 1,2-diacylglycerol activates protein kinase C [6–8].

The release of calcium from the internal stores by the agonist is both rapid and transient. Calcium release occurs within one to a few seconds, depending on the concentration of agonist [10–14]. The release results in a rapid rise in free intracellular [Ca^{2+}] ([Ca^{2+}]i) [10–14]. The increase in [Ca^{2+}]i causes activation of a plasma membrane Ca^{2+} ATPase, resulting in Ca^{2+} efflux from the cell and a return of [Ca^{2+}]i toward resting levels [15]. Return of [Ca^{2+}]i toward resting level takes place over an interval of three to five minutes [10–14].

Abbreviations: CCK-OP: cholecystokinin-octapeptide IP_4: inositol 1,3,4,5-tetrakisphosphate IP_3: inositol 1,4,5-trisphosphate

After release of the intracellular pool of Ca^{2+}, there is activation of Ca^{2+} entry across the plasma membrane [3,16]. This calcium entry results in a sustained level of $[Ca^{2+}]i$ during stimulation after the initial internal release. The level of $[Ca^{2+}]i$ during sustained stimulation depends on the concentration of extracellular $CaCl_2$ [17]. Both the internal Ca^{2+} release and Ca^{2+} entry have essential roles in mediating enzyme secretion. The release of intracellular Ca^{2+} by agonists or Ca^{2+} ionophores causes a burst in enzyme secretion, lasting about as long as the transient increase in $[Ca^{2+}]i$ [6,10,17]. These results indicate that intracellular Ca^{2+} release alone causes secretion. During sustained agonist stimulation, a continued increase in enzyme secretion is dependent on extracellular $CaCl_2$. That is, in the absence of extracellular Ca^{2+}, enzyme secretory rates return to resting levels after the transient increase in $[Ca^{2+}]i$ [3,17].

Small concentrations of cholecystokinin-octapeptide, acetylcholine analogs, and maximally effective concentrations of the cholecystokinin analog, JMV-180, cause sustained oscillations of $[Ca^{2+}]i$ in the acinar cell [18–20]. Although the mechanism of the $[Ca^{2+}]i$ oscillations is controversial, one would expect that each oscillatory increase in $[Ca^{2+}]i$ would stimulate a burst in enzyme secretion.

QUANTAL CALCIUM RELEASE

Compared to a maximally effective dose, one would expect that release of calcium from intracellular stores by submaximally effective doses of agonist or inositol 1,4,5-trisphosphate would occur at a slower rate but result in a complete release. Observations from several laboratories indicate that this process is not the case [21–26]. Both submaximally effective and maximally effective concentrations cause rapid and transient release of calcium from the intracellular stores. The release by a submaximally effective concentration is partial despite continued presence of agonist or inositol 1,4,5-trisphosphate. This phenomenon has been called "quantal Ca^{2+} release."

There have been several models proposed to account for the cellular mechanism of quantal calcium release. To date, none have been generally accepted. In one model [21,23,27], it was proposed that the Ca^{2+} stores have varying sensitivities to inositol 1,4,5-trisphosphate-induced Ca^{2+} release; that is, some compartments have a high sensitivity to IP_3 and release all of their Ca^{2+} with a low concentration of IP_3. Other stores have lower sensitivities to IP_3 and require greater concentrations of IP_3 to release Ca^{2+}. A continuous gradient of sensitivities of the stores to IP_3, then, would account for the observed effects of both IP_3 and the agonist.

In a variant of the above model [28–31], Irvine has proposed that the Ca^{2+} content of the internal store regulates its sensitivity to IP_3-induced Ca^{2+} release. In this model, the entire pool is responsive to a submaximal concentration of IP_3. The resulting Ca^{2+} release decreases the pool Ca^{2+} content, which, in turn, decreases the responsiveness of the pool to IP_3-induced Ca^{2+} release. Thus, a greater concentration of IP_3 would be necessary to release the remainder of the Ca^{2+} from the store. In a third model [25,32], it has been proposed that, with a continuous IP_3 stimulation, there is a conversion of the Ca^{2+}-releasing channel on the pool from an active to an inactive state, independent of the Ca^{2+} content of the pool.

Although published reports describe the phenomenon of quantal Ca^{2+} release in a variety of tissues, there has been no general agreement on the mechanism. The experimental strategy commonly used to determine whether Ca^{2+} depletion of the

pool results in a decrease in the sensitivity of the pool to IP_3 has been first to partially deplete the pool of Ca^{2+} with Ca^{2+} ionophores or IP_3, followed by a measurement of the potency of IP_3 to release the remaining Ca^{2+} [26,27,29–31]. In some [26,30,31] but not all [27] of these studies, results have been presented suggesting that luminal Ca^{2+} regulates the sensitivity of Ca^{2+} release to IP_3.

Using dispersed pancreatic acini, we found that the potency of cholecystokinin-octapeptide (CCK-OP) to release Ca^{2+} from intracellular stores was unaltered by partial Ca^{2+} depletion of the stores by a pre-stimulation with carbachol [33]. Because carbachol and CCK-OP released Ca^{2+} from the same pool [10], these results suggested that quantal release in the pancreatic acinar cell was not due to either changing sensitivities of the pool to IP_3 as a function of Ca^{2+} content or various compartments with different sensitivities to IP_3.

Of particular interest to this field was a recent experiment demonstrating that quantal release occurred in lipid vesicles containing only purified IP_3 receptors [25]. The quantal release phenomenon was independent of vesicular Ca^{2+} content. Because the IP_3 receptor contains the Ca^{2+} channel mediating IP_3 effects on Ca^{2+} release [34], these results indicated that quantal release is an intrinsic characteristic of the receptor.

As illustrated above, the biochemical mechanism of quantal Ca^{2+} release has not been completely determined; however, the phenomenon of quantal release may provide an important physiologic control. For example, as discussed earlier, the pancreatic acinar cell contains several receptor classes that mediate Ca^{2+} release. Successive applications of submaximally effective (physiologic) concentrations of agonists interacting with a different class of receptors on the cell would result in transient increases in $[Ca^{2+}]i$, and each rise in $[Ca^{2+}]i$ could cause a cellular response. A demonstration of such an effect has not yet been provided in the literature.

CALCIUM ENTRY

For cells containing non-voltage-regulated Ca^{2+} entry mechanisms, it is now generally accepted that the plasma membrane Ca^{2+} transport is regulated by the intracellular Ca^{2+} store [35,36]. Specifically, depletion of the intracellular store by IP_3-induced Ca^{2+} release causes activation of the plasma membrane influx mechanism. This mechanism has been referred to as the "capacitative model" by Putney [35,36].

This model was proposed to account for the generally observed coupling between intracellular Ca^{2+} release and cell Ca^{2+} entry in a variety of tissues [37–39]. Soon after the discovery that the initial action of the agonist was to cause formation of IP_3 which, in turn, released intracellular Ca^{2+} stores [40,41], Putney attempted to explain the coupling in his first variation of the "capacitance" model [35]. In this model, he proposed that depletion of the pool activated the pathway for Ca^{2+} entry because the pool signaled the entry mechanism, using a close anatomic relationship between the pool and the plasma membrane Ca^{2+} influx mechanism. The Ca^{2+} entered the pool directly after crossing the plasma membrane. The depletion-activated Ca^{2+} influx, would, in turn, provide Ca^{2+} for refilling the intracellular store. During continued stimulation, Ca^{2+} release from the store would provide a source of Ca^{2+} to be released into the cytoplasm.

Observations since Putney's first proposal suggested that Ca^{2+} does not enter the

pool directly after influx across the plasma membrane. Experiments in both pancreatic acinar cells and parietal cells demonstrated that there was a mechanism for store uptake of Ca^{2+} from the cytoplasm [41–44]. In these experiments, agonist action was terminated just after release of Ca^{2+} from the store and before the Ca^{2+} was effluxed from the cell. The termination of agonist action resulted in complete reloading of the stores from the cytoplasmic Ca^{2+}. Muallem et al. [43] demonstrated that intracellular Ca^{2+} buffers decreased the rate of refilling of the intracellular Ca^{2+} pools in pancreatic acini, also suggesting that the route of refilling was cytoplasmic.

Experiments using the tumor promoter, thapsigargin, also suggested that the depleted pool stimulated Ca^{2+} entry directly into the cytoplasm. Thapsigargin is a pharmacologic tool that depletes intracellular stores by inhibiting the Ca^{2+}-ATPase responsible for loading the stores [45] without raising the levels of intracellular inositol phosphates [46]. When thapsigargin was used to deplete intracellular stores, $[Ca^{2+}]i$ increased when extracellular Ca^{2+} was present [47]. In addition to indicating that Ca^{2+} entry was into the cytoplasm, these results suggested that inositol phosphates are not necessary for Ca^{2+} entry.

The most challenging issue at present is the elucidation of the mechanism by which depletion of the internal stores signals the plasma membrane Ca^{2+} influx mechanism. We have presented evidence that cyclic GMP may act to mediate activation of the Ca^{2+} entry mechanism in the pancreatic acinar cell [48]. In brief, in pancreatic acinar cells, as well as in other tissues, agonists that mobilize intracellular Ca^{2+} also cause an increase in cyclic GMP [49,50]. We found that a pharmacologic agent, LY83583, could inhibit the ability of the agonist, carbachol, to increase cyclic GMP without altering Ca^{2+} mobilization by carbachol [48]; however, LY83583 inhibited Ca^{2+} entry during carbachol stimulation and refilling of the intracellular pools at the termination of carbachol stimulation. The inhibition of refilling was due to a blockade of the plasma membrane Ca^{2+} entry mechanism. Recent experiments suggest that depletion of the intracellular Ca^{2+} stores by thapsigargin results in increased cellular cyclic GMP [51]. The studies to date do not indicate how intracellular stores regulate cyclic GMP formation or how cyclic GMP activates the Ca^{2+} entry mechanism. The findings suggest, however, that the increase in cyclic GMP during agonist stimulation is both necessary and sufficient to activate the Ca^{2+} entry mechanism.

Irvine has proposed that the phosphorylated metabolite of IP_3, inositol 1,3,4,5-tetrakisphosphate (IP_4), has a role in mediating Ca^{2+} entry [52–54]. This proposal came initially from observations in sea urchin eggs, where it was found that the full fertilization response could be elicited with a combination of IP_3 and IP_4 in the presence of external Ca^{2+} [52]. IP_3 alone was insufficient. Subsequently, electrophysiologic studies of Ca^{2+}-activated K^+ channels in lacrimal cells demonstrated that IP_3 alone caused only a transient activation [53,54]. The addition of IP_4 and external Ca^{2+} were necessary for sustained activation [53,54]. Although these results suggest a possible role for IP_4 in mediating Ca^{2+} influx, they contradict the interpretation of findings with thapsigargin discussed earlier. That is, thapsigargin depletes internal stores and activates Ca^{2+} influx without changing cellular inositol phosphates. Thus, it is probable that IP_4 is not necessary for regulation of the influx mechanism.

In conclusion, the weight of the evidence suggests that Ca^{2+} influx in non-excitable cells is activated by IP_3-induced depletion of intracellular Ca^{2+} stores. The Ca^{2+} enters across the plasma membrane into the cytoplasm, where it maintains $[Ca^{2+}]i$ and provides a source of Ca^{2+} for reloading the internal stores. The nature of the

mechanism mediating the communication between the stores and the plasma membrane has not been established. We have proposed a role for cyclic GMP in this process. Further work is necessary to complete this story.

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