J. Membrane Biol. 124, 189–197 (1991)

The Journal of
Membrane Biology

Topical Review

Calcium Release and Internal Calcium Regulation in Acinar Cells of Exocrine Glands

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Introduction

Recent years have witnessed intensive efforts in unravelling the mechanisms by which Ca^{2+} is regulated inside exocrine acinar cells following the stimulation of receptors for secretagogues such as acetylcholine (ACh). The main steps that may be distinguished in this process are: (1) inositoltrisphosphate-dependent Ca^{2+} release from internal nonmitochondrial pools; (2) activation of regulatory feedbacks resulting in Ca^{2+} fluctuations and propagated Ca^{2+} waves; (3) entry of Ca^{2+} ions from the external solution; and (4) restoration of the initial Ca^{2+} level by transfer of residual cytosolic Ca^{2+} ions to internal stores and to the extracellular solution. The first of these steps is by far that which has been most extensively investigated and which is best understood. It is the main topic of the present review. Some specific aspects of steps 2 and 3 will also be discussed. In particular, it will be seen that there is a close association between the initiation of the Ca^{2+} release and the generation of Ca^{2+} fluctuations during sustained agonist application. Step 4, which has not been studied in any detail at the cellular level in this type of preparation, will not be discussed.

Ca^{2+} Signaling Pathway

There is a large body of evidence, not be reviewed here, indicating that Ca^{2+} release in exocrine glands follows the classical signaling pathway, involving an intermediate G protein, phospholipase C activation and inositoltrisphosphate-sensitive Ca^{2+} stores (reviewed in Berridge & Irvine, 1989; Rana & Hokin, 1990). From the physiologist's standpoint which is taken here, such a scheme implies that stimulating an acinar cell with GTPγS or with inositoltrisphosphate should result in an external Ca-independent rise of Ca_i. In agreement with these predictions, Ca-dependent K^+ and Cl^- currents could be stimulated by applying either compound via a patch-clamp pipette in the lacrimal gland (Evans & Marty, 1986; Llano & Marty, 1987), in the exocrine pancreas (Maruyama, 1989; Wakui, Potter & Petersen, 1989) and in the mandibular gland (Cook et al., 1988). The pathway leading to Ca^{2+} release offers several potential feedback mechanisms. First, phospholipase C is known in other systems to be potentiated by an elevation of Ca_i. The available evidence suggests that this potential positive feedback is not operating in exocrine glands (reviewed in Petersen & Wakui, 1990), but more studies on more gland types are needed before a definitive statement can be made. Secondly, protein kinase C is known in several systems, including parotid glands (Sugiya, Obie & Putney, 1988) to inhibit $InsP_3$ production (reviewed in Rana & Hokin, 1990). As protein kinase C is itself activated by phospholipase C-produced diacylglycerol and by Ca_i, this provides a slow inhibitory feedback. Inhibition of ACh-induced responses is indeed observed following protein kinase stimulation in rat lacrimal glands (Llano & Marty, 1987) and in rat pancreas (Maruyama, 1989), but not in the rat parotid (Gray, 1988). In rat lacrimal glands, the primary target of the inhibition appears to be G protein or phospholipase C, whereas it is the muscarinic receptor which is inhibited in pancreatic cells (Maruyama, 1989). Thus the results appear to depend critically on the gland type considered. In lacrimal glands, it was shown that the protein kinase C feedback is responsible for the desensitization of the response—a process which develops and fades on a time scale of minutes (Tan & Marty, 1991).

Arachidonic acid production accompanies the activation of the phosphoinositide pathway (Rana & Hokin, 1990). Maruyama (1990) recently showed

Key Words exocrine glands · secretory granules · calcium stores · endoplasmic reticulum · inositoltrisphosphate · ion channels

that arachidonic acid application depresses the response to ACh in the exocrine pancreas, and he further obtained evidence indicating that this inhibition is caused by a loss of sensitivity of $InsP_3$ receptors for the agonist. The inhibition of $InsP_3$ receptors by arachidonic acid suggests the existence of a negative feedback which could operate in parallel or in synergy with that of protein kinase C. The relevance of the former feedback remains, however, unclear (*see* Tan & Marty, 1991).

Ca^{2+} Release Channels

Ca^{2+} release channels may be studied after fusion of microsomes with planar artificial membranes. Such techniques have been successfully applied to muscle tissue, where a Ca^{2+}-dependent Ca^{2+} channel (the ryanodine receptor) and an $InsP_3$-dependent Ca^{2+} channel (Ehrlich & Watras, 1988) have been characterized. Similar reconstitution methods have only recently been applied to exocrine tissue. A Ca^{2+}-selective channel was found which is, like the ryanodine receptor, activated by caffeine and inhibited by ruthenium red (Schmid et al., 1990). However, this channel is not affected by ryanodine or by Ca^{2+} and in addition displays a sensitivity to potential which is lacking in the ryanodine receptor. The channel described by Schmid and coworkers is not sensitive to $InsP_3$ or to heparin and is therefore also distinct from the $InsP_3$-gated channel from muscle tissue. Clearly more work is needed to characterize the properties of Ca^{2+} channels located in the intracellular organelles of acinar cells and to compare them with those of the sarcoplasmic reticulum of muscle cells.

The Initiation of Ca^{2+} Release

Upon application of ACh, Ca_i rises rather abruptly following a latency on the order of one to several seconds (Merritt & Rink, 1987; Horn & Marty, 1988; Fig. 1). A high resolution recording of the Ca^{2+} signal together with the Ca^{2+}-dependent current showed that the latter leads the former by about 0.1 sec (Foskett et al., 1989). This rather paradoxical result was interpreted on the basis of a Ca^{2+} signal propagated from the periphery to the center of the cell. As the signal starts at the periphery, the Ca^{2+} signal averaged on the entire cell volume is too small to be detected. Large Ca^{2+}-dependent currents are, however, observed because the Ca^{2+} concentration near the plasma membrane is high.

The delay decreases with increasing agonist concentrations, until it reaches a minimum value

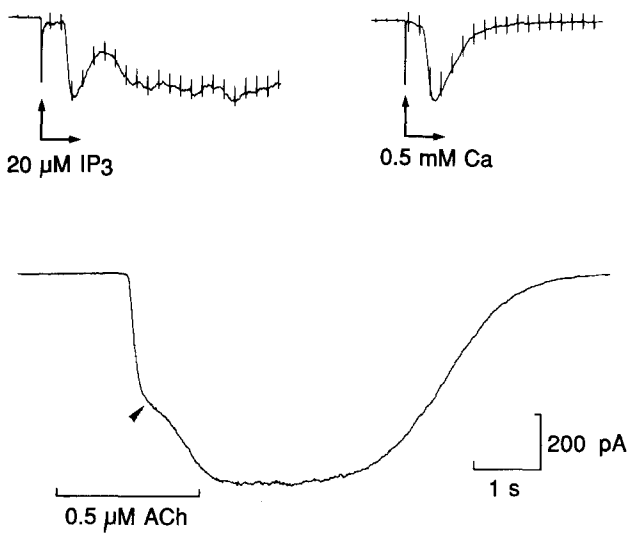

Fig. 1. The initiation of ACh-induced current. All recordings were taken at −60 mV (from three different cells). *Upper traces*: Responses to sudden applications of 20 μM $InsP_3$ (left) or 0.5 mM Ca^{2+} (right) through the recording pipette. Downward deflections reflect inward Cl^- currents. The pipette-cell connections were established at the vertical arrows. *Lower trace*: ACh-induced Cl^- current. Note the longer latency of the response and the biphasic time course of the current rise. The initial phase (arrow head) has a time course similar to those obtained with $InsP_3$ or Ca^{2+}. Reproduced from Marty et al. (1989) with permission of the publisher.

which cannot be overcome. In rat lacrimal glands, the relation between the delay, d, and the agonist concentration, A, was shown to follow the relation

$$1/d = 1/d_\infty + mA \quad (1)$$

where d_∞ is the delay at saturating agonist concentration and m is a constant. It was further shown that such a relation is expected from a simple kinetic model supposing a linear accumulation of active phospholipase C molecules with time and a regenerative Ca signal occurring once a threshold value for the number of active molecules of phospholipase C is reached (Marty et al., 1989).

An analysis of the response kinetics as a function of membrane potential showed that the slope m of Eq. (1) is voltage dependent, such that depolarizing the membrane is equivalent to reducing the ACh concentration. This result was interpreted as being due to a sensitivity of ACh binding to the membrane potential (Marty & Tan, 1989). Such sensitivity is expected if the agonist binding site is located in a deep region of the receptor molecule, where the membrane potential is susceptible to modify the local electric field.

Ca^{2+}-Induced Ca^{2+} Release

The shape of the initiation of the Ca-induced response is very suggestive of a regenerative Ca^{2+} signal. Its sigmoid time course could alternatively be explained on the basis of cooperative binding of several $InsP_3$ molecules on the same receptor (Meyer, Holowka & Stryer, 1988), but the number of binding sites necessary to account for the very abrupt take-off of the response after the latent period (Fig. 1) would be exceedingly high. Additional evidence in favor of a regenerative Ca^{2+} signal comes from experiments where either Ca^{2+} or $InsP_3$ were injected into acinar cells, resulting in all or none Ca^{2+} spikes (Marty & Tan, 1989; Osipchuk et al., 1990). In lacrimal glands, Ca^{2+} applications were performed by breaking into an acinar cell with a pipette loaded with a high Ca^{2+} concentration (0.1–1 mM). This resulted in transient responses which could be blocked by ruthenium red, a blocker of the channel responsible for Ca^{2+}-induced Ca^{2+} release of the sarcoplasmic reticulum (Marty & Tan, 1989; Fig. 1). Similar transients were also obtained with $InsP_3$ stimulations. These experiments led to the suggestion that the initiation of the response corresponded to the triggering of Ca-induced Ca release following the local accumulation of $InsP_3$ up to a certain threshold (Marty & Tan, 1989; Marty et al., 1989). In mouse pancreatic cells, similar transients were observed with Ca^{2+} and $InsP_3$ applications (Osipchuk et al., 1990). In addition, it was shown that both responses were potentiated by caffeine, and that the response to $InsP_3$, but not that to Ca^{2+}, was blocked by heparin (Wakui, Osipchuk & Petersen, 1990). Taken together, the results from both preparations strongly support a scheme by which the Ca^{2+} signal is initiated by a local Ca^{2+} release triggered by $InsP_3$ which is suddenly amplified and propagated due to Ca^{2+}-induced Ca^{2+} release. Thus, the finding that caffeine potentiates both Ca^{2+} and $InsP_3$-induced responses is in agreement with the notion that both responses employ the caffeine-sensitive Ca^{2+} channels involved in Ca^{2+}-induced Ca^{2+} release. Likewise, the finding that heparin, a blocker of the $InsP_3$-sensitive channel of smooth muscle cells (Ehrlich & Watras, 1988), only blocks the $InsP_3$-induced transient is as predicted by the notion that large Ca^{2+} concentrations can bypass the initial $InsP_3$-sensitive step.

Ca^{2+} Fluctuations

Ca^{2+} fluctuations in exocrine glands and in other nonexcitable tissues have been covered by two excellent recent reviews (Berridge, 1990; Petersen & Wakui, 1990) and will therefore not be discussed in detail here. One important point which has recently emerged from the measurement of Ca-dependent currents is that there are two distinct modes of Ca^{2+} fluctuations. The first mode consists of fast current transients (lasting about 1 sec each at room temperature) which are irregular in size and which are usually not organized in a periodic fashion. This "chaotic" mode is preferentially obtained when stimulating with $InsP_3$ (Evans & Marty, 1986) or with low ACh concentrations (Marty & Tan, 1989; Osipchuk et al., 1990). In pancreatic acinar cells, it can also be obtained with Ca injections (Osipchuk et al., 1990). The second mode consists of regular and periodic transients (Evans & Marty, 1986) with a period that depends more on temperature than on agonist concentration (Gray, 1988). This oscillating mode is selectively obtained when stimulating with moderate ACh concentrations (Evans & Marty, 1986; Marty & Tan, 1989; Osipchuk et al., 1990). In the exocrine pancreas, it was recently demonstrated using simultaneous recordings of whole-cell Cl^- currents and of Fura-2 associated fluorescence that the "chaotic" mode is associated with a minimal cellular Ca^{2+} rise, implying that the underlying Ca^{2+} signal is not spread out in the entire cell and that it is instead a local signal arising primarily from regions of the cell close to the plasma membrane (Osipchuk et al., 1990). In rat lacrimal glands, it was noted that the transients observed during the "chaotic" fluctuations were similar to the first phase of the responses obtained when applying moderate or large ACh concentrations (Marty & Tan, 1989). It thus seems reasonable to assume that the first phase of the response to ACh consists of a special kind of Ca^{2+} transient which is localized and which resembles the repetitive transients observable in the "chaotic" fluctuation mode.

Regenerative Responses of $InsP_3$-Sensitive Stores

It appears from the previous sections that the acinar cell has two Ca^{2+} amplification mechanisms. The first mechanism is triggered either by $InsP_3$ or by Ca^{2+} and leads to short and localized Ca^{2+} transients. The second mechanism leads to the propagation of the Ca^{2+} signal to the entire cell. Assuming that $InsP_3$-sensitive Ca^{2+} stores are involved in the former case, the results suggest that such stores are actually able to produce regenerative Ca^{2+} signals by themselves.

Additional evidence from other preparations (rat mast cells: Neher, 1988; guinea-pig hepatocytes: Ogden et al., 1990; *Xenopus* oocytes: Parker & Miledi, 1989; hamster eggs: Miyazaki, 1988) also

suggests that $InsP_3$-sensitive Ca^{2+} stores can discharge their content in a regenerative way. Thus Parker and Ivorra (1990) recently demonstrated that restricted regions in *Xenopus* oocytes release Ca^{2+} after photolysis of caged $InsP_3$ in an all-or-none manner. The time course of the Ca^{2+} transients observed in the oocytes is similar to the fast transients corresponding to the "chaotic" mode of Ca^{2+} fluctuations in exocrine glands. It is also similar to that of the single Ca^{2+} transients which may be obtained upon intracellular $InsP_3$ application in rat peritoneal mast cells (Neher, 1988). As in the other preparations, $InsP_3$-induced transients of mast cells are all or none. In this preparation, the presence of large Ca^{2+} transients in average Ca^{2+} measurements implies that the $InsP_3$-induced Ca^{2+} transients invade the entire cell.

The mechanism underlying the regenerative Ca^{2+} signals triggered by $InsP_3$-sensitive stores is not known. The evidence obtained from previously mentioned experiments in lacrimal glands suggests that Ca^{2+}-induced-Ca^{2+} release may be involved, since caffeine and ruthenium red appear to have potentiating and inhibiting effects on fast transients, respectively. On the other hand, two very recent reports indicate that $InsP_3$-sensitive Ca^{2+} channels from the brain display a strong sensitivity to the cytosolic Ca^{2+} concentration (Bezprozvanny, Watras & Ehrlich, 1991; Finch, Turner & Goldin, 1991). In each report the dependence of $InsP_3$-induced Ca^{2+} release on Ca^{2+} concentration was bell-shaped, with a strong potentiation elicited by increasing the Ca^{2+} concentration in the submicromolar range. This Ca^{2+} sensitivity could underlie the fast local Ca^{2+} transients elicited by $InsP_3$. To sum up, it appears likely that two separate positive feedback mechanisms are involved in the Ca^{2+} response: a local regenerative response possibly reflecting the properties of $InsP_3$-sensitive channels and a propagated Ca^{2+} wave presumably resulting from Ca^{2+}-induced Ca^{2+} release in the endoplasmic reticulum (Fig. 2).

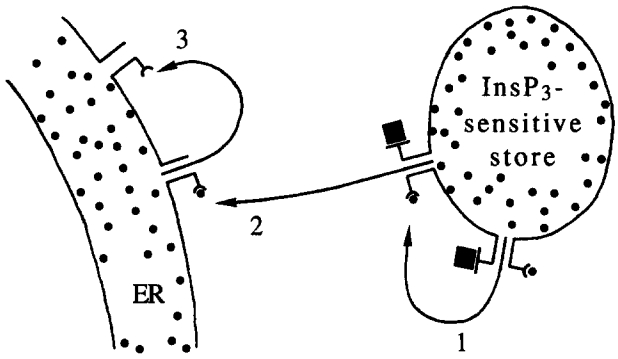

Fig. 2. Two mechanisms of amplification of Ca^{2+} signals. $InsP_3$-sensitive channels of $InsP_3$-releasable stores are supposed to be sensitive both to $InsP_3$ (squares) and to Ca^{2+} (dots), such that application of $InsP_3$ leads to a first type of generative Ca^{2+} signal (*1*). This first amplification mechanism is assumed to be localized. Once a certain level of activation of $InsP_3$-sensitive stores has been reached, Ca^{2+} ions are able to bind to Ca^{2+}-sensitive Ca^{2+} channels located on the endoplasmic reticulum (*2*). This starts a new type of Ca^{2+} amplification, which is propagated along the endoplasmic reticulum (*3*).

Ca^{2+} Imaging

Ca^{2+} imaging has been recently used to study the initiation of Ca^{2+} release in rat exocrine pancreas (Kasai & Augustine, 1990) and in rat parotids (Dissing, Nauntofte & Sten-Knudsen, 1990) using small aggregates of partially dissociated acinar cells. Such clusters keep a luminal/basolateral polarity, the regions of the cell which are close to the area of contact appearing rich in secretion granules, which are normally found on the luminal aspect of the cells. In one study, no spatial heterogeneity could be resolved during the onset of ACh application (Dissing et al., 1990). But in the other study, which benefited from a better time resolution and from more favorable temperature conditions (room temperature instead of 38°C), it was found that the first sign of a response is a localized Ca^{2+} rise restricted to the luminal region and that this is followed after a lag of around 1 sec by a homogeneous Ca^{2+} rise in the entire cell (Kasai & Augustine, 1990; *see* Fig. 3). Cell currents were not measured during the Ca^{2+} imaging experiments. In further experiments using simultaneous measurements of whole-cell current and of the average Ca^{2+} concentration, two phases in the response to ACh were again distinguished. It was shown that the first phase of current was accompanied by a minimal change of the average Ca^{2+} concentration and that the ionic selectivity of the conductance underlying the two phases were different. The first phase was exclusively selective for Cl^- ions while the second phase comprised both Cl^-- and cation-selective currents. These results suggest the presence of distinct membrane domains, the luminal membrane containing mainly Ca^{2+}-dependent Cl^- channels and the basolateral membrane containing both Ca^{2+}-dependent Cl^- channels and Ca^{2+}-dependent cation-selective channels. To interpret their imaging results, Kasai and Augustine (1990) further suggested that $InsP_3$-sensitive stores are primarily localized in the luminal part of the cell.

In view of the results described so far, the general scheme illustrated in Fig. 4 can be put forward. $InsP_3$ is produced near the basolateral membrane,

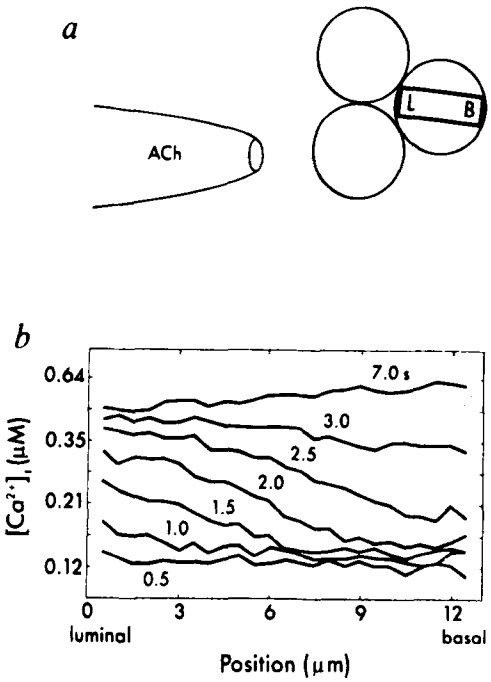

Fig. 3. Luminal initiation of Ca^{2+} release. (*a*) Experimental arrangement. A pipette containing 10 μM ACh was positioned next to a trio of pancreatic acinar cells. Luminal (*L*) versus basolateral (*B*) poles of each cell were distinguished on the basis of the presence of secretory granules near the regions of contact. (*b*) Ca^{2+} profiles along the *L-B* axis (box in *a*) at various times after the onset of ACh application. For Ca^{2+} measurement, cells were loaded with fura-2, fluorescence images were obtained using 390 nm excitation, and Ca^{2+} concentrations were calculated off-line using a reference image taken at 360 nm excitation before ACh application. Reprinted by permission from *Nature* Vol. 348, pp. 735–738. Copyright © 1991 Macmillan Magazines Ltd.

where ACh receptors are located (left panel), and diffuses to the luminal pole of the cell, where it liberates Ca^{2+} ions (middle panel). The Ca^{2+} signal is then propagated to the entire cell by way of Ca^{2+}-induced-Ca^{2+} release (right panel). This model, however, raises a paradox. $InsP_3$ has to diffuse from the basolateral to the luminal pole of the cell before it elicits any Ca^{2+} release. Yet the basolateral aspect of exocrine acinar cells is extremely rich in endoplasmic reticulum, so that it is surprising that no release should be evoked there if the endoplasmic reticulum is the source of $InsP_3$-induced Ca^{2+} release. One possible interpretation is presented in the next section.

$InsP_3$-Sensitive Ca^{2+} Stores May Be Associated to Exocytotic Vesicles in Certain Preparations

In cerebellar Purkinje cells, immunochemical techniques have demonstrated a rather wide distribution of $InsP_3$ receptors in the entire cell (Mignery et al., 1989; Satoh et al., 1990). Immunogold labelling indicates that the receptors are associated with the smooth endoplasmic reticulum, particularly with stacks of parallel cisternae (Mignery et al., 1989; Satoh et al., 1990). Unfortunately, similar results are not available in exocrine glands. It will be interesting to know whether $InsP_3$ receptors are in fact localized in the luminal pole of the cell and also to what subcellular membranes the receptors are associated. Recent evidence indicates that $InsP_3$-sensitive pools of retinal photoreceptors and bipolar cells of vertebrates are specifically localized in presynaptic terminals (Peng et al., 1991). These results suggest that in some cells, $InsP_3$ receptors are associated with unknown structures of presynaptic sites, which may be distinct from the endoplasmic reticulum. In fact, a recent report indicates that chromaffin granules respond better to $InsP_3$ than endoplasmic reticulum-derived vesicles (Yoo & Albanesi, 1990). Likewise, the sensitivity to $InsP_3$ of microsomal fractions from brain synaptosomes is closely associated to synaptic vesicles (Finch et al., 1991). These findings suggest that, in certain preparations, $InsP_3$-sensitive stores might be located on secretory vesicles in addition to, or in place of, the endoplasmic reticulum.

$InsP_3$-Sensitive Stores in Exocrine Glands

The hypothesis of a selective location of $InsP_3$ receptors on secretory granules would be readily compatible with the evidence illustrated in Fig. 3 suggesting that $InsP_3$-sensitive Ca^{2+} stores are located on the luminal end of the acinar cells, because this region of the cell is packed with secretory granules. These granules are a major Ca^{2+} storing site in secreting cells, but there is no known function for their Ca^{2+} storage capability. Subfractionation experiments from vesicular membranes of rat pancreatic acinar cells have been used to suggest that the $InsP_3$-sensitive store is a fraction of the endoplasmic reticulum (Streb et al., 1984). However, the evidence is only indicative since (1) fractions enriched in endoplasmic reticulum may have contained a contamination of lysed and resealed granules and (2) heavy fractions containing intact granules were in fact able to release Ca^{2+} upon application of $InsP_3$. Thus the proposal of a granule localization of $InsP_3$-sensitive stores cannot be excluded on the basis of the subfractionation experiments. More recently, several investigations have been directed at characterizing the mechanism of Ca^{2+} uptake of $InsP_3$-sensitive pools. These pools are not associated with a vanadate-sensitive Ca^{2+} ATPase but with a Ca^{2+} uptake system consisting of a Mg^{2+}, ATP-driven H^+ uptake and of a Ca^{2+}-H^+ exchanger (Thévenod & Schulz,

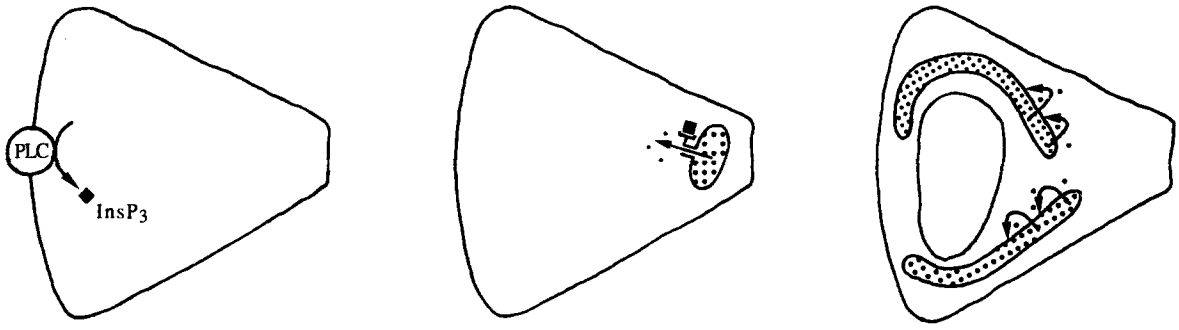

Fig. 4. Ca^{2+} signaling and cell polarity. $InsP_3$ (square) is produced near the basolateral membrane as a result of the activation of phospholipase C (left). $InsP_3$ then diffuses through the entire cell and binds to specific receptors linked to luminal Ca^{2+} stores. As a result of $InsP_3$-receptor binding, Ca^{2+} ions are liberated (middle panel). The ensuing cytoplasmic Ca^{2+} rise initiates Ca^{2+}-induced Ca^{2+} release in the endoplasmic reticulum (right panel), and the Ca^{2+} signal propagates back to the basolateral pole of the cell.

1988; Thévenod et al., 1989; Dehlinger-Kremer, Zeuzem & Schulz, 1991). This evidence is not easily compatible with the generally accepted view that the $InsP_3$ receptors are located primarily on the endoplasmic reticulum, since the Ca^{2+} uptake system of the endoplasmic reticulum is vanadate sensitive and pH insensitive. It remains to be seen whether the uptake characteristics of $InsP_3$-sensitive stores are compatible with an identification with secretory granules. In summary, such an identification appears presently as an attractive hypothesis, which, however, remains to be tested. This hypothesis would explain on a very simple basis the appearance of a Ca^{2+} conductance in the plasma membrane during the second phase of sustained stimulation, as explained below.

There Is No Accepted Mechanism for Secondary Ca^{2+} Entry

Even though the first phase of the response to secretion-inducing agents is independent of the external Ca^{2+} concentration, this is followed by a second phase where Ca^{2+} entry also participates in the response (reviewed by Putney, 1986). The second phase may be detected by measuring Ca^{2+}-dependent currents, which include a component depending on external Ca^{2+} after prolonged acetylcholine applications (Morris et al., 1987), but not during the first minute of exposure to the agonist (Marty et al., 1986). A similar dependency on external Ca^{2+} develops after several minutes of $InsP_3$ injection (Llano, Marty & Tanguy, 1987). These experiments demonstrate that neither receptor occupancy nor the activation of the intermediate G protein are responsible for the triggering of the Ca^{2+} conductance. In spite of intensive investigations, the nature of the pathway controlling this conductance remains to a large extent obscure. Particularly, the reason for the delayed action of ACh or of $InsP_3$ on the Ca^{2+} permeability of the plasma membrane is still unknown.

Putney (1986) pointed out that the Ca^{2+} conductance seemed to be activated in order to maintain a high internal Ca_i after depletion of internal stores during prolonged stimulation and therefore proposed that the depletion of $InsP_3$-sensitive stores is the signal for the opening of this conductance. The difficulty with this hypothesis is to delineate a specific mechanism which would signal the depletion of the Ca^{2+} store and would transmit the information to the plasma membrane. Irvine (1990) suggested that the depletion of the Ca^{2+} store modifies the properties of the $InsP_3$ receptor and that this modification alters the probability of the $InsP_3$ receptor to interact with a specific protein on the plasma membrane. This protein-protein interaction would be analogous to the coupling between the ryanodine receptor of the sarcoplasmic reticulum and the voltage sensor of the tubular system. Increasing the cytoplasmic $InsP_3$ concentration and/or decreasing the intraluminal Ca^{2+} concentration would lead to a dissociation of the receptors on the reticular and cellular membranes and to the activation of Ca^{2+} release and Ca^{2+} influx. These suggestions, interesting as they are, have now to be submitted to experimental tests. A second hypothesis, which is not exclusive of the first, is that the signal for the Ca^{2+} conductance is provided by a concomitant rise of the $InsP_3$ concentration and of its phosphorylated derivative, $InsP_4$ (Irvine & Moor, 1986; Irvine, 1990). Experiments in sea urchin eggs (Irvine & Moor, 1986) and in mouse lacrimal glands (Morris et al. 1987; Changya et al.,

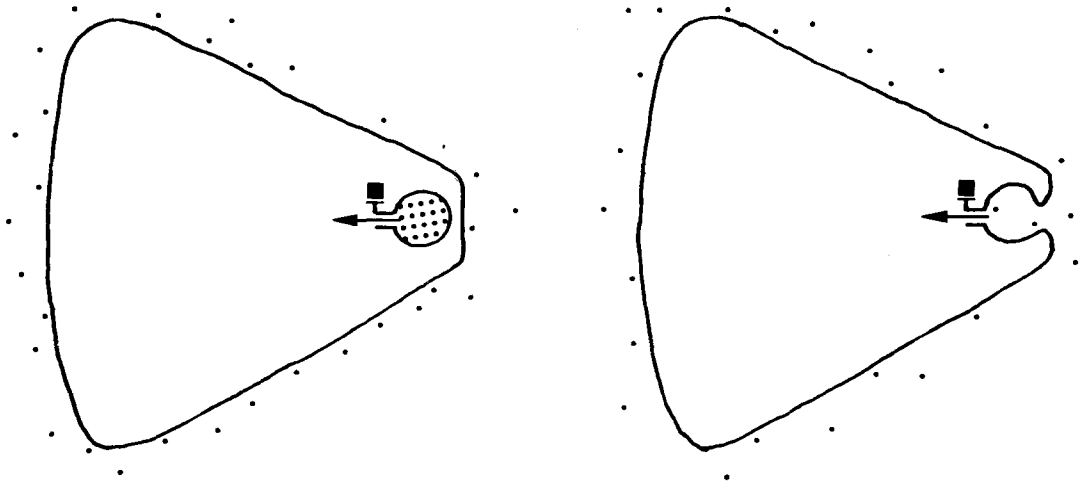

Fig. 5. A possible mechanism for secondary calcium entry. This mechanism assumes that $InsP_3$ (squares) binds to a receptor located on secretory granules. Ca^{2+} ions (dots) are liberated upon binding to the receptor (left panel). Once exocytosis has taken place, the same $InsP_3$-sensitive channels may be used to transfer Ca^{2+} from the extracellular solution (right panel).

1989) indicated that Ca^{2+} entry could be elicited by a combination of the two inositol nucleotides, while each of them taken separately was ineffective. However, later work indicated that $InsP_4$ was not consistently related to Ca^{2+} entry in sea urchin eggs (Crossley et al., 1988; Irvine et al., 1988). Our own (*unpublished*) experiments on rat lacrimal glands failed to reveal a specific role of $InsP_4$-$InsP_3$ combination in eliciting Ca^{2+} entry. In rat pancreatic acinar cells, Maruyama (1989) found a potentiation by $InsP_4$ of the $InsP_3$-induced current, but this potentiation was only obtained in cells where the sensitivity to $InsP_3$ was reduced as a result of being left for several hours at room temperature. Finally, experiments in rat peritoneal mast cells (Matthews, Neher & Penner, 1989) failed to correlate Ca^{2+} entry with the $InsP_3$-$InsP_4$ combination. Thus support in favor of the $InsP_4$ hypothesis has so far been obtained only in a restricted number of preparations and/or under rather special experimental conditions, and the question of the mechanism of control of Ca^{2+} entry should still be considered open.

A New Model for Secondary Ca Entry Based on an Association of $InsP_3$ Receptors to Secretory Granules

A new and particularly simple model for secondary Ca entry is illustrated in Fig. 5. If $InsP_3$-sensitive stores are, in fact, localized on the secretory granules, then their activation by internal $InsP_3$ should lead to Ca^{2+} entry once the granules have fused with the plasma membrane. Thus the delay of the secondary Ca^{2+} entry may be explained by the time necessary to undergo exocytosis and may not require any other internal messenger than $InsP_3$. Clearly the model of Fig. 5 will need experimental tests and should presently be considered as speculative.

What is the Permeation Mechanism Associated with Secondary Ca Entry?

The exact nature of the Ca^{2+} entry pathway has remained somewhat elusive. This conductance is clearly different from those of excitable tissue. In nonexcitable cells, Ca^{2+} entry is weakly enhanced by hyperpolarization (Llano et al., 1987; Matthews et al., 1989), whereas the Ca^{2+} conductance systems of excitable cells are steeply activated by depolarization. In mast cells, the whole-cell current underlying Ca^{2+} entry is only of 1–2 pA or less, and it was not possible to associate specific single-channel currents with this mean current (Matthews et al., 1989). In a T-lymphocyte-derived cell line, Lewis and Cahalan (1989) clearly demonstrated the activation of a cell current correlated with the secondary Ca^{2+} entry triggered by phytohemagglutinin, a Ca^{2+} mobilizing agent. This current was roughly linearly related to the membrane potential and had an amplitude on the order of 10 pA near the cell resting potential. Again, no current fluctuations were observed, suggesting that the underlying permeation mechanism employed either low unit conductance channels or carriers. Evidence was obtained indicating that the conductance had a reversal potential positive to +20 mV when using normal external saline and a Cs-rich

internal solution, indicating a large selectivity for Ca^{2+} over monovalent cations.

Conclusion

Recent results call for a reinterpretation of the mechanisms underlying the recruitment of intracellular Ca^{2+} in exocrine glands. One new hypothesis suggested by these developments is that $InsP_3$-sensitive channels liberate Ca^{2+} ions from secretory vesicles, as illustrated in Fig. 5.

I would like to thank Drs. E. Neher and A. Trautmann for useful discussions during the preparation of this manuscript.

References

Berridge, M.J. 1990. Calcium oscillations. *J. Biol. Chem.* **265:**9583–9586

Berridge, M.J., Irvine, R.F. 1989. Inositol phosphates and cell signalling. *Nature* **341:**197–204

Bezprozvanny, I., Watras, J., Ehrlich, B.E. 1991. Bell-shaped calcium responses of inositol 1,4,5-trisphosphate-gated and calcium-gated channels from endoplasmic reticulum of cerebellum. *Nature* **351:**751–754

Changya, L., Gallacher, D.V., Irvine, R.F., Potter, B.V.L., Petersen, O.H. 1989. Inositol 1,3,4,5-tetrakisphosphate is essential for sustained activation of the Ca^{2+}-dependent K^+ current in single internally perfused mouse lacrimal acinar cells. *J. Membrane Biol.* **109:**85–93

Cook, D.I., Gard, G.B., Champion, M., Young, J.A. 1988. Patch-clamp studies of the electrolyte secretory mechanism of rat mandibular gland cells stimulated with acetylcholine or isoproterenol. *In*: Molecular Mechanisms in Secretion. Alfred Benzon Symposium 25, p. 133–145. N.A. Thorn, M. Treiman, and O.H. Petersen, editors. Munksgaard, Copenhagen

Crossley, I., Swann, K., Chamben, E., Whitaker, M. 1988. Activation of sea urchin eggs is independent of external calcium ions. *Biochem. J.* **252:**257–262

Dehlinger-Kremer, M., Zeuzem, S., Schulz, I. 1991. Interaction of caffeine, IP_3- and vanadate-sensitive Ca^{2+} pools in acinar cells of the exocrine pancreas. *J. Membrane Biol.* **119:**85–100

Dissing, S., Nauntofte, B., Sten-Knudsen, O. 1990. Spatial distribution of intracellular, free Ca^{2+} in isolated rat parotid acini. *Pfluegers Arch.* **417:**1–12

Ehrlich, B.E., Watras, J. 1988. Inositol 1,4,5-trisphosphate activates a channel from smooth muscle sarcoplasmic reticulum. *Nature* **336:**583–586

Evans, M.G., Marty, A. 1986. Potentiation of muscarinic and alpha-adrenergic responses by an analogue of guanosine triphosphate. *Proc. Natl. Acad. Sci. USA* **83:**4099–4103

Finch, E.A., Turner, T.J., Goldin, S.M. 1991. Calcium as a coagonist of inositol 1,4,5-trisphosphate-induced calcium release. *Science* **252:**443–446

Foskett, J.K., Gunter-Smith, P.J., Melvin, J.E., Turner, R.J. 1989. Physiological localisation of an agonist-sensitive pool of Ca^{2+} in parotid acinar cells. *Proc. Natl. Acad. Sci. USA* **86:**167–171

Gray, P.T.A. 1988. Oscillations of free cytosolic calcium evoked by cholinergic and catecholaminergic agonists in rat parotid acinar cells. *J. Physiol.* **406:**35–53

Horn, R., Marty, A. 1989. Muscarinic activation of ionic currents measured by a new whole-cell recording method. *J. Gen. Physiol.* **92:**145–159

Irvine, R.F. 1990. 'Quantal' release and the control of Ca^{2+} entry by inositol phosphates—a possible mechanism. *FEBS Lett.* **263:**5–9

Irvine, R.F., Moor, R.M. 1986. Micro-injection of inositol 1,3,4,5-tetrakisphosphate activates sea urchin eggs by a mechanism dependent on external Ca^{2+}. *Biochem. J.* **240:**917–920

Irvine, R.F., Moor, R.M., Pollock, W.K., Smith, P.M., Wreggett, K.A. 1988. Inositol phosphates: Proliferation, metabolism and function. *Phil. Trans. R. Soc. London B* **320:**281–298

Kasai, H., Augustine, G.J. 1990. Cytosolic Ca^{2+} gradients triggering unidirectional fluid secretion from exocrine pancreas. *Nature* **348:**735–738

Lewis, R.S., Cahalan, M.D. 1989. Mitogen-induced oscillations of cytosolic Ca^{2+} and transmembrane Ca^{2+} current in human leukemic T cell. *Cell Regul.* **1:**99–112

Llano, I., Marty, A. 1987. Protein kinase C activators inhibit the inositol-trisphosphate-mediated muscarinic current responses in rat lacrimal cells. *J. Physiol.* **394:**239–248

Llano, I., Marty, A., Tanguy, J. 1987. Dependence of intracellular effects of GTPγS and inositoltrisphosphate on cell membrane potential and on external Ca ions. *Pfluegers Arch.* **409:**499–506

Marty, A., Evans, M.G., Tan, Y.P., Trautmann, A. 1986. Muscarinic response in rat lacrimal glands. *J. Exp. Biol.* **124:**15–32

Marty, A., Horn, R., Tan, Y.P., Zimmerberg, J. 1989. The delay of the Ca mobilization response to muscarinic stimulation. *In:* Secretion and Its Control. 42nd Annual Symposium of the Society of General Physiologists. C.M. Armstrong, and G.S. Oxford, editors. pp. 97–110. Rockefeller University Press, New York

Marty, A., Tan, Y.P. 1989. The initiation of calcium release following muscarinic stimulation in rat lacrimal glands. *J. Physiol.* **419:**665–687

Maruyama, Y. 1989. Activation and desensitization mechanisms of muscarinic current response in single pancreatic acinar cells of rats. *J. Physiol.* **417:**343–359

Maruyama, Y. 1990. Inhibitory effects of arachidonic acid on muscarinic current response in single pancreatic acinar cells of rats. *J. Physiol.* **430:**471–482

Matthews, G., Neher, E., Penner, R. 1989. Second messenger-activated calcium influx in rat peritoneal mast cells. *J. Physiol.* **418:**105–130

Merritt, J.E., Rink, T.J. 1987. Rapid increases in cytosolic free calcium in response to muscarinic stimulation of rat parotid acinar cells. *J. Biol. Chem.* **262:**4958–4960

Meyer, T., Holowka, D., Stryer, L. 1988. Highly cooperative opening of calcium channels by inositol 1,4,5 trisphosphate. *Science* **240:**653–656

Mignery, G.A., Südhof, T.C., Takei, K., De Camilli, P. 1989. Putative receptor for inositol 1,4,5-trisphosphate similar to ryanodine receptor. *Nature* **342:**192–195

Miyazaki, S. 1988. Inositol 1,4,5 trisphosphate-induced calcium release and guanine nucleotide-binding protein-mediated periodic calcium rises in golden hamster eggs. *J. Cell Biol.* **106:**345–353

Morris, A.P., Gallacher, D.V., Irvine, R.F., Petersen, O.H. 1987. Synergism of inositol trisphosphate and tetrakisphosphate in activating Ca^{2+}-dependent K^+ channels. *Nature* **330:**653–655

Neher, E. 1988. The influence of intracellular calcium concentra-

tion on degranulation of dialysed mast cells from rat peritoneum. *J. Physiol.* **395:**193–214

Ogden, D.C., Capiod, T., Walker, J.W., Trentham, D.R. 1990. Kinetics of the conductance evoked by noradrenaline, inositol trisphosphate or Ca^{2+} in guinea-pig isolated hepatocytes. *J. Physiol.* **422:**585–602

Osipchuk, Y.V., Wakui, M., Yule, D.I., Gallacher, D.V., Petersen, O.H. 1990. Cytoplasmic Ca^{2+} oscillations evoked by receptor stimulation, G-protein activation, internal application of inositoltrisphosphate or Ca^{2+}: Simultaneous microfluorimetry and Ca^{2+} dependent Cl^- current recording in single pancreatic acinar cells. *EMBO J.* **9:**697–704

Parker, I., Ivorra, I. 1990. Localized all or none calcium liberation by inositol trisphosphate. *Science* **250:**977–979

Parker, I., Miledi, R. 1989. Nonlinearity and facilitation in phosphoinositide signaling studied by the use of caged inositoltrisphosphate in *Xenopus* oocytes. *J. Neurosci.* **9:**4068–4077

Peng, Y.-W., Sharp, A.H., Snyder, S.H., Yau, K.-W. 1991. Localization of the inositol 1,4,5-trisphosphate receptor in synaptic terminals in the vertebrate retina. *Neuron* **6:**525–531

Petersen, O.H., Wakui, M. 1990. Oscillating intracellular Ca^{2+} signals evoked by activation of receptors linked to inositol lipid hydrolysis: Mechanism of generation. *J. Membrane Biol.* **118:**93–105

Putney, J.W., Jr. 1986. A model for receptor-regulated calcium entry. *Cell Calcium* **7:**1–12

Rana, R.S., Hokin, L.E. 1990. Role of phosphoinositides in transmembrane signaling. *Physiol. Rev.* **70:**115–163

Satoh, T., Ross, G.A., Villa, A., Supattapone, S., Pozzan, T., Snyder, S.H., Meldolesi, J. 1990. The inositol 1,4,5-trisphosphate receptor in cerebellar Purkinje cells. Quantitative immunogold labeling reveals concentration in an endoplasmic reticulum compartment. *J. Cell Biol.* **111:**615–624

Schmid, A., Dehlinger-Kremer, M., Shulz, I., Gögelein, H. 1990. Voltage-dependent $InsP_3$-insensitive calcium channels in membranes of pancreatic endoplasmic reticulum vesicles. *Nature* **346:**374–376

Streb, H., Bayerdörffer, E., Haase, W., Irvine, R.F., Schulz, I. 1984. Effect of inositol-1,4,5-trisphosphate on isolated subcellular fractions of rat pancreas. *J. Membrane Biol.* **81:**241–253

Sugiya, H., Obie, J.F., Putney, J.W., Jr. 1988. Two modes of regulation of the phospholipase C-linked substance-P receptor in rat parotid acinar cells. *Biochem. J.* **253:**459–466

Tan, Y.P., Marty, A. 1991. Protein kinase C-mediated desensitization of the muscarinic response in rat lacrimal gland cells. *J. Physiol.* **433:**357–371

Thévenod, F., Dehlinger-Kremer, M., Kemmer, T.P., Christian, A.-L., Potter, B.V.L., Schulz, I. 1989. Characterization of inositol 1,4,5-trisphosphate-sensitive (IsCaP) and -insensitive (IisCaP) nonmitochondrial Ca^{2+} pools in rat pancreatic acinar cells. *J. Membrane Biol.* **109:**173–186

Thévenod, F., Schulz, I. 1989. H^+-ion dependent calcium uptake into an inositol 1,4,5-trisphosphate sensitive calcium pool from rat parotid gland. *Am. J. Physiol.* **255:**G429–G440

Wakui, M., Osipchuk, Y.V., Petersen, O.H. 1990. Receptor-activated cytoplasmic Ca^{2+} spiking mediated by inositol trisphosphate is due to Ca^{2+}-induced Ca^{2+} release. *Cell* **63:**1025–1032

Wakui, M., Potter, B.V.L., Petersen, O.H. 1989. Pulsatile intracellular calcium release does not depend on fluctuations in inositoltrisphosphate concentration. *Nature* **339:**317–320

Yoo, S.H., Albanesi, J.P. 1990. Inositol 1,4,5-trisphosphate-triggered Ca^{2+} release from bovine adrenal medullary secretory vesicles. *J. Biol. Chem.* **265:**13446–13448

Received 8 May 1991; revised 11 July 1991