Mediators of Ca\(^{2+}\)-Dependent Secretion
by Archana Chaudhry* and Ronald P. Rubin*

Ca\(^{2+}\), an obligatory mediator of the secretory process, acts in concert with other second messengers that further amplify or inhibit the secretory response. In this overview, we will consider the relative roles of diacylglycerol (DAG), arachidonic acid, and cyclic AMP (cAMP) in modulating Ca\(^{2+}\)-dependent secretion in nonexcitable cells. DAG, a product of phospholipase C (PLC)-catalyzed breakdown of phosphoinositides, stimulates protein kinase C; Ca\(^{2+}\) ionophores and phorbol esters (or DAG analogues) elicit a synergistic secretory response in the exocrine pancreas and parotid gland. These findings suggest that the complete activation of secretion requires stimulation of both Ca\(^{2+}\)-dependent and protein kinase C-dependent pathways. Hydrolysis of phospholipids can also lead to the liberation of arachidonic acid in secretory cells. Endogenously generated arachidonic acid inhibits polyphosphoinositide synthesis in exocrine pancreas, leading to inhibition of agonist-induced IP\(_3\) formation, Ca\(^{2+}\)-mobilization and amylase secretion. By contrast, arachidonic acid and its metabolites stimulate PLC in the rabbit peritoneal neutrophil, causing Ca\(^{2+}\)-mobilization and lysosomal enzyme secretion. Arachidonic acid can thus serve as a positive or negative feedback regulator of secretion induced by Ca\(^{2+}\)-mobilizing agonists. Finally, in the parotid gland, stimulation of amylase secretion by norepinephrine, the physiological mediator, which stimulates both the \(\alpha\) and \(\beta\) adrenoceptors, requires the interaction of both Ca\(^{2+}\) and cAMP pathways to produce a full secretory response. These studies, taken together, indicate that phosphoinositide and cAMP-dependent pathways play coordinate roles in signal transduction, leading to the Ca\(^{2+}\)-mediated secretion.

The role of calcium (Ca\(^{2+}\)) in stimulus-secretion coupling has been unequivocally established. In electrically excitable cells such as the neuron, adrenal medullary chromaffin cell, the \(\beta\) cell of the endocrine pancreas, and cells of the adeno- and neurohypophysis, the rise in cellular Ca\(^{2+}\) following stimulation is derived to a large extent from influx of cation through voltage-sensitive channels (1). In nonexcitable secretory cells, such as those of exocrine glands and neutrophils, cellular stores of Ca\(^{2+}\) play a more predominant role in regulating secretion, although Ca\(^{2+}\) influx through receptor-operated channels also increases cellular Ca\(^{2+}\) availability (Fig. 1). The initial secretory response seems dependent on Ca\(^{2+}\) released from intracellular stores, but prolonged secretion requires the presence of extracellular Ca\(^{2+}\) (2).

The concept that increases in intracellular ionic Ca\(^{2+}\) stimulate secretion in nonexcitable cells is supported by the following pieces of evidence: a) secretagogues evoke increases in cytosolic Ca\(^{2+}\) (3,4) and cause a rapid efflux of \(^{45}\)Ca from cells (2); b) Ca\(^{2+}\) ionophores which bypass receptors to raise cytoplasmic Ca\(^{2+}\) stimulate enzyme secretion (4); c) depletion of cellular Ca\(^{2+}\) inhibits secretion (2); and d) amylase secretion is stimulated when increasing concentrations of buffered Ca\(^{2+}\) are introduced into the cytosol of electropermeabilized cells (5).

Ca\(^{2+}\)-mobilizing agonists stimulate phospholipase C (PLC), which catalyzes the phosphodiesteratic cleavage of phosphatidylinositol 4,5-bisphosphate (PIP\(_2\)) (Fig. 1). This leads to the formation of 1,4,5-inositol trisphosphate (IP\(_3\)) and diacylglycerol (DAG), both of which have important second messenger roles. 1,4,5-IP\(_3\) releases cellular Ca\(^{2+}\) by interacting with a specific receptor site on the endoplasmic reticulum (6), and DAG activates protein kinase C, a key regulatory enzyme (7). Arachidonic acid is also liberated from phosphoinositides during stimulation by secretagogues, and free arachidonic acid and/or its metabolites may also serve as cellular messengers to modulate the secretory response (8). Also, some secretory cells possess a signaling system that uses cyclic AMP (cAMP) as a second messenger. In such systems Ca\(^{2+}\) and cAMP may act either sequentially or in concert to regulate secretion. Simultaneous changes in the intracellular concentrations of cytosolic Ca\(^{2+}\) and cAMP have been reported after stimulation of secretory cells by a variety of secretagogues (9,10). However, in contrast to Ca\(^{2+}\), cAMP has not been characterized as a direct mediator of exocytosis.

In this brief overview, we will consider the concept that the second messengers DAG, arachidonic acid,
and cAMP interact with Ca\textsuperscript{2+} to modulate the secretory response (Fig. 1). We will employ the parotid and pancreatic acinar cells, as well as the rabbit neutrophil, to offer evidence to support this thesis.

A large body of evidence suggests that in many tissues optimal secretion requires both Ca\textsuperscript{2+} and DAG (Fig. 1). Nishizuka and his colleagues (7) first demonstrated that DAG activates a phospholipid-dependent kinase (protein kinase C) by increasing the affinity of the kinase for Ca\textsuperscript{2+}. Thus, in the presence of DAG, protein kinase C can be maximally stimulated at submicromolar concentrations of Ca\textsuperscript{2+}. The interactions between Ca\textsuperscript{2+} and protein kinase C in cellular secretion have been probed by using calcium ionophores (which bypass receptors to raise cytoplasmic Ca\textsuperscript{2+}) and phorbol esters (which substitute for DAG) to activate the Ca\textsuperscript{2+}-dependent and protein kinase C-dependent pathways separately. In isolated pancreatic acini, phorbol 12,13-dibutyrate (PDBu) when added together with a threshold concentration of the Ca\textsuperscript{2+} ionophore, ionomycin, causes a synergistic potentiation of amylase secretion, with no further elevation in cytoplasmic Ca\textsuperscript{2+} than the one elicited by ionomycin alone (Fig. 2). Diacylglycerols containing unsaturated fatty acids also stimulate amylase secretion and exhibit synergistic effects on secretion in combination with ionomycin (4). These results suggest that complete activation of amylase secretion by the pancreas requires stimulation of both Ca\textsuperscript{2+}-dependent and protein kinase C-dependent pathways. Similar synergistic effects of ionophores and phorbol esters have been reported in other model secretory systems (11, 12).

Apart from interacting with the protein kinase C pathway, Ca\textsuperscript{2+} may interact with the arachidonic acid messenger system to modulate secretion (18). Mammalian phospholipids are enriched in arachidonic acid, and Ca\textsuperscript{2+}-mobilizing agonists liberate free arachidonic acid either through activation of phospholipase A\textsubscript{2}, the sequential activation of PLC and DAG lipase, or phosphatidate-specific phospholipase A\textsubscript{2} (18). For example, the mukaric agonist carbachol elevates free arachidonate levels in pancreatic acinar cells (14). The time course of this event parallels that of other cellular responses to carbachol, including IP\textsubscript{3} accumulation and amylase

![Figure 1. A hypothetical model depicting the various second messengers that can regulate cellular secretion. Occupation of receptors (R) by calcium-mobilizing agonists (Ag\textsubscript{Ca}) activates phospholipase C (PLC) through a G protein (G\textsubscript{i}, G\textsubscript{q}). PLC-mediated breakdown of phosphatidylinositol 4,5-bisphosphate (PIP\textsubscript{2}) leads to the formation of inositol trisphosphate (IP\textsubscript{3}) and diacylglycerol (DAG), both of which act as second messengers—IP\textsubscript{3} by mobilizing intracellular Ca\textsuperscript{2+} and DAG by activating protein kinase C. Ca\textsuperscript{2+} and protein kinase C act synergistically to promote a full secretory response. The increase in intracellular Ca\textsuperscript{2+} levels brought about by 1,4,5-IP\textsubscript{3}, or via influx through receptor-operated calcium channels, can stimulate phospholipase A\textsubscript{2} and lead to arachidonic acid release (AA). Arachidonic acid and its metabolites can either amplify or inhibit agonist-induced rises in cytosolic Ca\textsuperscript{2+} thus modulating secretion. Agonists that cause secretion by modulating cyclic AMP (A\textsubscript{GAMP}) express their actions via stimulatory and inhibitory G proteins (G\textsubscript{i}, and G\textsubscript{q}) to activate and inhibit adenylyl cyclase, respectively. Cyclic AMP can act in concert with Ca\textsuperscript{2+} to promote secretion, perhaps by enhancing Ca\textsuperscript{2+} availability.](image1)

![Figure 2. Potentiation by phorbol dibutyrate of ionomycin-induced amylase secretion without a further rise in [Ca\textsuperscript{2+}]. Rat pancreatic acini were incubated in the presence of either phorbol dibutyrate (PDBu) (1 \textmu M), or ionomycin (0.3 \textmu M), or PDBu plus ionomycin (P+1) [Ca\textsuperscript{2+}], (Quin-2 fluorescence) and amylase secretion were determined after 10 and 30 min, respectively. Double asterisks (***) indicate significantly different from control value (p < 0.05). Modified from Merritt and Rubin (4).](image2)
secretion \((15,16)\). Additionally, exogenous arachidonic acid causes a concentration-dependent reduction in the steady-state levels of \[^{32P}\text{PIP}_2\] (14). The decrease in the \[^{32P}\text{PIP}_2\] pool is not due to PLC-stimulated \[^{32P}\text{PIP}_2\] hydrolysis, since arachidonic acid alone does not promote the accumulation of \[^{32P}\text{IP}_3\]. In fact, pretreatment of \[^3H\]myo-inositol labeled cells with arachidonic acid abolishes subsequent \[^{32P}\text{IP}_3\] accumulation in response to carbachol (Fig. 3), and arachidonic acid blocks the enhanced incorporation of \[^3H\]myo-inositol into phospholipids elicited by carbachol (14). These findings indicate that in exocrine pancreas, arachidonic acid inhibits the synthesis of the polyphosphoinositide pool used by Ca\(^{2+}\)-mobilizing agonists.

To confirm the inhibitory role of endogenously released arachidonic acid on phosphoinositide turnover and, consequently, on agonist-mediated Ca\(^{2+}\) mobilization as well as amylase secretion, we utilized tetrahydrocannabinol (THC) to increase endogenous levels of unesterified arachidonic acid. THC is an inhibitor of acyl-CoA transferase and stimulates arachidonic acid release from cells by activating phospholipase \(A_2\) \((17-19)\). In acinar cells prelabeled with both \[^3H\]arachidonic acid and \[^{32P}\text{IP}_3\], THC causes a significant increase in levels of free \[^3H\]arachidonic acid that correlates with a corresponding decrease in the steady-state levels of \[^{32P}\text{PIP}_2\] (Fig. 4). The effects of THC on arachidonate release and \[^{32P}\text{IP}_3\] levels are dose-related over the concentration range of 1 to 20 \(\mu\)M (unpublished observations). Pretreatment with THC causes a dose-related inhibition of \[^3H\]IP3 accumulation (unpublished observations), as well as cytosolic Ca\(^{2+}\) and amylase secretion (Table 1) elicited by cerulein in the exocrine pancreas. These results indicate that endogenously generated arachidonic acid and/or its metabolites can serve as a negative feedback regulator of phosphoinositide turnover and, thus, inhibit agonist-induced rises in cytosolic \([\text{Ca}^{2+}]\) and amylase secretion.

By contrast, in the rabbit neutrophil, the lipoygenase metabolite leukotriene \(B_4\) (LTB\(_4\)) stimulates \[^{32P}\text{IP}_3\] accumulation, Ca\(^{2+}\) mobilization, and enzyme degranulation \((20,21)\). Thus, in the neutrophil, arachidonate metabolites may amplify the agonist-
induced breakdown of polyphosphoinositides by being exported from the cell and, subsequently, acting as receptor agonists on neighboring cells to stimulate PLC and mobilize cellular Ca\textsuperscript{2+}. A positive feedback mechanism also appears operative in the blood platelet (22) and rat corpus luteum (23). Thus it appears that agonist-induced liberation of arachidonic acid can either amplify Ca\textsuperscript{2+}-induced secretion or inhibit it, depending upon the secretory system under scrutiny.

Recent evidence suggests that Ca\textsuperscript{2+} can also interact with the cAMP-dependent pathway to enhance the secretory responses of secretagogues. Cyclic AMP generated through beta adrenoceptor action appears to be a critical modulator of amylase secretion by the rat parotid gland. By contrast, Ca\textsuperscript{2+}-mobilizing agonists which express their actions through muscarinic and alpha-adrenergic receptors cause a predominance of water and electrolyte release (24). Norepinephrine (NE), the physiological neurotransmitter for salivary amylase secretion, which stimulates both beta and alpha adrenoceptors, requires participation of both the cAMP and Ca\textsuperscript{2+} pathways to produce a full secretory response. Figure 5 shows that amylase secretion induced by NE is greater than the sum of the release

| [Ca\textsuperscript{2+}], nM | Amylase release, % total tissue content |
|-----------------------------|-------------------------------------|
| Cerulein, 0.1 \mu M          | 294 ± 54                             |
| Cerulein + THC, 5 \mu M     | 158 ± 30*                            |

*Fura-2 loaded acinar cells were exposed to cerulein in the presence and absence of THC and [Ca\textsuperscript{2+}], was calculated as previously described (25). Amylase secretion was determined in acini incubated for 30 min with cerulein in the presence and absence of THC. Basal values for [Ca\textsuperscript{2+}] and amylase secretion have been subtracted from the data that is shown here as mean ± SE (n = 3-5).

\*Significantly different from samples treated with cerulein alone (p < 0.05).

obtained when NE is used as an alpha adrenoceptor agonist (in the presence of propranolol) and a beta adrenoceptor agonist (in the presence of prazosin).

Further support for an interaction between the Ca\textsuperscript{2+} and cAMP pathways comes from our observation that a sub threshold concentration of carbachol causes a significant enhancement of isoproterenol-induced secretion (Fig. 6). The site of interaction between the two transduction systems is distal to the catalytic site of adenylate cyclase because carbachol failed to elevate isoproterenol-stimulated cAMP levels (unpublished observations). One possible explanation for the above findings is that there is an enhancement of Ca\textsuperscript{2+} availability produced by the coordinate interactions of the two pathways. Parotid acinar cells exposed to

Figure 5. Effect of prazosin or propranolol on norepinephrine (NE)-induced salivary amylase release. Rat parotid acinar cells were exposed for 15 min to 500 nM NE in the presence and absence of either 100 nM prazosin or 1 \mu M propranolol. Values are means ± SE with basal release subtracted (n = 3).


**REFERENCES**

1. Ozawa, S., and Sand, O. Electrophysiology of excitatory endocrine cells. Physiol. Rev. 66: 887-952 (1986).
2. Williams, J. A. Regulation of pancreatic acinar cell function by intracellular calcium. Am. J. Physiol. 238: G269-G279 (1980).
3. O'Doherty, J., and Stark, R. J. Stimulation of pancreatic cell secretion and increases in cytosolic calcium and sodium. Am. J. Physiol. 242: G513-G521 (1982).
4. Merritt, J. E., and Rubin, R. P. Pancreatic amylase secretion and cytoplasmic free calcium: effects of ionomycin, phorbol dibutyrate and diacylglycerol alone and in combination. Biochem. J. 230: 151-159 (1985).
5. Knight, D. E., and Koh, E. Ca++ and cyclic nucleotide dependence of amylase release from isolated rat pancreatic acinar cells rendered permeable by intense electric fields. Cell Calcium 5: 401-418 (1984).
6. Spät, A., Bradford, P. G., McKinney, J. S., Rubin, R. P., and Putney, J. W., Jr. A saturable receptor for $^{32}$P]inositol (1,4,5)triphosphate in guinea pig hepatocytes and rabbit neutrophils. Nature 319: 514-516 (1986).
7. Nishizuka, Y. Studies and perspectives of protein kinase C. Science 233: 305-312 (1986).
8. Laychock, S. G., and Putney, J. W., Jr. Roles of phospholipid metabolism in secretory cells. In: Cellular Regulation of Secretion and Release (P. M. Conn, Ed.), Academic Press, New York, 1982, pp. 53-105.
9. Rubin, R. P. Calcium and Cellular Secretion. Plenum Press, New York, 1982.
10. Rasmussen, H., and Barrett, P. Q. Calcium messenger system: an integrated view. Physiol. Rev. 64: 938-984 (1984).
11. Putney, J. W., Jr., McKinney, J. S., Aub, D. L., and Leslie, B. A. Phorbol ester-induced protein secretion in rat parotid gland. Relationship to the role of inositol lipid breakdown and protein kinase C activation in stimulus-secretion coupling. Mol. Pharmacol. 26: 261-266 (1984).
12. Knight, D. E. Calcium and exocytosis. In: Calcium and the Cell. John Wiley and Sons, Sussex, England, 1986, pp. 250-270.
13. Rubin, R. P. Inositol lipids and cell secretion. In: Phosphoinositides and Receptor Mechanisms (J. W. Putney, Jr., Ed.), Alan R. Liss, Inc., New York, 1986, pp. 149-162.
14. Chaudhry, A., Laychock, S. G., and Rubin, R. P. The effects of fatty acids on phosphoinositol synthesis and myo-inositol accumulation in exocrine pancreas. J. Biol. Chem. 262: 17426-17431 (1987).
15. Halenda, S. P., and Rubin, R. P. Phospholipid turnover in isolated rat pancreatic acini. Consideration of the relative roles of phospholipase A$_2$ and phospholipase C. Biochem. J. 208: 713-721 (1982).
16. Rubin, R. P., Godfrey, P. P., Chapman, D. A., and Putney, J. W., Jr. Secretagogue induced formation of inositol phosphates in rat exocrine pancreas. Implications for a messenger role for inositol phosphates. Biochem. J. 212: 655-659 (1984).
17. Greenberg J. H., and Mellors, A. Specific inhibition of an acyltransferase by $\Delta^4$-tetrahydrocannabinol. Biochem. Pharmacol. 27: 329-333 (1978).
18. Laychock, S. G., Hoffman, J. M., Meisel, E., Bilgin, S. Pancreatic islet arachidonic acid turnover and metabolism and insulin release in response to delta-9-tetrahydrocannabinol. Biochem. Pharmacol. 35: 2003-2008 (1986).
19. Burstein, S., and Hunter, S. A., Prostaglandins and cannabinoids—VIII. Elevation of phospholipase A$_2$ activity by cannabinoids in whole cells and subcellular preparations. J. Clin. Pharmacol. 21: 2405-2408 (1981).
20. Bradford, P. G., and Rubin, R. P. Characterization of formylmethionyl-leucyl-phenylalanine stimulation of inositol trisphosphate accumulation in rabbit neutrophils. Mol. Pharmacol. 27: 74-78 (1985).
21. Bradford, P. G., and Rubin, R. P. Leukotriene B$_4$ stimulates phospholipase-C-mediated formation of inositol trisphosphate accumulation in neutrophils: implications for convergence of the phospholipase A$_2$ and C pathways. In: Advances in Prostaglandin, Thromboxane, and Leukotriene Research, Vol. 15 (O. Hayaishi and S. Yamamoto, Eds.), Raven Press, New York, 1985, pp. 105-108.
22. Siess, W., Siegel, F. L., and Lapetina, E. G. Arachidonic acid stimulates the formation of 1,2-diacylglycerol and phosphatidic acid in human platelets. J. Biol. Chem. 258: 11236-11242 (1983).
23. Leung, P. C. K., Minegishi, T., Ma, F., Zhou, F., and Ho-Yuen, B. Induction of polypehosphoinositide breakdown in rat corpus luteum by prostaglandin F$_{2\alpha}$. Endocrinology 119: 12-18 (1986).
24. Putney, J. W., Jr. Identification of cellular activation mechanisms associated with salivary secretion. Ann. Rev. Physiol. 48: 75-88 (1986).
25. Grynkiewicz, G., Poenie, M., and Tsien, R. Y. A new generation of Ca$^{2+}$ indicators with greatly improved fluorescence properties. J. Biol. Chem. 260: 3440-3450 (1985).