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J. W. Putney, J. Poggioli and S. J. Weiss

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Receptor regulation of calcium release and calcium permeability in parotid gland cells

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The mechanism by which hormones and neurotransmitters regulate fluid secretion in exocrine glands apparently involves the regulation of transmembrane movements of electrolytes, a process for which Ca serves as a second messenger. Analysis of the kinetics of efflux of $^{86}Rb^+$ (a marker for K^+) indicates that the initial phase of the response to secretagogues is mediated through the release of Ca from a cellular pool inaccessible to chelating agents. By investigating the movements of ^{45}Ca under nearly steady-state conditions, we find that this cellular pool can be filled from the extracellular space without a concomitant elevation in ionized intracellular Ca^{2+}. This suggests that the cellular pool is probably associated with the plasma membrane.

We have also investigated the possible role of phosphatidic acid in the mechanism by which receptors mobilize Ca^{2+}. Our results suggest that phosphatidic acid, formed on receptor activation, may directly mediate Ca influx into the acinar cell.

Introduction

The importance of Ca in the responses of exocrine glands to neurotransmitters and hormones has been appreciated for quite some time (Douglas & Poisner 1963; Hokin 1966; Petersen *et al.* 1967). The actions of Ca may be subdivided according to the nature of the response involved. Calcium is intimately involved in stimulus–secretion coupling: the stimulation of exocytosis of stored enzymes and other macromolecules. Calcium is also necessary for the generation of transepithelial water flow in the exocrine glands, believed to arise secondarily as a result of altered fluxes of osmotically important ions. The term stimulus–permeability coupling (Putney 1978, 1981) has been used to describe this role of Ca in coupling membrane receptor activation to alterations in monovalent ion permeability and transport.

There is also a general consensus that the role of Ca in these processes involves a receptor-mediated increase in the concentration of ionized Ca in the cytosol. It is the source of that Ca, however, which has been the source of some controversy and confusion. Thus, the apparent dependency of the K^+ release response of the parotid gland upon extracellular Ca (Selinger *et al.* 1973) suggests that receptor activation in the parotid gland primarily increases the permeability of the plasma membrane to Ca. On the other hand, the release of α-amylase from the exocrine pancreas can be quite efficiently activated by secretagogues in the complete absence of external Ca (Williams 1980). This is also true for the Ca-mediated increase in membrane conductance to Na^+ and Cl^- (Iwatsuki & Petersen 1977). The interpretation of these and other results is that the exocrine pancreas operates largely by releasing Ca from some cellular pool, bound or sequestered in some intracellular organelle (Williams 1980).

In recent years, careful kinetic analysis of these responses has shown that the differences among exocrine glands with regard to their mechanisms of Ca mobilization may be only

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quantitative. There is, for example, a brief initial phase of the K^+ release (measured as $^{86}Rb^+$ efflux) response in the parotid which is clearly independent of extracellular Ca (Putney 1976) and is apparently mediated by Ca released from some cellular pool (Putney 1977; Haddas *et al* 1979). Comparable results were obtained for the rat lacrimal gland (Putney *et al.* 1977; Parod & Putney 1978*a*, *b*, 1979). Similarly, for the exocrine pancreas, both the amylase secretion and membrane conductance responses are eventually lost in Ca-free media (Petersen 1978; Laugier & Petersen 1980). After the cellular pool is depleted, both responses are absolutely dependent on extracellular Ca (Petersen 1978; Laugier & Petersen 1980). Collectively, these results suggest that in the pancreas, salivary and lacrimal glands, receptor activation causes Ca mobilization by two (probably simultaneous) mechanisms: a release of Ca from some cellular pool inaccessible to chelating agents and the opening of surface membrane Ca channels or gates. In this report we summarize some recent experimental findings on the nature of these processes in the rat parotid salivary gland.

Calcium release

Much of our understanding of Ca release and Ca influx in the pancreas is derived from analysis of ^{45}Ca movements under nearly steady-state conditions (Schulz & Stolze 1980; Stolze & Schulz 1980). These techniques, described in more detail by Schulz *et al.* (this symposium), clearly demonstrate the two phases of Ca mobilization in the pancreas: net release followed by a sustained net influx. In the parotid, these same two phases are obtained as well, but in keeping with the known brevity of Ca-independent responses in the parotid, the net ^{45}Ca release is small and difficult to detect (figure 1*a*). The augmented efflux is easily seen if the rather large ^{45}Ca influx is prevented by addition of excess ethyleneglycol-bis-(aminoethylether)-*N*,*N*-tetraacetic acid (EGTA) (figure 1*b*). Also shown in figure 1*a* is the effect of removing the cholinergic stimulus with the muscarinic antagonist atropine. As was originally shown by Schulz & Stolze (1980), rather than an immediate return of the net influx to control level, there is first a rapid, transient net influx of ^{45}Ca. The net fluxes of ^{45}Ca calculated from the experiments in figure 1 are shown in figure 2. The net influx due to atropine is greater than the resting influx, the carbachol-activated influx, or even the apparent initial influx during the first 1 min of ^{45}Ca loading (about 0.16 nmol min^{-1} mg^{-1} protein). After the application of atropine, a second release of ^{45}Ca can be elicited by adrenalin. The addition of EGTA just before the addition of atropine prevents the rapid influx of ^{45}Ca and a subsequent release by adrenalin. Similar results with ^{86}Rb efflux experiments support the interpretation that on removal of the stimulus (with atropine) the receptor-sensitive Ca pool reloads extremely rapidly from the extracellular space. When ^{86}Rb efflux or the release of tritiated protein are measured during this reloading period, no transient elevation in response is seen (figure 3). This indicates that the pool reloads from the extracellular space without a concomitant elevation in ionized Ca in the cytoplasm.

Clearly the hypothesis most consistent with these observations is that the receptor-regulated Ca pool resides in, or in close association with, the plasma membrane. This idea must also take account of the observations (unpublished) that release of Ca from this pool is ATP dependent and that divalent cationophores can release this Ca. It is hoped that continued investigation will serve to define more clearly the molecular and morphological characteristics of this pool, which plays a central role in regulation of exocrine glands and other Ca-regulated tissues.

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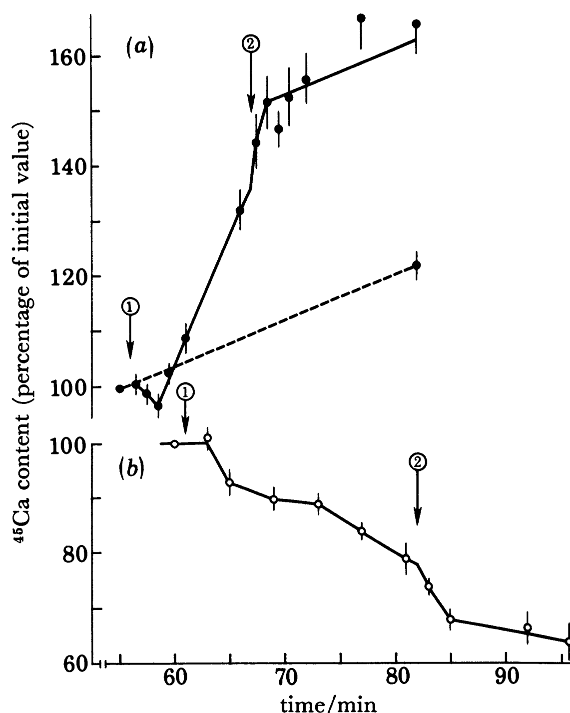

FIGURE 1. Effects of agonists and antagonists on ^{45}Ca content of rat parotid acinar cells. Values are expressed as percentages of initial value, obtained just before the addition of a particular agent ((*a*) 2.96 ± 0.12 nmol/mg protein, $n = 20$; (*b*) 2.53 ± 0.06, $n = 8$). ●—●, (1) 0.1 mM carbachol; (2) 0.1 mM atropine. ●--●, Basal (unstimulated) uptake. ○—○, (1) 10 mM EGTA; (2) 0.1 mM carbachol. (From Poggioli & Putney (1982), with permission.)

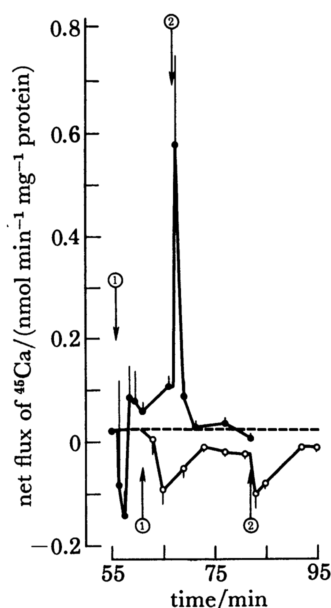

FIGURE 2. Net ^{45}Ca fluxes from the experiments shown in figure 1. The broken line indicates the basal influx (0.025 ± 0.004 nmol min^{-1} mg^{-1} protein). ●—●, (1) 0.1 mM carbachol; (2) 0.1 mM atropine. ○—○, (1) 10 mM EGTA; (2) 0.1 mM carbachol. (From Poggioli & Putney (1982), with permission.)

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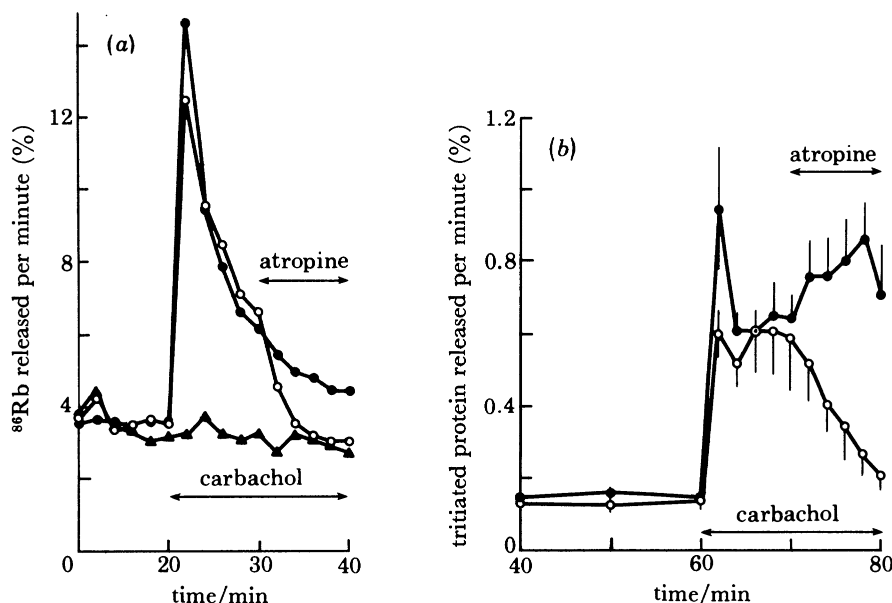

FIGURE 3. Effect of atropine on carbachol-stimulated responses of parotid tissue. (*a*) Efflux of ^{86}Rb is used as an index of membrane permeability to potassium (Putney 1978): ▲–▲, no additions (control); ●–●, 0.1 mM carbachol, 20–40 min; ○–○, 0.1 mM carbachol, 20–40 min, 0.1 mM atropine, 30–40 min. (*b*) Release of secretory proteins, previously labelled with [^{3}H]leucine: ●–●, 0.1 mM carbachol, 60–80 min; ○–○, 0.1 mM carbachol, 60–80 min, 0.1 mM atropine, 70–80 min. (From Poggioli & Putney (1982), with permission.)

CALCIUM GATING

As to the locus of the second phase of Ca mobilization, the enhanced uptake of Ca, there can be little doubt that this occurs in the plasmalemma. In recent years, there has been considerable interest in the molecular events involved in this process, particularly in the purported role of phosphatidylinositol metabolism. The development of this theory is attributable primarily to the experiments and writings of Michell; as the fundamental evidence for a role of phosphatidylinositol turnover is covered by Michell *et al.* (this symposium), those issues will not be dealt with further here.

The biochemical reactions involved in the phosphatidylinositol effect are believed to be initiated by a receptor-mediated breakdown of phosphatidylinositol at the plasma membrane by a phospholipase C to yield diacylglycerol (Michell 1975). The diacylglycerol is rapidly phosphorylated by diacylglycerol kinase to form phosphatidic acid. Presumably, although direct evidence is lacking, the phosphatidic acid is then transported to the endoplasmic reticulum where phosphatidylinositol is resynthesized and returned to the plasmalemma (Michell 1979; Putney 1981*b*). The net result of these reactions is usually a decrease in phosphatidylinositol, an increase in phosphatidic acid and, if $^{32}PO_4$ is present, an increase in the incorporation of radioactive phosphorus into both phospholipids.

One proposed role of these reactions in Ca gating is that the phosphatidic acid synthesized at the plasma membrane might function as a Ca ionophore permitting Ca entry into the cell (Salmon & Honeyman 1979, 1980; Putney *et al.* 1980). In platelets (Lapetina & Cuatrecasas 1979) and in the parotid gland (unpublished observations), significant stimulation of phosphatidate synthesis is detectable within a few seconds after the application of a stimulus.

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Phosphatidic acid mimics the actions of receptor activation in some (Salmon & Honeyman 1980; Putney *et al.* 1980; Harris *et al.* 1981) but not all (Foreman & Mongar 1972; Mongar & Svec 1972) tissues. Phosphatidic acid will transport Ca across an organic interphase in a Pressman chamber (Tyson *et al.* 1976) and across artificial bilayers in liposomes (Serhan *et al.* 1981). Cullis *et al.* (1981) have suggested that the mechanism by which phosphatidate (and also cadiolipin) could transport Ca might involve the rapid formation and collapse of non-bilayer membrane structures containing Ca bound to phosphatidate.

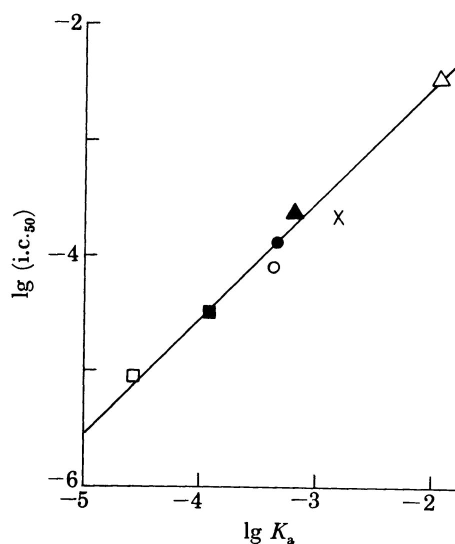

FIGURE 4. Relation between inhibition of ^{45}Ca-partitioning activity of phosphatidic acid and inhibition of receptor-activated Ca-dependent ^{86}Rb release by various agents. The ordinate values are the logarithms of concentrations of agent necessary to inhibit ^{45}Ca transport by phosphatidic acid into $CHCl_3$ by 50 %, and the abscissae are the logarithms of apparent dissociation constants (K_a) for antagonism of Ca gating (measured as ^{86}Rb release due to carbachol) in parotid slices. The agents were: □, La^{3+}; ■, Tm^{3+}; ○, neomycin; ●, Co^{2+}; ▲, Ni^{2+}; △, Mg^{2+}; ×, Ca^{2+} (K as agonist estimated). The line was fitted by least-squares analysis. For details see Putney *et al.* (1980), from which this figure is reproduced with permission.

The pharmacology of the Ca channels in the rat parotid gland provides additional evidence for a role of phosphatidic acid. In parotid gland cells and other non-excitable cells, the organic Ca antagonists such as verapamil and D-600 have no specific actions on the Ca channels (Marier *et al.* 1977; Putney 1981*c*). Agents that act as Ca antagonists are strongly cationic substances such as divalent cations, trivalent lanthanides, and aminoglycoside antibiotics (Putney 1981*c*). A series of these was tested for ability to prevent Ca from binding to phosphatidate, assayed as phosphatidate-dependent extraction of ^{45}Ca into $CHCl_3$. The results showed a strong correlation between the potency of these substances as Ca antagonists in the parotid gland and their ability to interfere with Ca binding to phosphatidic acid (Putney *et al.* 1980) (figure 4).

Thus, three points of evidence favour the idea that phosphatidic acid mediates Ca gating by receptor activation in the parotid gland: (1) phosphatidic acid is rapidly (in seconds) synthesized on receptor activation; (2) phosphatidic acid is a Ca ionophore in artificial membrane systems; (3) agents that block the inward movement of Ca by receptor activation also block the binding of Ca to phosphatidic acid, and with similar potencies. On the other hand, there do appear to be situations where increased phosphatidate synthesis does not apparently lead to an increase in membrane permeability to Ca (Michell *et al.* 1977). This may

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indicate an additional degree of complexity or selectivity imposed by membranes of living cells that does not come into play in artificial bilayers. Obviously our complete understanding of the role of phosphatidic acid awaits the results of continued investigations at the biochemical and cellular levels.

Conclusions

In the parotid and other exocrine glands, a number of hormones and neurotransmitters produce their effects through surface membrane receptors. Many of these receptors are coupled to mechanisms of Ca mobilization, leading to an increase in the concentration of ionized Ca within the cell. The general case appears to be a biphasic Ca mobilizing mechanism; a transient phase of cellular Ca release is followed by a sustained phase of augmented Ca influx. The relative contributions of Ca release and Ca influx vary from one gland to another.

The mechanism involved in the Ca release is unknown. Our recent results suggest that in the parotid gland, on removal of a stimulus, the releasable pool of Ca reloads rapidly from the extracellular space without a concomitant elevation in ionized intracellular Ca. One hypothesis consistent with these findings is that the releasable Ca pool is located in the plasma membrane of the cell.

There is considerable circumstantial evidence that the mechanism by which receptors activate Ca influx or Ca gating may involve the turnover of phosphatidylinositol. One means by which this could be accomplished is through the synthesis of phosphatidic acid at the plasma membrane. Phosphatidic acid would mediate inward Ca movement by functioning as a Ca ionophore.

References (Putney *et al.*)

Cullis, P. R., deKruijff, B., Hope, M. J., Nayar, R. & Schmid, S. L. 1980 Phospholipids and membrane transport. *Can. J. Biochem.* **58**, 1091–1100.

Douglas, W. W. 1974 Involvement of calcium in exocytosis and the exocytosis–vesiculation sequence. *Biochem. Soc. Symp.* **39**, 1–28.

Douglas, W. W. & Poisner, A. M. 1963 The influence of calcium on the secretory response of the submaxillary gland to acetylcholine or to noradrenaline. *J. Physiol., Lond.* **165**, 528–541.

Foreman, J. C. & Mongar, J. L. 1972 Effect of calcium on dextran-induced histamine release from isolated mast cells. *Br. J. Pharmac.* **46**, 767–769.

Haddas, R. A., Landis, C. A. & Putney, J. W., Jr 1979 Relationship between calcium release and potassium release in rat parotid gland. *J. Physiol., London.* **291**, 457–465.

Harris, R. A., Schmidt, J., Hitzemann, B. A. & Hitzemann, R. J. 1981 Phosphatidate as a molecular link between depolarization and neurotransmitter release in brain. *Science N.Y.* **212**, 1290–1291.

Hokin, L. E. 1966 Effects of calcium omission on acetylcholine-stimulated amylase secretion and phospholipid synthesis in pigeon pancreas slices. *Biochim. biophys. Acta* **115**, 219–221.

Iwatsuki, N. & Petersen, O. H. 1977 Pancreatic acinar cells: localization of acetylcholine receptors and the importance of chloride and calcium for acetylcholine-evoked depolarization. *J. Physiol., Lond.* **269**, 723–733.

Lapetina, E. G. & Cuatrecasas, P. 1979 Stimulation of phosphatidic acid production in platelets precedes the formation of arachidonate and parallels the release of serotonin. *Biochim. biophys. Acta* **573**, 394–402.

Laugier, R. & Petersen, O. H. 1980 Pancreatic acinar cells: electrophysiological evidence for stimulant-evoked increase in membrane calcium permeability in the mouse. *J. Physiol., Lond.* **303**, 61–72.

Marier, S. H., Putney, J. W., Jr & Van De Walle, C. M. 1978 Control of calcium channels by membrane receptors in the rat parotid gland. *J. Physiol., Lond.* **279**, 141–151.

Michell, R. H. 1975 Inositol phospholipids and cell surface receptor functions. *Biochim. biophys. Acta* **415**, 81–147.

Michell, R. H. 1979 Inositol phospholipids in membrane function. *Trends biochem. Sci.* **4**, 128–131.

Michell, R. H., Jafferji, S. S. & Jones, L. M. 1977 The possible involvement of phosphatidylinositol breakdown in the mechanism of stimulus-response coupling at receptors which control cell-surface calcium gates. *Adv. exp. Biol. Med.* **83**, 447–464.

Mongar, J. L. & Svec, P. 1972 The effect of phospholipids on anaphylactic histamine release. *Br. J. Pharmac.* **46**, 741–752.

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Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Parod, R. J. & Putney, J. W., Jr 1978*a* An alpha-adrenergic receptor mechanism controlling potassium permeability in the rat lacrimal gland acinar cell. *J. Physiol., Lond.* **281**, 359–369.
Parod, R. J. & Putney, J. W., Jr 1978*b* Role of calcium in the receptor mediated control of potassium permeability in the rat lacrimal gland. *J. Physiol., Lond.* **281,** 371–381.
Parod, R. J. & Putney, J. W., Jr 1979 Stimulation of ^{45}Ca efflux from rat lacrimal gland slices by carbachol and epinephrine. *Life Sci.* **25**, 2211–2215.
Petersen, O. H. 1978 Calcium dependence of bombesin-evoked pancreatic amylase secretion. *J. Physiol., Lond.* **285**, 30–31P.
Petersen, O. H., Poulsen, J. H. & Thorn, N. A. 1967 Secretory potentials, secretory rate, and water permeability of the duct system in the cat submandibular gland during perfusion with calcium-free Locke's solution. *Acta physiol. scand.* **71**, 203–210.
Poggioli, J. & Putney, J. W., Jr 1982 Net calcium fluxes in rat parotid acinar cells. Evidence for a hormone-sensitive calcium pool in or near the plasma membrane. *Pflügers Arch. ges. Physiol.* (In the press.)
Putney, J. W., Jr 1976 Biphasic modulation of potassium release in rat parotid gland by carbachol and phenylephrine. *J. Pharmac. exp. Ther.* **198**, 375–384.
Putney, J. W., Jr 1977 Muscarinic, α-adrenergic and peptide receptors regulate the same calcium influx sites in the parotid gland. *J. Physiol., Lond.* **268**, 139–149.
Putney, J. W., Jr 1978 Stimulus-permeability coupling: role of calcium in the receptor regulation of membrane permeability. *Pharmac. Rev.* **30**, 209–245.
Putney, J. W., Jr 1981*a* Calcium in the regulation of membrane permeability in the exocrine glands. In *Calcium in normal and pathological biological systems* (ed. L. J. Anghileri). Boca Raton, Florida: C.R.C. Press. (In the press.)
Putney, J. W., Jr 1981*b* Recent hypotheses regarding the phosphatidylinositol effect. *Life Sci.* **29**, 1183–1194.
Putney, J. W., Jr 1981*c* Calcium antagonists and calcium gating mechanisms in the exocrine glands. In *New perspectives on calcium antagonists* (ed. G. B. Weiss). Baltimore: Waverly Press. (In the press.)
Putney, J. W., Jr, Parod, R. J. & Marier, S. H. 1977 Control by calcium of exocytosis and membrane permeability to potassium in the rat lacrimal gland. *Life Sci.* **20**, 1905–1912.
Putney, J. W., Jr, Weiss, S. J., Van De Walle, C. M. & Haddas, R. A. 1980 Is phosphatidic acid a calcium ionophore under neurohumoral control? *Nature, Lond.* **284**, 345–347.
Rubin, R. P. 1974 *Calcium and the secretory process.* New York: Plenum Press.
Salmon, D. M. & Honeyman, T. W. 1979 Increased phosphatidate accumulation during single contractions of isolated smooth muscle cells. *Biochem. Soc. Trans.* **7**, 986–988.
Salmon, D. M. & Honeyman, T. W. 1980 Proposed mechanism of cholinergic action in smooth muscle. *Nature, Lond.* **284**, 344–345.
Schulz, I. & Stolze, H. H. 1980 The exocrine pancreas: the role of secretagogues, cyclic nucleotides, and calcium in enzyme secretion. *A. Rev. Physiol.* **42**, 127–156.
Selinger, Z., Batzri, S., Eimerl, S. & Schramm, M. 1973 Calcium and energy requirements for K^+ release mediated by the epinephrine α-receptor in rat parotid slices. *J. biol. Chem.* **248**, 369–372.
Serhan, C., Anderson, P., Goodman, E., Durham, P. & Weissmann, G. 1981 Phosphatidate and oxidized fatty acids are calcium ionophores: studies employing arsenazo III in liposomes. *J. biol. Chem.* **256**, 2736–2741.
Stolze, H. & Schulz, I. 1980 Effect of atropine, ouabain, antimycin A and A23187 on 'trigger Ca^{2+} pool' in exocrine pancreas. *Am. J. Physiol.* **238**, G338–348.
Tyson, C. A., Zande, H. V. & Green, D. E. 1976 Phospholipids as ionophores. *J. biol. Chem.* **251**, 1326–1332.
Williams, J. A. 1980 Regulation of pancreatic acinar cell function by intracellular calcium. *Am. J. Physiol.* **238**, G269–279.

Discussion

B. D. Gomperts (*Department of Experimental Pathology, University College London, U.K.*). Dr Putney has proposed that phosphatidic acid, generated as a consequence of physiological stimulation of the parotid gland, acts as an ionophore for Ca^{2+}. This would then permit the movement of Ca^{2+} into the cytosol, which mediates cellular activation. A similar proposal based on studies with bullfrog gastric smooth muscle has been made by Salmon & Honeyman (1980).

Before accepting such a role for phosphatidic acid, we should consider with care some of the features that characterize A23187 and ionomycin as Ca^{2+} ionophores. These compounds are now widely used in both model and biological systems in the full confidence that they will always transfer Ca^{2+} across membranes. Does phosphatidic acid actually live up to the designation of an ionophore?

The crudest test of a potential ionophore for Ca^{2+} is its ability to solvate Ca^{2+} ions in bulk

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phases of low dielectric constant. While phosphatidic acid shares this property (Tyson *et al.* 1976; Putney *et al.* 1980) one should stress that this evidence alone is insufficient to qualify it as an ionophore: as is well known, $Ca(OH)_2$ has a measurable solubility in organic solvents, and nobody would seriously claim ionophoric status for the OH^- ion.

An excellent system for demonstrating ionophoric activity is the promotion of Ca^{2+} movements into or out of liposomes, and such a test has now been carried out for phosphatidic acid (Serhan *et al.* 1981). Its ability to carry Ca^{2+} across phospholipid bilayer membranes appears to be slight. Even when incorporated into the liposomal membranes at the time of their formation at 1.5 mol % (probably a reasonable estimate of the maximum proportion of phospholipid that could arise in a tissue from the breakdown of phosphatidylinositol), the measured Ca^{2+} fluxes were considered to be orders of magnitude less than those achieved by ionomycin or A23187. That such activity as was detected might be due not to phosphatidic acid, but to an impurity (a lyso compound possibly?) is suggested by the observation of low-level Ca^{2+} fluxes when phosphatidic acid was added externally to preformed liposomes. The work of Uemura & Kinsky (1972) has convincingly demonstrated the non-feasibility of incorporating diacylphospholipids into preformed bilayers.

In reconstituted sarcoplasmic reticulum, which is another relevant model system, the vesicles still accumulate Ca^{2+} when supplied with ATP even if the pump protein is in an environment consisting almost entirely of phosphatidic acid (Bennett *et al.* 1978). In contrast, the effect of the ionophores A23187 and ionomycin is to uncouple the pump completely (Caswell & Pressman 1972; Beeler *et al.* 1979).

Applied to mast cells, phosphatidic acid neither promotes the Ca^{2+}-dependent exocytotic secretion of histamine, nor does it enhance secretion due to an anaphylactoid reaction (Foreman & Mongar 1972). The latter process can be potentiated by externally applied phosphatidylserine (though it is likely that it is the lyso derivative that is the active form (Martin & Lagunoff 1979; Smith *et al.* 1979)), but it has never been suggested that this lipid is acting as an ionophore for Ca^{2+}. The mast cell was of course the first tissue in which the Ca^{2+} ionophores were tested in cell biology. A23187 (Foreman *et al.* 1973), ionomycin (Bennett *et al.* 1979), and more recently a synthetic ionophore (Gomperts *et al.* 1981) have all been found to stimulate Ca^{2+}-dependent histamine secretion, and to fulfil the criteria for ionophores in model systems.

In the knowledge that A23187 and ionomycin are Ca^{2+} ionophores, they have been used with confidence that they will carry Ca^{2+} in all systems in which they are applied. In every situation in which the physiological response is mediated by an increase in intracellular Ca^{2+} concentration, the application of ionophore in the presence of Ca^{2+} will bring about that response. The same cannot be said of phosphatidic acid, and for this reason I would doubt that its reported acitivities can be ascribed to its behaving as an ionophore.

References

Beeler, T. J., Jona, I. & Martonosi, A. 1979 *J. biol. Chem.* **254**, 6229–6231.

Bennett, J. P., Smith, G. A., Houslay, M. D., Hesketh, T. R., Metcalfe, J. C. & Warren, G. B. 1978 *Biochim. biophys. Acta* **513**, 310–320.

Bennett, J. P., Cockcroft, S. & Gomperts, B. D. 1979 *Nature, Lond.* **282**, 851–853.

Caswell, A. H. & Pressman, B. C. 1972 *Biochem. biophys. Res. Commun.* **49**, 292–298.

Foreman, J. C. & Mongar, J. L. 1972 *Br. J. Pharmac.* **46**, 767–769.

Foreman, J. C., Mongar, J. L. & Gomperts, B. D. 1973 *Nature, Lond.* **245**, 249–251.

Gomperts, B. D., Bennett, J. P. & Allan, D. 1981 *Eur. J. Biochem.* (In the press.)

Martin, T. W. & Lagunoff, D. 1979 *Nature, Lond.* **279**, 250–252.

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Putney, J. W., Weiss, S. J., van de Walle, C. M. & Haddas, R. A. 1980 *Nature, Lond.* **284**, 345–347.
Salmon, D. M. & Honeyman, T. W. 1980 *Nature, Lond.* **284**, 344–345.
Serhan, C., Anderson, P., Goodman, E., Dunham, P. & Weissmann, G. 1981 *J. biol. Chem.* **256**, 2736–2741.
Tyson, C. A., van de Zande, H. & Green, D. E. 1976 *J. biol. Chem.* **251**, 1326–1332.
Uemura, K. & Kinsky, S. C. 1972 *Biochemistry, Wash.* **11**, 4085–4093.

J. W. PUTNEY, JR. Dr Gomperts has raised the point (acknowledged by us above) that there is evidence that phosphatidic acid may not act as a Ca ionophore in all biological systems. This could be due to any of several reasons; I shall briefly consider three.

First, it is possible, as Dr Gomperts implies, that phosphatidic acids cannot, under any circumstances, translocate Ca across biological membranes in quantities of any physiological significance. The difficulty with this is that one must (at least for the parotid gland) dispose of considerable pharmacological and biochemical evidence already discussed above (and which I will not reiterate here) as being due to coincidence.

A second and more palatable suggestion is simply that the expression of ionophoric activity may vary from one situation to another depending on a number of as yet undetermined factors. The simplest of such factors could relate to the lipid environment, but effects of ions, proteins and membrane carbohydrates could be important as well. Even the more 'established' ionophores may differ in potency by an order of magnitude from one tissue to another. For the phosphatidic acids, the complete failure to transport Ca in some systems raises the semantic question as to the appropriateness of the term 'ionophore', although I can think of no other particularly suitable term. Within the context of this point, however, it should be pointed out that there has been no demonstrated failure of phosphatidic acids to translocate Ca in cells with receptors that both gate Ca and activate Ca-independent phosphatidylinositol turnover (see paper by R. H. Michell *et al.* later in this volume).

A third point which is seldom considered (largely due to technical inconvenience) concerns the different molecular species of the phosphatidic acids involved. If, in fact, phosphatidylinositol is degraded solely to generate phosphatidic acid at the plasma membrane, then it may be significant that specifically phosphatidylinositol, a minor phospholipid with a uniquely characteristic 1-stearoyl, 2-arachidonoyl fatty acid composition, is singled out for this purpose. Accordingly, the precise molecule that is formed, therefore, would be *sn*-1-stearoyl-2-arachidonoyl phosphatidic acid. This is not likely to be the molecule involved in the experimental situations discussed by Dr Gomperts.

A last point that I should like to make concerns the suitability of the antibiotic ionophores as models for putative endogenous ionophores in mammalian cells. We must bear in mind, I think, that as these compounds are antibiotics, they have presumably been designed (through evolution) for the broadest possible spectrum (i.e. low specificity) and for the ability to act on cells when applied externally. A phospholipid-type ionophore, on the other hand, would be synthesized *in situ* and never destined to leave the bilayer environment except through specialized, controlled mechanisms. It is therefore not surprising that attempts to mimic neurotransmitter action by the application of phosphatidate to the aqueous medium bathing cells have resulted in variable degrees of success.