J. exp. Biol. **124**, 323–335 (1986)
Printed in Great Britain © *The Company of Biologists Limited 1986*

REGULATION OF ION CHANNELS BY INOSITOL TRISPHOSPHATE AND DIACYLGLYCEROL

BY MICHAEL J. BERRIDGE

AFRC Unit of Insect Neurophysiology and Pharmacology, Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ

SUMMARY

Calcium-mobilizing receptors function to regulate ion channels located not only in the plasma membrane but also across the membranes of intracellular organelles, particularly the endoplasmic reticulum. A characteristic feature of such receptors is that they stimulate the hydrolysis of an inositol lipid to generate a pair of second messengers. Diacylglycerol remains within the plasma membrane where it activates protein kinase C leading to the phosphorylation of proteins some of which may regulate specific ionic channels, such as the calcium-dependent potassium channel or the Na^+/H^+ exchanger which regulates intracellular pH. The inositol trisphosphate (Ins 1,4,5P_3) released to the cytosol functions as a second messenger to release calcium from the endoplasmic reticulum. The Ins 1,4,5P_3 acts on a specific receptor to enhance the passive efflux of calcium while having no effect on the active calcium pump. There are indications that this Ins 1,4,5P_3-induced release of calcium from an internal membrane store might provide an explanation of excitation–contraction coupling in skeletal muscle. Skinned skeletal muscle cells can be induced to contract by adding Ins 1,4,5P_3. Mobilization of calcium from intracellular reservoirs by Ins 1,4,5P_3 may thus prove to be a ubiquitous and fundamental mechanism for regulating cellular activity.

INTRODUCTION

Many cellular processes are regulated by calcium-mobilizing receptors which can use the hydrolysis of an inositol lipid as part of a transduction mechanism for generating second messengers. The inositol lipid used by the receptor is phosphatidylinositol 4,5-bisphosphate (PtdIns 4,5P_2) which is cleaved to diacylglycerol (DG) and inositol 1,4,5-trisphosphate (Ins 1,4,5P_3) (Berridge, 1984; Berridge & Irvine, 1984; Hokin, 1985; Downes & Michell, 1985). Both products seem to function as second messengers in that DG activates protein kinase C (C-kinase) (Nishizuka, 1984) whereas Ins 1,4,5P_3 diffuses into the cytosol to release calcium from the endoplasmic reticulum (Berridge, 1984; Berridge & Irvine, 1984). By stimulating the hydrolysis of PtdIns 4,5P_2, a whole variety of hormones and neurotransmitters initiate a bifurcating signal pathway which functions to control numerous cellular processes including secretion, contraction, metabolism, fertilization, phototransduction and cell proliferation. In this article I shall concentrate on the role of

Key words: inositol trisphosphate, phosphatidylinositol, diacylglycerol.

Ins 1,4,5P_3 in regulating calcium channels within the endoplasmic reticulum. I shall also consider how the DG/C-kinase limb of this bifurcating signal pathway functions to modulate ion channels within the plasma membrane. Before considering how these two signals pathways regulate ion channels it is necessary to describe the biochemistry underlying inositol lipid signal transduction.

AGONIST-DEPENDENT PtdIns 4,5P_2 HYDROLYSIS TO DG AND Ins 1,4,5P_3

The PtdIns 4,5P_2 used by the receptor mechanism to generate intracellular second messengers is a relatively minor membrane component comprising less than 0·5 % of the total cellular phospholipids. It is derived from phosphatidylinositol (PtdIns) by two phosphorylation reactions. First, a PtdIns kinase phosphorylates PtdIns to phosphatidylinositol 4-phosphate (PtdIns 4P) which is then converted to PtdIns 4,5P_2 by a second kinase (Fig. 1). These two kinases appear to be extremely active and thus ensure a ready supply of the PtdIns 4,5P_2 required by the receptor as a precursor to generate second messengers. Occupation of an appropriate receptor results in the stimulation of a phosphodiesterase which then cleaves PtdIns 4,5P_2 to DG and Ins 1,4,5P_3. This transduction mechanism thus has much in common with that used to form cyclic AMP. In both cases, a highly phosphorylated precursor is cleaved by an enzyme (which functions as an amplification unit) to release second messengers. Further similarities exist with regard to the way in which receptors are coupled to the underlying amplification unit. In the cyclic AMP system, receptors are coupled to adenylate cyclase by way of specific GTP-binding proteins (G-proteins). A G-protein may also serve to couple receptors to the phosphodiesterase which cleaves PtdIns 4,5P_2 to DG and Ins 1,4,5P_3 (Haslam & Davidson, 1984; Cockcroft & Gomperts, 1985).

In keeping with the proposed role of DG and Ins 1,4,5P_3 as second messengers, they are rapidly inactivated once the external signal is withdrawn. The neutral DG which operates within the plane of the membrane can either be converted to phosphatidic acid by a DG kinase or it can be fed to a DG lipase which will release arachidonic acid (Fig. 1). There also are two pathways for degrading Ins 1,4,5P_3. First, it can be dephosphorylated to free inositol through a stepwise series of phosphatases (Storey, Shears, Kirk & Michell, 1984). Alternatively, it can be phosphorylated through a newly discovered kinase to form inositol 1,3,4,5-tetrakisphosphate (Ins 1,3,4,5P_4) (Irvine, Letcher, Heslop & Berridge, 1986). The latter was first identified in brain cortical slices and appears to be the precursor of Ins 1,3,4P_3 (Batty, Nahorski & Irvine, 1985). When cells are stimulated not only do they produce Ins 1,4,5P_3 but, after a short lag, they also begin to form Ins 1,3,4P_3 (Irvine, Letcher, Lander & Downes, 1984*b*, 1985; Burgess, McKinney, Irvine & Putney, 1985). When Ins 1,4,5P_3 is released from the membrane it can thus flow along two separate routes, a degradative pathway to free inositol or *via* a novel pathway to generate other inositol polyphosphates which could have additional messenger functions (Irvine *et al.* 1984*b*; Batty *et al.* 1985). At present the only

inositol phosphate for which a second messenger function has been identified is Ins 1,4,5P_3 (Berridge, 1984; Berridge & Irvine, 1984).

The role of Ins 1,4,5P_3 as an intracellular second messenger is further complicated by the possible existence of a cyclic form (Wilson *et al.* 1985*a*; Wilson, Neufeld & Majerus, 1985*b*). When PtdIns 4,5P_2 is cleaved *in vitro* by a purified phosphodiesterase not only is Ins 1,4,5P_3 produced but so is inositol 1,2-cyclic 4,5-trisphosphate (cyclic Ins 1,4,5P_3). Although the latter was less active than Ins 1,4,5P_3 in releasing calcium from permeabilized blood platelets it was more active in stimulating membrane depolarization in *Limulus* photoreceptors (Wilson *et al.* 1985*b*). Wilson *et al.* (1985*b*) have speculated that Ins 1,4,5P_3 and cyclic Ins 1,4,5P_3 may perform separate transducing functions thus stressing the versatility of this signalling system. Since the presence of cyclic Ins 1,4,5P_3 in intact cells has yet to be described,

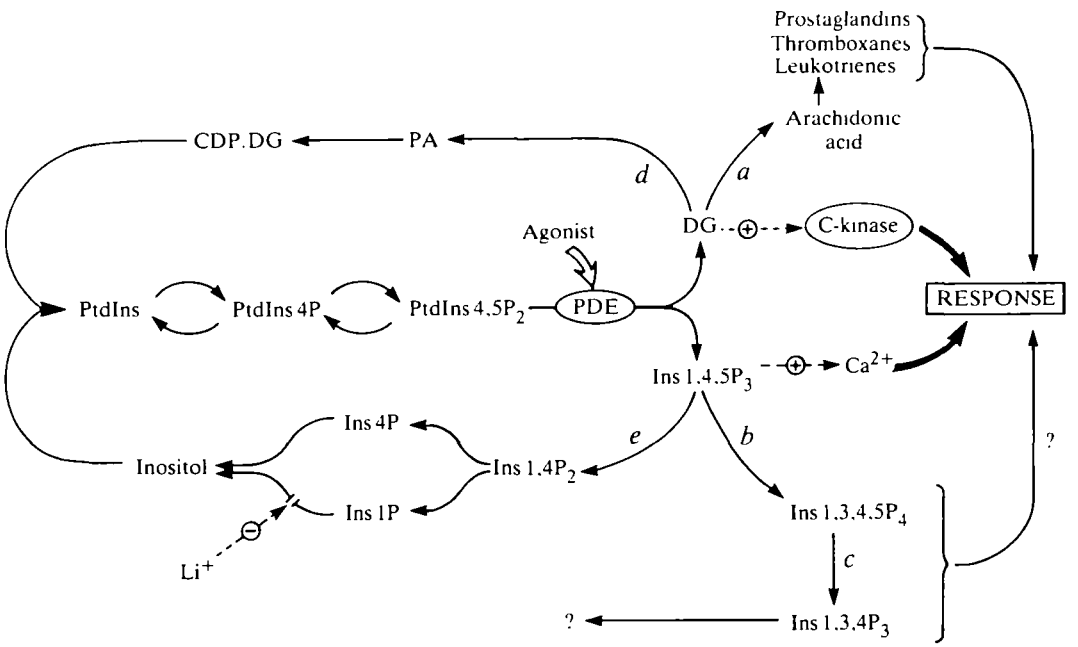

Fig. 1. Summary of agonist-dependent inositol lipid metabolism. The key event is the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PtdIns 4,5P_2) to give diacylglycerol (DG) and inositol 1,4,5-trisphosphate (Ins 1,4,5P_3), both of which function as second messengers. In addition, these two products may be converted to other metabolites which could have additional messenger functions. A DG kinase (*a*) removes arachidonic acid which can be converted into a variety of metabolites. On the other hand, Ins 1,4,5P_3 can be converted to inositol 1,3,4,5-tetrakisphosphate (Ins 1,3,4,5P_4) by means of an inositol trisphosphate 3-kinase (*b*). A phosphatase (*c*) removes the phosphate from the 5-position of Ins 1,3,4,5P_4 to give Ins 1,3,4P_3 which might have a second messenger function. The other pathways for metabolizing DG and Ins 1,4,5P_3 ultimately combine to reform phosphatidylinositol (PtdIns). The inositol lipid cycle begins with a DG kinase (*d*) which converts DG to phosphatidic acid (PA) whereas the inositol phosphate cycle begins with the dephosphorylation of Ins 1,4,5P_3 to Ins 1,4P_2 by means of an inositol trisphosphatase (*e*). PDE, phosphodiesterase; CDP.DG, cytidine diphosphodiacylglycerol; C-kinase, protein kinase C.

I shall concentrate on the evidence that Ins 1,4,5P_3 is a calcium-mobilizing second messenger.

MOBILIZATION OF INTRACELLULAR CALCIUM BY Ins 1,4,5P_3

The importance of Ins 1,4,5P_3 as an intracellular second messenger is clearly demonstrated by the ability of this molecule to stimulate a number of complex physiological processes when injected into intact cells (Table 1). Many of these processes are regulated by calcium. Using appropriate techniques, increases in intracellular calcium have been recorded following injection of Ins 1,4,5P_3 into either *Limulus* photoreceptors (Brown & Rubin, 1984) or *Xenopus* oocytes (Busa *et al.* 1985). The properties of this Ins 1,4,5P_3-induced release of intracellular calcium have been studied using either permeabilized cells (Table 2) or membrane fractions (Table 3). All the calcium released by Ins 1,4,5P_3 seems to originate solely from the endoplasmic reticulum. Once the plasma membrane has been permeabilized either by incubation in low calcium media, treatment with detergents or by means of high electric discharges, the internal organelles become accessible to molecules supplied in the bathing medium. Such permeabilized cells sequester calcium into both the endoplasmic reticulum and mitochondria but only the former is sensitive to Ins 1,4,5P_3. If the uptake of calcium into the endoplasmic reticulum is inhibited by vanadate, the subsequent release of calcium by Ins 1,4,5P_3 is severely curtailed (Streb, Irvine, Berridge & Schulz, 1983). Blocking the uptake of calcium into the mitochondria using various inhibitors (e.g. oligomycin, antimycin A, ruthenium red) had no effect on uptake into the endoplasmic reticulum, nor did it inhibit the subsequent release of calcium following addition of Ins 1,4,5P_3 (Streb *et al.* 1983; Biden *et al.* 1984; Burgess *et al.* 1984*a*,*b*; Gershengorn, Geras, Purrello & Rebecchi, 1984; Joseph *et al.* 1984*a*,*b*). Conversely, if the mitochondria of permeabilized liver cells are intentionally loaded with calcium by incubating them in

Table 1. *Effects of injecting Ins 1,4,5P_3 into intact cells*

Tissue	Effect	References
Limulus	Phototransduction and adaptation	Brown *et al.* (1984) Fein *et al.* (1984)
Limulus	Calcium mobilization	Brown & Rubin (1984) Wilson, Neufeld & Majerus (1985*b*)*
Salamander rods	Modulation of light response	Waloga & Anderson (1985)
Sea urchin oocytes	Fertilization membrane	Whitaker & Irvine (1984) Turner, Jaffe & Fein (1985)
Xenopus oocytes	Depolarizing Cl^- current	Oron, Dascal, Nadler & Lupu (1985) Busa *et al.* (1985)
Xenopus oocytes	Fertilization membrane	Picard *et al.* (1985) Busa *et al.* (1985)

* Used cyclic Ins 1,4,5P_3.

Table 2. *Effects of adding Ins 1,4,5P_3 to permeabilized cells*

Tissue	Effect	References
Pancreas	Calcium mobilization	Streb, Irvine, Berridge & Schulz (1983)
Liver	Calcium mobilization	Burgess *et al.* (1984*a*,*b*)
		Joseph *et al.* (1984*a*)
Insulinoma	Calcium mobilization	Biden *et al.* (1984)
		Joseph *et al.* (1984*b*)
Neutrophils	Calcium mobilization	Prentki, Wollheim & Lew (1984*b*)
Pituitary	Calcium mobilization	Gershengorn, Geras, Purrello & Rebecchi (1984)
Adrenal glomerulosa	Calcium mobilization	Kojima, Kojima, Kreutter & Rasmussen (1984)
Macrophages	Calcium mobilization	Hirata *et al.* (1984)
	Calcium mobilization	Hirata *et al.* (1985*a*)
Swiss 3T3	Calcium mobilization	Berridge, Heslop, Irvine & Brown (1984)
Parathyroid	Calcium mobilization	Epstein, Prentki & Attie (1985)
Blood platelet	Calcium mobilization	Wilson, Neufeld & Majerus (1985*b*)
T-cell line	Calcium mobilization	Imboden & Stobo (1985)
Smooth muscle	Calcium mobilization	Suematsu, Hirata, Hashimoto & Kuriyama (1984)
Smooth muscle	Calcium mobilization	Yamamoto & van Breemen (1985)
Smooth muscle	Calcium mobilization	Hashimoto, Hirata & Ito (1985)
Smooth muscle	Calcium mobilization	Smith, Smith & Higgins (1985)
Smooth muscle	Contraction	Somlyo, Bond, Somlyo & Scarpa (1985)
Skeletal muscle	Contraction	Vergara, Tsien & Delay (1985)
Skeletal muscle	Contraction	Volpe, Salviati, Di Virgilio & Pozzan (1985)
Dictyostelium	Cyclic GMP formation	Europe-Finner & Newell (1985)
Blood platelet	Exocytosis	Brass & Joseph (1985)
Sea urchin egg	Exocytosis	Clapper & Lee (1985)

Table 3. *Summary of tissues where calcium mobilization in response to Ins 1,4,5P_3 has been demonstrated in microsomal preparations*

Tissue	Reference
Pancreas	Streb *et al.* (1984)
Insulinoma	Prentki *et al.* (1984*a*)
	Joseph *et al.* (1984*b*)
Liver	Dawson & Irvine (1984)
	Joseph *et al.* (1984*b*)
	Dawson (1985)
	Muallem, Schoeffield, Pandol & Sachs (1985)
Platelets	Adunyah & Dean (1985)
	O'Rourke, Halenda, Zavoico & Feinstein (1985)
Smooth muscle	Carsten & Miller (1985)
Plant hypocotyl	Drøbak & Ferguson (1985)
Skeletal muscle	Volpe, Salviati, Di Virgilio & Pozzan (1985)
Sea urchin egg	Clapper & Lee (1985)

media containing a high concentration of calcium, there was no release upon addition of Ins 1,4,5P_3 (Burgess *et al.* 1984*a*). Separation of organelles and membrane fractions by differential centrifugation supports the notion that Ins 1,4,5P_3 acts predominantly to release calcium from the endoplasmic reticulum (Table 3). While release can clearly be seen after addition of Ins 1,4,5P_3 to microsomes, there was no release from either mitochondria or secretion granules (Prentki *et al.* 1984*a*; Prentki, Wollheim & Lew, 1984*b*).

There is a constant cycling of calcium across the endoplasmic reticulum which under steady-state conditions reflects a balance between passive efflux and ATP-dependent influx. The calcium pump is electrogenic and requires a compensating flow of potassium in the opposite direction to maintain electroneutrality (Muallem, Schoeffield, Pandol & Sachs, 1985). A constant supply of potassium counter ions within the endoplasmic reticulum is provided by a furosemide-sensitive potassium/chloride cotransporter. Release of calcium induced by Ins 1,4,5P_3 also requires an opposite flow of potassium as a counter ion (Muallem *et al.* 1985).

A net release of calcium from the endoplasmic reticulum could occur either by promoting the efflux pathway or by inhibiting the active pump. All the evidence points to an action of Ins 1,4,5P_3 on the passive efflux pathway. Once the endoplasmic reticulum has sequestered calcium, blocking the pump by the addition of vanadate or the removal of ATP does not mimic the effect of Ins 1,4,5P_3, nor does it prevent this agent from releasing calcium (Prentki *et al.* 1984*b*; Clapper & Lee, 1985; Hirata *et al.* 1985*a*; Muallem *et al.* 1985). Release of calcium from the endoplasmic reticulum by Ins 1,4,5P_3 is independent of temperature, again suggesting a passive phenomenon (Brass & Joseph, 1985; Hirata *et al.* 1985*a*; Smith, Smith & Higgins, 1985). Smith *et al.* (1985) have argued that this temperature-independence suggests an action of Ins 1,4,5P_3 on a channel rather than a carrier.

In response to the sudden addition of Ins 1,4,5P_3 more than 50% of the stored calcium is released within seconds but, on continued incubation, the calcium is taken up again, so restoring the initial steady-state condition (Joseph *et al.* 1984*a*; Biden *et al.* 1984). The re-uptake of calcium occurs because the pulse of Ins 1,4,5P_3 is degraded by an inositol trisphosphatase (Joseph *et al.* 1984*a*). In these permeabilized cells, the release of calcium from the endoplasmic reticulum by Ins 1,4,5P_3 occurs fast enough for it to be able to stimulate various physiological processes such as the cortical reaction in sea urchin eggs (Clapper & Lee, 1985), exocytosis in blood platelets (Brass & Joseph, 1985) or contraction of smooth (Somylo, Bond, Somylo & Scarpa, 1985) and skeletal muscle (Vergara, Tsien & Delay, 1985; Volpe, Salviati, Di Virgilio & Pozzan, 1985). It has been suggested that Ins 1,4,5P_3 could regulate the intracellular level of calcium, not only during cell stimulation but also when the cell is at rest, by controlling the rate of calcium cycling across the endoplasmic reticulum (Prentki, Corkey & Matschinsky, 1985). By varying the rate of infusions of Ins 1,4,5P_3 to counteract losses *via* the degradation enzyme, Prentki *et al.* (1985) have achieved a series of steady-state calcium levels.

Release of calcium from the endoplasmic reticulum by enhancing the passive efflux component seems to occur through a specific receptor for Ins 1,4,5P_3. No

release occurs upon addition of inositol, inositol 1-phosphate, inositol 2-phosphate, inositol 1,2-cyclic phosphate, inositol 1,4-bisphosphate, 2,3,diphosphoglyceric acid, fructose 2,6-bisphosphate, fructose 1,6-bisphosphate (Streb *et al.* 1983; Joseph *et al.* 1984*a*,*b*; Berridge, Heslop, Irvine & Brown, 1984; Biden *et al.* 1984; Burgess *et al.* 1984*a*,*b*; Irvine *et al.* 1984*b*). The specificity of the response is highlighted by the fact that there is no release following stimulation with inositol 1,4-bisphosphate which is the natural breakdown product resulting from the dephosphorylation of Ins 1,4,5P_3 by the inositol trisphosphatase. Preliminary measurements suggest that Ins 1,3,4,5P_4 is incapable of releasing calcium (M. J. Berridge & R. F. Irvine, unpublished observation). Preliminary structure–activity studies have begun to identify those aspects of the molecule which are crucial for release to occur (Irvine *et al.* 1984*b*; Burgess *et al.* 1984*b*). All analogues capable of stimulating release have phosphates in the 4- and 5-positions of the molecules. The phosphate on the 1-position is not necessary because inositol 4,5-bisphosphate can release calcium, albeit at much higher concentrations, suggesting that this phosphate plays a role in enhancing the affinity of the molecule for its receptor. First attempts to isolate and purify the Ins 1,4,5P_3 receptor have begun with the identification of a specific binding site in permeabilized cells and microsomes using [^{32}P]Ins 1,4,5P_3 (Baukal *et al.* 1985; Spät *et al.* 1986*a*; Spät, Fabiato & Rubin, 1986*b*). This binding site has a similar pharmacological profile to that detected in the calcium release experiments. Another approach has been to develop a photoaffinity label which could be used specifically to tag the receptor and thus to facilitate its identification during purification procedures. Hirata *et al.* (1985*b*) have described an arylazide derivative of Ins 1,4,5P_3 which can irreversibly inhibit the release of calcium induced by Ins 1,4,5P_3 in macrophages. Since the inhibitory effect of this derivative can be prevented by adding a 10-fold excess of Ins 1,4,5P_3, it would seem that the normal receptor is being labelled. The aim of all these studies will be the identification of the Ins 1,4,5P_3 receptor as a first step in working out how it functions to trigger the explosive release of calcium from the endoplasmic reticulum.

Ins 1,4,5P_3 AND EXCITATION–CONTRACTION COUPLING IN MUSCLE

A major unsolved problem in physiology concerns the nature of the mechanism whereby excitation of the sarcolemma of skeletal muscle results in a release of calcium from the sarcoplasmic reticulum (Caille, Ildefonse & Rougier, 1985). The two membranes are separated by cytosol so it is necessary to explain how information is transferred from the surface membranes to the sarcoplasmic reticulum. There is growing evidence that Ins 1,4,5P_3 may function to relay information between the two membranes in smooth muscle and perhaps also in skeletal muscle. Somlyo & Somlyo (1968) coined the term 'pharmacomechanical coupling' to describe the phenomenon whereby smooth muscle cells contract in response to transmitters such as acetylcholine, norepinephrine, vasopressin and angiotensin II. It has been known for some time that such transmitters act on smooth muscle to stimulate the hydrolysis of PtdIns 4,5P_2 to give Ins 1,4,5P_3 (Akhtar & Abdel-Latif, 1980, 1984; Smith *et al.*

1984; Nabika, Velletri, Lovenberg & Beaven, 1985). That Ins 1,4,5P_3 might function as a second messenger in smooth muscle is supported by the finding that it will stimulate the release of calcium when applied to skinned muscle cells (Suematsu *et al.* 1984; Somylo *et al.* 1985; Yamamoto & van Breemen, 1985) or from microsomes derived from uterine sarcoplasmic reticulum (Carsten & Miller, 1985). What is even more interesting is that Ins 1,4,5P_3 can trigger vascular smooth muscle to contract (Somylo *et al.* 1985). Similar evidence has been advanced to support the proposal that Ins 1,4,5P_3 may function in excitation–contraction coupling in skeletal muscle (Vergara *et al.* 1985; Volpe *et al.* 1985). In addition, Ins 1,4,5P_3 can release calcium from the sarcoplasmic reticulum, and when the latter was separated into two parts much more calcium was released from the terminal cisternae than from the longitudinal tubular region (Volpe *et al.* 1985). The current hypothesis is that depolarization of the T-tubule membrane somehow stimulates the hydrolysis of PtdIns 4,5P_2 to release Ins 1,4,5P_3, which then diffuses across the 20-nm gap to trigger the release of calcium from the sarcoplasmic reticulum. Preliminary measurements of Ins 1,4,5P_3 in skeletal muscle indicate that the level does increase in response to electrical stimulation (Vergara *et al.* 1985). While many questions remain unanswered, the possibility that Ins 1,4,5P_3 might function in skeletal muscle is certainly worth pursuing further as it may provide an elegant solution to a problem that has puzzled physiologists for decades.

ROLE OF Ins 1,4,5P_3 AND DG IN REGULATING ION TRANSPORT

Release of calcium from the endoplasmic reticulum by Ins 1,4,5P_3 is but one limb of a bifurcating signal pathway. The other component is DG, which operates within the plane of the membrane to activate protein kinase C (Fig. 1) (Nishizuka, 1984). The importance of this bifurcating signal pathway lies in the fact that both limbs cooperate with each other to control a whole host of cellular processes. I have already described the role of Ins 1,4,5P_3 in releasing intracellular calcium and now shall concentrate on the role of the DG/C-kinase pathway in regulating ion transport. In addition to acting independently of each other, an important aspect of this bifurcating pathway is that the two limbs often act synergistically with each other (Kaibuchi *et al.* 1983; Nishizuka, 1984).

This synergism was uncovered by devising methods for stimulating each signal pathway independently of the other. Calcium ionophores could be used to bypass the action of Ins 1,4,5P_3 in elevating the intracellular level of calcium, whereas phorbol esters were found to duplicate the ability of DG to activate protein kinase C (Castagna *et al.* 1982). When these two agents were administered together they could stimulate cells maximally at concentrations which had no effect when given alone. In the case of blood platelets, such synergism can be explained on the basis of the DG/C-kinase pathway enhancing the sensitivity of the exocytotic process to the stimulatory action of calcium (Knight & Scrutton, 1984). Another example of the interaction between the DG/C-kinase and calcium pathways has been uncovered in *Aplysia* neurones, where the voltage-sensitive calcium current in bag cell neurones is

enhanced following stimulation with a phorbol ester or after injection of purified protein kinase C (De Riemer *et al.* 1985).

Modulation of calcium-sensitive processes may be a particularly important function of the DG/C-kinase pathway. An interesting example has been uncovered in pyramidal hippocampal neurones, where the administration of a phorbol ester blocked accommodation which normally sets in due to the opening of a calcium-dependent potassium channel (Baraban, Snyder & Alger, 1985). The idea is that the DG/C-kinase pathway may act by reducing the sensitivity of potassium channels to the stimulatory action of calcium. Such a mechanism may account for the normal inhibition of accommodation brought about by acetylcholine because the latter is known to stimulate inositol lipid hydrolysis in the hippocampus (Gonzales & Crews, 1984; Fisher & Bartus, 1985). The DG/C-kinase pathway may also control two distinct potassium currents and a voltage-dependent calcium current in photoreceptors of *Hermissenda* (Farley & Auerbach, 1986). Regulation of neuronal excitability by adjusting potassium channels may turn out to be a general mode of action of receptors which operate through the inositol lipids. For example, substance P excites magnocellular cholinergic neurones (Stanfield, Nakajima & Yamaguchi, 1985), whereas a transient outward current in dorsal raphe serotonergic neurones is stimulated by α_1-adrenoreceptors (Aghajanian, 1985). Both these transmitters are known to stimulate inositol lipid hydrolysis in the brain, but it is not clear yet whether either Ins 1,4,5P_3 or DG has any role to play in regulating potassium channels in either of these tissues.

Another important ionic change regulated by this receptor mechanism is the Na^+/H^+ exchanger which has an important role in regulating intracellular pH, especially during the action of mitogenic stimuli. There are large increases in intracellular pH following fertilization (Swann & Whitaker, 1985) or during the action of growth factors on fibroblasts (Moolenaar, Tsien, van der Saag & de Laat, 1983; L'Allemain, Paris & Pouysségur, 1984). Since these changes in both oocytes and fibroblasts can be duplicated by the addition of a phorbol ester the idea has developed that the DG/C-kinase pathway may act to stimulate the Na^+/H^+ exchanger. The two major ionic events which occur when cells are induced to grow may thus be under the independent control of the two separate limbs of the inositol lipid signalling system. DG acts to stimulate the Na^+/H^+ exchanger to increase intracellular pH whereas the Ins 1,4,5P_3 released to the cytosol stimulates the release of calcium to account for the large increase in intracellular calcium recorded in cells responding to growth factors (Hesketh *et al.* 1985; Moolenaar, Tertoolen & de Laat, 1984).

REFERENCES

ADUNYAH, S. E. & DEAN, W. L. (1985). Inositol trisphosphate-induced Ca^{2+} release from human platelet membranes. *Biochem. biophys. Res. Commun.* **128**, 1274–1280.

AGHAJANIAN, G. K. (1985). Modulation of a transient outward current in serotonergic neurones by α_1-adrenoreceptors. *Nature, Lond.* **315**, 501–503.

AKHTAR, R. A. & ABDEL-LATIF, A. A. (1980). Requirements for calcium ions in acetylcholine-stimulated phosphodiesteratic cleavage of phosphatidyl-*myo*-inositol 4,5-bisphosphate in rabbit iris smooth muscle. *Biochem. J.* **192**, 783–791.

AKHTAR, R. A. & ABDEL-LATIF, A. A. (1984). Carbachol causes rapid phosphodiesteratic cleavage of phosphatidylinositol 4,5-bisphosphate and accumulation of inositol phosphates in rabbit iris smooth muscle: prazosin inhibits noradrenaline- and ionophore A23187-stimulated accumulation of inositol phosphates. *Biochem. J.* **224**, 291–300.

BARABAN, J. M., SNYDER, S. H. & ALGER, B. E. (1985). Protein kinase C regulates ionic conductance in hippocampal pyramidal neurones: electrophysiological effects of phorbol esters. *Proc. natn. Acad. Sci. U.S.A.* **82**, 2538–2542.

BATTY, I. R., NAHORSKI, S. R. & IRVINE, R. F. (1985). Rapid formation of inositol (1,3,4,5) tetrakisphosphate following muscarinic stimulation of rat cerebral cortical slices. *Biochem. J.* **232**, 211–215.

BAUKAL, A. J., GUILLEMETTE, G., RUBIN, R., SPÄT, A. & CATT, K. J. (1985). Binding sites for inositol trisphosphate in the bovine adrenal cortex. *Biochem. biophys. Res. Commun.* **133**, 532–538.

BERRIDGE, M. J. (1984). Inositol trisphosphate and diacylglycerol as second messengers. *Biochem. J.* **220**, 345–360.

BERRIDGE, M. J., HESLOP, J. P., IRVINE, R. F. & BROWN, K. D. (1984). Inositol trisphosphate formation and calcium mobilization in Swiss 3T3 cells in response to platelet-derived growth factor. *Biochem. J.* **222**, 195–201.

BERRIDGE, M. J. & IRVINE, R. F. (1984). Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature, Lond.* **312**, 315–321.

BIDEN, T. J., PRENTKI, M., IRVINE, R. F., BERRIDGE, M. J. & WOLLHEIM, C. B. (1984). Inositol 1,4,5-trisphosphate mobilizes intracellular Ca^{2+} from permeabilized insulin secreting cells. *Biochem. J.* **223**, 237–248.

BRASS, L. F. & JOSEPH, S. K. (1985). A role for inositol trisphosphate in intracellular Ca^{2+} mobilization and granule secretion in platelets. *J. biol. Chem.* **260**, 15 172–15 179.

BROWN, J. E. & RUBIN, L. J. (1984). A direct demonstration that inositol-trisphosphate induces an increase in intracellular calcium in *Limulus* photoreceptors. *Biochem. biophys. Res. Commun.* **125**, 1137–1142.

BROWN, J. E., RUBIN, L. J., GHALAYINI, A. J., TARVER, A. P., IRVINE, R. F., BERRIDGE, M. J. & ANDERSON, R. E. (1984). *Myo*-inositol polyphosphate may be a messenger for visual excitation in *Limulus* photoreceptors. *Nature, Lond.* **311**, 160–163.

BURGESS, G. M., GODFREY, P. P., MCKINNEY, J. S., BERRIDGE, M. J., IRVINE, R. F. & PUTNEY, J. W. (1984*a*). The second messenger linking receptor activation to internal Ca release in liver. *Nature, Lond.* **309**, 63–66.

BURGESS, G. M., IRVINE, R. F., BERRIDGE, M. J., MCKINNEY, J. S. & PUTNEY, J. W. (1984*b*). Actions of inositol phosphates on Ca pools in guinea-pig hepatocytes. *Biochem. J.* **224**, 741–746.

BURGESS, G. M., MCKINNEY, J. S., IRVINE, R. F. & PUTNEY, J. W. (1985). Inositol (1,3,4) trisphosphate and inositol (1,4,5) trisphosphate formation in Ca-mobilizing hormone activated cells. *Biochem. J.* **232**, 237–248.

BUSA, W. B., FERGUSON, J. E., JOSEPH, S. K., WILLIAMSON, J. R. & NUCCITELLI, R. (1985). Activation of frog (*Xenopus laevis*) eggs by inositol trisphosphate. I. Characterization of Ca^{2+} release from intracellular stores. *J. Cell Biol.* **101**, 677–682.

CAILLE, J., ILDEFONSE, M. & ROUGIER, O. (1985). Excitation–contraction coupling in skeletal muscle. *Prog. Biophys. molec. Biol.* **46**, 185–239.

CARSTEN, M. E. & MILLER, J. D. (1985). Ca^{2+} release by inositol trisphosphate from Ca^{2+}-transporting microsomes derived from uterine sarcoplasmic reticulum. *Biochem. biophys. Res. Commun.* **130**, 1027–1031.

CASTAGNA, M., TAKAI, Y., KAIBUCHI, K., SANO, K., KIKKAWA, U. & NISHIZUKA, Y. (1982). Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumour-promoting phorbol esters. *J. biol. Chem.* **257**, 7847–7851.

CLAPPER, D. L. & LEE, H. C. (1985). Inositol trisphosphate induces calcium release from nonmitochondrial stores in sea urchin egg homogenates. *J. biol. Chem.* **260**, 13 947–13 954.

COCKCROFT, S. & GOMPERTS, B. D. (1985). Role of guanine nucleotide binding protein in the activation of polyphosphoinositide phosphodiesterase. *Nature, Lond.* **314**, 534–536.

DAWSON, A. P. (1985). GTP enhances inositol trisphosphate-stimulated Ca^{2+} release from rat liver microsomes. *FEBS Letts* **185**, 147–150.

DAWSON, A. P. & IRVINE, R. F. (1984). Inositol (1,4,5) trisphosphate-promoted Ca^{2+} release from microsomal fractions of rat liver. *Biochem. biophys. Res. Commun.* **120**, 858–864.

DE RIEMER, S. A., STRONG, J. A., ALBERT, K. A., GREENGARD, P. & KACZMAREK, L. K. (1985). Enhancement of calcium current in *Aplysia* neurones by phorbol ester and protein kinase C. *Nature, Lond.* **313**, 313–316.

DOWNES, C. P. & MICHELL, R. H. (1985). Inositol phospholipid breakdown as a receptor-controlled generator of second messengers. In *Molecular Mechanisms of Transmembrane Signalling* (ed. P. Cohen & M. Houslay), pp. 3–56. Amsterdam: Elsevier Science Publishers.

DRØBAK, B. K. & FERGUSON, I. B. (1985). Release of Ca^{2+} from plant hypocotyl microsomes by inositol-1,4,5-trisphosphate. *Biochem. biophys. Res. Commun.* **130**, 1241–1246.

EPSTEIN, P. A., PRENTKI, M. & ATTIE, M. F. (1985). Modulation of intracellular Ca^{2+} in the parathyroid cell: release of Ca^{2+} from non-mitochondrial pools by inositol trisphosphate. *FEBS Letts* **188**, 141–144.

EUROPE-FINNER, G. N. & NEWELL, P. C. (1985). Inositol 1,4,5-trisphosphate induces cyclic GMP formation in *Dictyostelium discoideum*. *Biochem. biophys. Res. Commun.* **130**, 1115–1122.

FARLEY, J. & AUERBACH, S. (1986). Protein kinase C activation induces conductance changes in *Hermissenda* photoreceptors like those seen in associative learning. *Nature, Lond.* **319**, 220–223.

FEIN, A., PAYNE, R., CORSON, D. W., BERRIDGE, M. J. & IRVINE, R. F. (1984). Photoreceptor excitation and adaptation by inositol 1,4,5-trisphosphate. *Nature, Lond.* **311**, 157–160.

FISHER, S. K. & BARTUS, R. T. (1985). Regional differences in the coupling of muscarinic receptors to inositol phospholipid hydrolysis in guinea pig brain. *J. Neurochem.* **45**, 1085–1095.

GERSHENGORN, M. C., GERAS, E., PURRELLO, V. S. & REBECCHI, M. J. (1984). Inositol trisphosphate mediates thyrotropin-releasing hormone mobilization of nonmitochondrial calcium in rat mammatropic pituitary cells. *J. biol. Chem.* **259**, 1067–1068.

GONZALEZ, R. A. & CREWS, F. T. (1984). Characterization of the cholinergic stimulation of phosphoinositide hydrolysis in rat brain slices. *J. Neurosci.* **4**, 3120–3127.

HASHIMOTO, T., HIRATA, M. & ITO, Y. (1985). A role for inositol 1,4,5-trisphosphate in the initiation of agonist-induced contractions of dog tracheal smooth muscle. *Br. J. Pharmac.* **86**, 191–199.

HASLAM, R. J. & DAVIDSON, M. M. L. (1984). Receptor-induced diacylglycerol formation in permeabilized platelets: possible role for a GTP-binding protein. *J. Receptor Res.* **4**, 605–629.

HESKETH, T. R., MOORE, J. P., MORRIS, J. D. H., TAYLOR, M. V., ROGERS, J., SMITH, G. A. & METCALFE, J. C. (1985). A common sequence of calcium and pH signals in the mitogenic stimulation of eukaryotic cells. *Nature, Lond.* **313**, 481–484.

HIRATA, M., KUKITA, M., SASAGURI, T., SUEMATSU, E., HASHIMOTO, T. & KOGA, T. (1985*a*). Increase in Ca^{2+} permeability of intracellular Ca^{2+} store membrane of saponin-treated guinea pig peritoneal macrophages by inositol 1,4,5-trisphosphate. *J. Biochem., Tokyo* **97**, 1575–1582.

HIRATA, M., SASAGURI, T., HAMACHI, T., HASHIMOTO, T., KUKITA, M. & KOGA, T. (1985*b*). Irreversible inhibition of Ca^{2+} release in saponin-treated macrophages by the photoaffinity derivative of inositol-1,4,5-trisphosphate. *Nature, Lond.* **317**, 723–725.

HIRATA, M., SUEMATSU, E., HASHIMOTO, T., HAMACHI, T. & KOGA, T. (1984). Release of Ca^{2+} from a non-mitochondrial store site in peritoneal macrophages treated with saponin by inositol 1,4,5-trisphosphate. *Biochem. J.* **223**, 229–236.

HOKIN, L. E. (1985). Receptors and phosphoinositide-generated second messengers. *A. Rev. Biochem.* **54**, 205–235.

IMBODEN, J. B. & STOBO, J. D. (1985). Transmembrane signalling by the T cell antigen receptor. *J. exp. Med.* **161**, 446–456.

IRVINE, R. F., ÄNGGARD, E. A., LETCHER, A. J. & DOWNES, C. P. (1985). Metabolism of inositol (1,4,5) trisphosphate and inositol (1,3,4) trisphosphate in rat parotid glands. *Biochem. J.* **229**, 505–511.

IRVINE, R. F., BROWN, K. D. & BERRIDGE, M. J. (1984*a*). Specificity of inositol trisphosphate-induced calcium release from permeabilized Swiss-mouse 3T3 cells. *Biochem. J.* **221**, 269–272.

IRVINE, R. F., LETCHER, A. J., HESLOP, J. P. & BERRIDGE, M. J. (1986). The inositol tris/tetrakis phosphate pathway – demonstration of inositol (1,4,5)trisphosphate-3-kinase activity in animal tissue. *Nature, Lond.* (in press).

IRVINE, R. F., LETCHER, A. J., LANDER, D. J. & DOWNES, C. P. (1984*b*). Inositol trisphosphates in carbachol-stimulated rat parotid glands. *Biochem. J.* **223**, 237–243.

JOSEPH, S. K., THOMAS, A. P., WILLIAMS, R. J., IRVINE, R. F. & WILLIAMSON, J. R. (1984*a*). *Myo*-inositol 1,4,5-trisphosphate: a second messenger for the hormonal mobilization of intracellular Ca^{2+} in liver. *J. biol. Chem.* **259**, 3077–3081.

JOSEPH, S. K., WILLIAMS, R. J., CORKEY, B. E., MATSCHINSKY, F. M. & WILLIAMSON, J. R. (1984*b*). The effect of inositol trisphosphate on Ca^{2+} fluxes in insulin-secreting tumor cells. *J. biol. Chem.* **259**, 12952–12955.

KAIBUCHI, K., TAKAI, Y., SAWAMURA, M., HOSHIJIMA, M., FUJIKURA, T. & NISHIZUKA, Y. (1983). Synergistic functions of protein phosphorylation and calcium mobilization in platelet activation. *J. biol. Chem.* **258**, 6701–6704.

KNIGHT, D. E. & SCRUTTON, M. C. (1984). Cyclic nucleotides control a system which regulates Ca^{2+} sensitivity of platelet secretion. *Nature, Lond.* **309**, 66–68.

KOJIMA, I., KOJIMA, K., KREUTTER, D. & RASMUSSEN, H. (1984). The temporal integration of the aldosterone secretory response to angiotensin occurs via two intracellular pathways. *J. biol. Chem.* **259**, 14448–14457.

L'ALLEMAIN, G., PARIS, S. & POUYSSÉGUR, J. (1984). Growth factor action and intracellular pH regulation in fibroblasts. *J. biol. Chem.* **259**, 5809–5815.

MOOLENAAR, W. H., TERTOOLEN, L. G. J. & DE LAAT, S. W. (1984). Growth factors immediately raise cytoplasmic free Ca^{2+} in human fibroblasts. *J. biol. Chem.* **259**, 8066–8069.

MOOLENAAR, W. H., TSIEN, R. Y., VAN DER SAAG, P. T. & DE LAAT, S. W. (1983). Na^+/H^+ exchange and cytoplasmic pH in the action of growth factors in human fibroblasts. *Nature, Lond.* **304**, 645–648.

MUALLEM, S., SCHOEFFIELD, M., PANDOL, S. & SACHS, G. (1985). Inositol trisphosphate modification of ion transport in rough endoplasmic reticulum. *Proc. natn. Acad. Sci. U.S.A.* **82**, 4433–4437.

NABIKA, T., VELLETRI, P. A., LOVENBERG, W. & BEAVEN, M. A. (1985). Increase in cytosolic calcium and phosphoinositide metabolism induced by angiotensin II and arg-vasopressin in vascular smooth muscle cells. *J. biol. Chem.* **260**, 4661–4670.

NISHIZUKA, Y. (1984). The role of protein kinase C in cell surface signal transduction and tumor promotion. *Nature, Lond.* **308**, 693–697.

ORON, Y., DASCAL, N., NADLER, E. & LUPU, M. (1985). Inositol 1,4,5-trisphosphate mimics muscarinic response in *Xenopus* oocytes. *Nature, Lond.* **313**, 141–143.

O'ROURKE, F. A., HALENDA, S. P., ZAVOICO, G. B. & FEINSTEIN, M. B. (1985). Inositol 1,4,5-trisphosphate releases Ca^{2+} from a Ca^{2+}-transporting membrane vesicle fraction derived from human platelets. *J. biol. Chem.* **260**, 956–962.

PICARD, A., GIRAUD, F., LE BOUFFANT, F., SLADECZEK, F., LE PEUCH, C. & DOREE, M. (1985). Inositol 1,4,5-trisphosphate microinjection triggers activation, but not meiotic maturation in amphibian and starfish oocytes. *FEBS Letts* **182**, 446–480.

PRENTKI, M., BIDEN, T. J., JANJIC, D., IRVINE, R. F., BERRIDGE, M. J. & WOLLHEIM, C. B. (1984*a*). Rapid mobilization of Ca^{2+} from rat insulinoma microsomes by inositol-1,4,5-trisphosphate. *Nature, Lond.* **309**, 562–564.

PRENTKI, M., CORKEY, B. E. & MATSCHINSKY, F. M. (1985). Inositol 1,4,5-trisphosphate and the endoplasmic reticulum Ca^{2+} cycle of a rat insulinoma cell line. *J. biol. Chem.* **260**, 9185–9190.

PRENTKI, M., WOLLHEIM, C. B. & LEW, P. D. (1984*b*). Ca^{2+} homeostasis in permeabilized human neutrophils: characterization of Ca^{2+}-sequestering pools and the action of inositol 1,4,5-trisphosphate. *J. biol. Chem.* **259**, 1377–1378.

SMITH, J. B., SMITH, L., BROWN, E. R., BARNES, D., SABIR, M. A., DAVIS, J. S. & FARESE, R. V. (1984). Angiotensin II rapidly increases phosphatidate-phosphoinositide synthesis and phosphoinositide hydrolysis and mobilizes intracellular calcium in cultured arterial muscle cells. *Proc. natn. Acad. Sci. U.S.A.* **81**, 7812–7816.

SMITH, J. B., SMITH, L. & HIGGINS, B. L. (1985). Temperature and nucleotide dependence of calcium release by *myo*-inositol 1,4,5-trisphosphate in cultured vascular smooth muscle cells. *J. biol. Chem.* **260**, 14413–14416.

SOMLYO, A., BOND, M., SOMLYO, A. P. & SCARPA, A. (1985). Inositol trisphosphate-induced calcium release and contraction in vascular smooth muscle. *Proc. natn. Acad. Sci. U.S.A.* **82**, 5231–5235.

SOMLYO, A. V. & SOMLYO, A. P. (1968). Electromechanical and pharmacomechanical coupling in vascular smooth muscle. *J. Pharmac. exptl Ther.* **259**, 129–145.

SPÄT, A., BRADFORD, P. G., MCKINNEY, J. S., RUBIN, R. P. & PUTNEY, J. W. (1986*a*). A saturable receptor for ^{32}P-inositol-1,4,5-trisphosphate in hepatocytes and neutrophils. *Nature, Lond.* **319**, 514–516.

SPÄT, A., FABIATO, A. & RUBIN, R. P. (1986*b*). Binding of inositol trisphosphate by a liver microsomal fraction. *Biochem. J.* **233**, 929–932.

STANFIELD, P. R., NAKAJIMA, Y. & YAMAGUCHI, K. (1985). Substance P raises neuronal membrane excitability by reducing inward rectification. *Nature, Lond.* **315**, 498–501.

STOREY, D. J., SHEARS, S. B., KIRK, C. J. & MICHELL, R. H. (1984). Stepwise enzymatic dephosphorylation of inositol 1,4,5-trisphosphate to inositol in liver. *Nature, Lond.* **312**, 374–376.

STREB, H., BAYERDORFFER, 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.

STREB, H., IRVINE, R. F., BERRIDGE, M. J. & SCHULZ, I. (1983). Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate. *Nature, Lond.* **306**, 67–69.

SUEMATSU, E., HIRATA, M., HASHIMOTO, T. & KURIYAMA, H. (1984). Inositol 1,4,5-trisphosphate releases Ca^{2+} from intracellular store sites in skinned single cells of porcine coronary artery. *Biochem. biophys. Res. Commun.* **120**, 481–485.

SWANN, K. & WHITAKER, M. (1985). Stimulation of the Na/H exchanger of sea urchin eggs by phorbol ester. *Nature, Lond.* **314**, 274–277.

TURNER, P. R., JAFFE, L. A. & FEIN, A. (1985). Regulation of cortical vesicle exocytosis in sea urchin eggs by inositol 1,4,5-trisphosphate and GTP-binding protein. *J. Cell Biol.* **102**, 70–76.

VERGARA, J., TSIEN, R. Y. & DELAY, M. (1985). Inositol 1,4,5-trisphosphate: a possible chemical link in excitation–contraction coupling in muscle. *Proc. natn. Acad. Sci. U.S.A.* **82**, 6352–6356.

VOLPE, P., SALVIATI, G., DI VIRGILIO, F. & POZZAN, T. (1985). Inositol 1,4,5-trisphosphate induces calcium release from sarcoplasmic reticulum of skeletal muscle. *Nature, Lond.* **316**, 347–349.

WALOGA, G. & ANDERSON, R. E. (1985). Effects of inositol 1,4,5-trisphosphate injections into salamander rods. *Biochem. biophys. Res. Commun.* **126**, 59–62.

WHITAKER, M. & IRVINE, R. F. (1984). Inositol 1,4,5-trisphosphate microinjection activates sea urchin eggs. *Nature, Lond.* **312**, 636–639.

WILSON, D. B., CONNOLLY, T. M., BROSS, T. E., MAJERUS, P. W., SHERMAN, W. R., TYLER, A. N., RUBIN, L. J. & BROWN, J. E. (1985*a*). Isolation and characterization of the inositol cyclic phosphate products of polyphosphoinositide cleavage by phospholipase C. *J. biol. Chem.* **260**, 13 496–13 501.

WILSON, D. B., NEUFELD, E. J. & MAJERUS, P. W. (1985*b*). Phosphoinositide interconversion in thrombin-stimulated human platelets. *J. biol. Chem.* **260**, 1046–1051.

YAMAMOTO, H. & VAN BREEMEN, C. (1985). Inositol 1,4,5-trisphosphate releases calcium from skinned cultured smooth muscle cells. *Biochem. biophys. Res. Commun.* **130**, 270–274.