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REVIEW ARTICLE

Inositol trisphosphate and diacylglycerol as second messengers

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Introduction

Hormones and neurotransmitters that use calcium as a second messenger specifically hydrolyse membrane phosphoinositides. This perturbation of membrane phospholipids may represent a fundamental transducing mechanism that initiates a signal cascade resulting in the mobilization of calcium, the activation of protein kinase C, the release of arachidonic acid (for the synthesis of prostaglandins, thromboxanes and leukotrienes) and the stimulation of guanylate cyclase to form cyclic GMP. A characteristic feature of these multifunctional receptors, therefore, is that they are linked to a membrane transducing mechanism that spawns a number of putative second messengers (Michell, 1975, 1979; Irvine & Dawson, 1980; Berridge, 1981, 1982; Irvine *et al.*, 1982; Takai *et al.*, 1982; Nishizuka, 1983). The key reaction of this transducing mechanism is a receptor-mediated hydrolysis of the phosphoinositides to give two products (diacylglycerol and inositol trisphosphate), both of which may function as second messengers to initiate the signal cascade. Inositol trisphosphate seems to act by mobilizing intracellular calcium, whereas diacylglycerol stimulates protein phosphorylation. Before describing the biochemical details of this bifurcating signal pathway, I will briefly consider some general properties of calcium-mobilizing receptors that function by hydrolysing the phosphoinositides.

Abbreviations used: although this Review uses the abbreviations recommended by IUPAC–IUB, it was suggested at a recent meeting (the Chilton Conference on Inositol and Phosphoinositides, Dallas, January 1984) that these might be used interchangeably with simpler abbreviations (shown in parentheses) that have the advantage of being easier to pronounce. Thus: PtdIns (PI), phosphatidylinositol; PtdIns4*P* (PIP), phosphatidylinositol 4-phosphate; PtdIns4,5P_2 (PIP_2), phosphatidylinositol 4,5-bisphosphate; Ins1*P* (IP_1), inositol 1-phosphate; Ins1,4P_2 (IP_2), inositol 1,4-bisphosphate; Ins1,4,5P_3 (IP_3), inositol 1,4,5-trisphosphate; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

Some general properties of receptors that hydrolyse phosphoinositides

The initial discovery that hormones have effects on phosphoinositide metabolism was made by Hokin & Hokin (1953, 1955). Since then it has become apparent that a large number of different agonists can stimulate an increase in the metabolism of membrane phosphoinositides. These agonists include classical neurotransmitters such as acetylcholine (Weiss & Putney, 1981; Poggioli *et al.*, 1983; Fisher *et al.*, 1983; Brown & Brown, 1983), noradrenaline (Prpić *et al.*, 1982; Uchida *et al.*, 1982), histamine (Jones *et al.*, 1979), 5-hydroxytryptamine (Fain & Berridge, 1979*b*), in addition to more complex molecules such as the peptides vasopressin (Creba *et al.*, 1983; Rhodes *et al.*, 1983; Thomas *et al.*, 1983), substance P (Hanley *et al.*, 1980; Weiss *et al.*, 1982*a*), angiotensin (Billah & Michell, 1979; Prpić *et al.*, 1982), pancreozymin (Calderon *et al.*, 1980), caerulein (Putney *et al.*, 1983), thyrotropin-releasing hormone (Martin, 1983; Rebecchi *et al.*, 1983), platelet-derived growth factor (Habenicht *et al.*, 1981) and epidermal growth factor (Sawyer & Cohen, 1981). In those cases where the pharmacology is well-established, the change in phosphoinositide metabolism is always specific for one particular receptor class (Michell, 1975, 1979; Berridge, 1980; Michell & Kirk, 1981) such as the muscarinic cholinergic receptor, the α_1-adrenergic receptor, the H_1-histaminergic receptor or the V_1-vasopressin receptor. A characteristic feature of these receptors is that they are multifunctional in nature in that they have been implicated as part of a general transducing mechanism for the mobilization of calcium, for the activation of protein kinase C, for the release of arachidonic acid and for the activation of guanylate cyclase (Berridge, 1981, 1982). In order to generate this battery of signal molecules, these receptors employ a general transducing mechanism based on the hydrolysis of a specific membrane phosphoinositide.

Agonist-dependent phosphoinositide metabolism

Structure of the phosphoinositides

There are a number of reviews dealing with different aspects of agonist-dependent phosphoinositide metabolism (Hawthorne, 1964; Hawthorne & White, 1975; Michell, 1975, 1979, 1982*a*,*b*; Hawthorne & Pickard, 1979; Berridge, 1980, 1981, 1982; Michell & Kirk, 1981; Putney, 1981, 1982; Downes, 1982, 1983; Downes & Michell, 1982; Fain, 1982; Irvine *et al.*, 1982; Abdel-Latif, 1983; Agranoff, 1983; Fain *et al.*, 1983; Farese, 1983). So far, the collective term phosphoinositide has been used to describe the three anionic phospholipids that contain *myo*-inositol in their head groups (Hawthorne, 1964; Hawthorne & White, 1975; Michell, 1975; Berridge, 1981). The most abundant form is PtdIns that contains *myo*-inositol attached to phosphate through the hydroxyl on the 1-position (Fig. 1). The other two members are formed by sequential phosphorylation of hydroxyl groups on the 4- and 5-positions of PtdIns (Hawthorne & Pickard, 1979; Downs & Michell, 1982). Membranes contain a PtdIns kinase (Fig. 1*a*) that specifically phosphorylates the hydroxyl on the 4-position to produce PtdIns4*P*. A PtdIns4*P* kinase (Fig. 1*b*) phosphorylates the hydroxyl on the 5-position to form PtdIns4,5P_2. The conversion of PtdIns to these two polyphosphoinositides can be reversed by two phosphomonoesterases that specifically remove phosphate from the 5- and 4-positions (Figs. 1*c* and 1*d*).

While the polyphosphoinositides are usually present in cells in minor amounts compared with PtdIns, the exact proportions of these three phosphoinositides within the plasma membrane of most cells has not been determined. The important point to establish, however, is that the kinases and phosphomonoesterases that maintain this dynamic equilibrium between these three lipids are some of the most active enzymes in the cell (Hawthorne & Pickard, 1979). A change in the level of any one of the trio will be rapidly 'buffered' by interconversions of the other two participants. An important question to decide is which of these three lipids is used by the receptor mechanism. While most previous attention was focused on PtdIns, there is a growing acceptance that it is the polyphosphoinositides that are the immediate precursors used by receptors.

Agonist-dependent cleavage of the polyphosphoinositides

The first indication that the polyphosphoinositides might be hydrolysed as part of a receptor mechanism was obtained using synaptosomes that responded to acetylcholine with an increase in the level of Ins1,4P_2 (Durell *et al.*, 1968). Acetylcholine was also found to reduce the ^{32}P-labelling of the polyphosphoinositides (Schacht & Agranoff, 1972). Such an effect on the polyphosphoinositides was confirmed in iris smooth muscle where acetylcholine stimulated the breakdown of PtdIns4,5P_2 (Abdel-Latif *et al.*, 1977). This breakdown of labelled PtdIns4,5P_2 was mediated by a phospho-

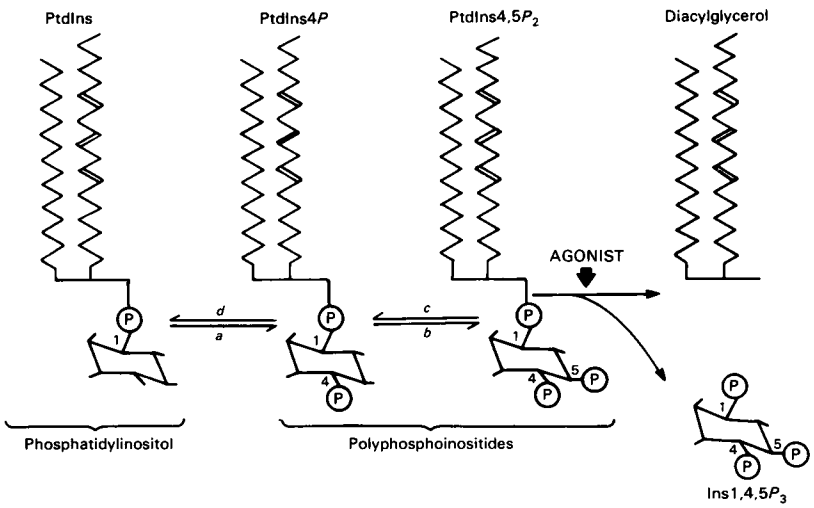

Fig. 1. *Phosphorylation–dephosphorylation pathways of phosphoinositide metabolism*
These phosphoinositides are mainly confined to the inner leaflet of the plasma membrane. Agonists act by stimulating the hydrolysis of PtdIns4,5P_2 by a phosphodiesterase enzyme to give diacylglycerol and Ins1,4,5P_3 (see the text for further details).

diesterase, because Akhtar & Abdel-Latif (1980) demonstrated that acetylcholine stimulated an accumulation of the expected water-soluble metabolite Ins1,4,5P_3 in addition to Ins1P. However, since this receptor-mediated cleavage of PtdIns4,5P_2 required calcium ions and could be mimicked by treating smooth muscle cells with the ionophore A23187 (Akhtar & Abdel-Latif, 1980), the possibility that it might play a role in generating calcium signals was ignored.

A re-awakening in the interest in the polyphosphoinositides began with studies on the liver that showed that calcium-mobilizing agonists such as vasopressin caused a very rapid breakdown particularly of PtdIns4,5P_2 (Kirk *et al.*, 1981; Michell *et al.*, 1981; Kirk, 1982; Creba *et al.*, 1983). Unlike the situation in smooth muscle described earlier, the breakdown of these polyphosphoinositides was only partially reduced by omitting calcium from the bathing medium and was not induced by the ionophore A23187. On the basis of these observations on liver, Michell *et al.* (1981) have proposed that the initial response to hormones is a breakdown of the polyphosphoinositides and that changes in the level of PtdIns occur indirectly as it is consumed to replace the polyphosphoinositides. The observation that agonists might act by stimulating a rapid breakdown of the polyphosphoinositides has been confirmed for liver (Rhodes *et al.*, 1983; Thomas *et al.*, 1983) and has been extended to a number of other cell types including the parotid gland (Weiss *et al.*, 1982*a*; Putney, 1982; Downes & Wusteman, 1983), blood platelets (Billah & Lapetina, 1982*b*; Agranoff *et al.*, 1983; Mauco *et al.*, 1983), pancreas (Putney *et al.*, 1983), neutrophils (Volpi *et al.*, 1983), cloned rat pituitary cells (Martin, 1983; Rebecchi & Gershengorn, 1983) and insect salivary gland (Berridge, 1983; Litosch *et al.*, 1984).

Agonist-dependent accumulation of inositol phosphates

Direct evidence that the breakdown of the polyphosphoinositides was carried out through the phosphodiesterase pathway was provided by studying the water-soluble inositol phosphates (Agranoff *et al.*, 1983; Berridge, 1983; Berridge *et al.*, 1983; Martin, 1983; Rebecchi & Gershengorn, 1983; Downes & Wusteman, 1983). Stimulation of blood platelets with thrombin has revealed a rapid increase in the level of Ins1,4,5P_3 coinciding with a fall in the level of PtdIns4,5P_2 (Agranoff *et al.*, 1983). Likewise, stimulation of the insect salivary gland, mammalian parotid and brain resulted in substantial accumulation of Ins1,4,5P_3, but there were also significant increases in the levels of Ins1,4P_2 and Ins1P (Berridge *et al.*, 1983). These last two inositol phosphates could be derived either from dephosphorylation of Ins1,4,5P_3 or from cleavage of the corresponding phospholipid. In order to decide between these two alternatives, a kinetic analysis was carried out on how fast these inositol phosphates appeared following stimulation. When the fly salivary gland was stimulated with 5-hydroxytryptamine, there was a very rapid accumulation of Ins1,4,5P_3 that increased 5-fold within 5s (Berridge, 1983). Over the same time period, there was also a rapid increase in Ins1,4P_2 but absolutely no change in the levels of either Ins1P or free inositol, although the latter did increase at later time periods. The time course of the appearance of these inositol phosphates, as just described for the insect gland, has been confirmed in pituitary cells responding to thyrotropin-releasing hormone (Martin, 1983; Rebecchi & Gershengorn, 1983; Drummond *et al.*, 1984).

The fact that Ins1,4,5P_3 and Ins1,4P_2 increased in the insect salivary gland prior to any change in the level of Ins1P or free inositol indicates that the primary action of 5-hydroxytryptamine is to stimulate the hydrolysis of the polyphosphoinositides rather than PtdIns (Berridge, 1983). While these studies on inositol phosphates clearly indicate that PtdIns4,5P_2 is hydrolysed as part of the receptor mechanism, there is some doubt concerning the participation of PtdIns4P. The rapid formation of Ins1,4P_2 might suggest that PtdIns4P is indeed hydrolysed, but as Ins1,4P_2 can also arise through dephosphorylation of Ins1,4,5P_3 (Fig. 2) there is doubt concerning the role of this lipid. Therefore, in the following discussion it will be assumed, for the sake of simplicity, that PtdIns4,5P_2 in the primary substrate used by the receptor mechanism (Fig. 2), bearing in mind that PtdIns4P may also be hydrolysed. These studies on phospholipid breakdown and release of inositol phosphates all point to the fact that a very early event in the action of calcium-mobilizing receptors is the hydrolysis of PtdIns4,5P_2, as outlined in Fig. 2.

The scheme shown in Fig. 2 illustrates how this proposed agonist-dependent hydrolysis of PtdIns4,5P_2 is related to phosphoinositide metabolism as a whole. The interaction of an agonist with its receptor induces the hydrolysis of PtdIns4,5P_2 by the relevant phosphodiesterase enzyme to yield two putative intracellular signals, diacylglycerol and Ins1,4,5P_3. Ultimately, of course, it is necessary to resynthesize the lipid precursor so that the cycle may continue. The two products formed during the hydrolysis of PtdIns4,5P_2 are mostly conserved by being fed into a lipid cycle and an inositol phosphate cycle that finally combine to reform PtdIns (Fig. 2). Studies on red blood cells has revealed the presence of an active inositol trisphosphatase that specifically dephosphorylates Ins1,4,5P_3 to Ins1,4P_2 (Downes *et al.*, 1982). A

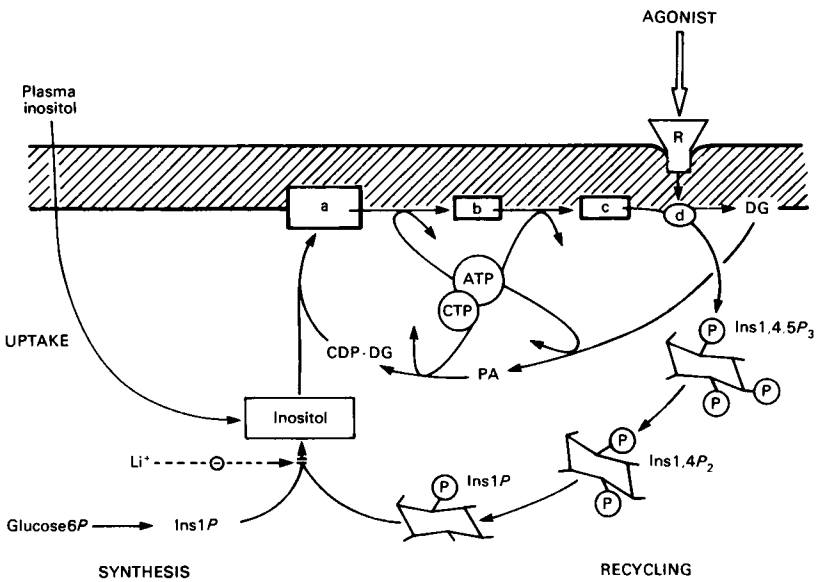

Fig. 2. *Agonist-dependent phosphoinositide metabolism*
A proportion of the membrane pool of PtdIns (a) is phosphorylated to PtdIns4P (b) and PtdIns4,5P_2 (c). The agonist acts on its receptor (R) to stimulate the hydrolysis of PtdIns4,5P_2 by a phosphodiesterase (d) to yield diacylglycerol (DG) and inositol trisphosphate (Ins1,4,5P_3). The latter is recycled back through an inositol phosphate cycle to free inositol for resynthesis to PtdIns. Lithium prevents the formation of inositol by inhibiting the inositol 1-phosphatase that dephosphorylates the Ins1P being recycled or originating from synthesis *de novo*. A diacylglycerol kinase phosphorylates diacylglycerol to form phosphatidic acid (PA) that is primed by interacting with CTP to form the cytidine diphosphate diacylglycerol (CDP·DG) that recombines with inositol to replenish the pool of PtdIns (a).

similar enzyme exists in the insect salivary gland, where there is also some evidence for an inositol bisphosphatase that converts Ins1,4P_2 to Ins1P (Berridge *et al.*, 1983). The final step is the conversion of Ins1P to free *myo*-inositol by an inositol 1-phosphatase (Hallcher & Sherman, 1980). A fascinating property of this enzyme is that it is inhibited by lithium (Fig. 2) (Hallcher & Sherman, 1980; Berridge *et al.*, 1982). By disrupting the inositol phosphate cycle, lithium results in a decrease in free inositol with a corresponding increase in the level of Ins1P (Allison & Stewart, 1971; Allison *et al.*, 1976; Sherman *et al.*, 1981; Berridge *et al.*, 1982). This inhibitory action of lithium greatly amplifies the response and can thus be employed as a new and sensitive assay to detect receptor-mediated inositol lipid breakdown in a wide range of different tissues, and particularly in brain (Berridge *et al.*, 1982; Downes, 1982; Brown *et al.*, 1984).

The immediate substrate for the receptor mechanism is PtdIns4,5P_2 that must be constantly replenished by phosphorylation of PtdIns using the kinase enzymes described earlier (Fig. 2). Since the formation of the phosphoinositides is dependent on ATP (Fig. 2), the receptor mechanism should be sensitive to changes in energy metabolism. When respiratory metabolism in the pancreas is inhibited by dinitrophenol, acetylcholine is no longer able to break down PtdIns (Hokin, 1974). In the case of blood platelets (Holmsen *et al.*, 1982), rat liver (Prpić *et al.*, 1982), parotid (Poggioli *et al.*, 1983; Downes & Wusteman, 1983) and insect salivary gland (Berridge *et al.*, 1984), the agonist-dependent breakdown of phosphoinositides was also shown to require energy. On the basis of the hypothesis outlined in Fig. 2, the energy-requiring processes can be identified as the two-step conversion of PtdIns to the PtdIns4,5P_2 that is used by the receptor process. The cell must expend energy to ensure that it has a signalling system that not only can respond rapidly to external signals but can maintain this responsiveness over an extended period of time.

Phosphoinositide levels and receptor sensitivity

There are some reports suggesting that changes in receptor sensitivity resulting from denervation may be caused by alterations in inositol lipid metabolism (Abdel-Latif *et al.*, 1979; Takenawa *et al.*, 1983; Downes *et al.*, 1983). On the basis of the model described in Fig. 2, it is possible to predict several ways by which the availability of

PtdIns4,5P_2 might be altered to give such changes in receptor sensitivity. Subtle alterations in the activities of the kinases or phosphomonoesterases (Fig. 1) will shift the position of the equilibrium that exists between the three phosphoinositides, resulting in a change in the size of the PtdIns4,5P_2 pool. Adrenocorticotropin may act in such a way in the brain, where it is thought to reduce the activity of the enzyme that converts PtdIns4*P* to PtdIns4,5P_2 (Jolles *et al.*, 1980, 1981). Aloyo *et al.* (1983) have proposed that this reduction in the level of PtdIns4,5P_2 might be mediated by C-kinase.

Another method whereby the level of PtdIns4,5P_2 might be varied is to alter the absolute level of all three phosphoinositides. Such a mechanism is made more likely because cells seem to contain a relatively small hormone-sensitive pool of inositol lipids, perhaps that located in the plasma membrane, that is separate from the remaining phosphoinositides (Fain & Berridge, 1979*b*; Monaco, 1982; Billah & Lapetina, 1982*a*; Shukla & Hanahan, 1982; Monaco & Woods, 1983). This hormone-sensitive pool amounts to about 8% in the insect salivary gland (Fain & Berridge, 1979*b*) and about 17% in rat mammary tumour cells (Monaco, 1982). In the case of the insect salivary gland, depletion of this hormone-sensitive pool resulted in inactivation of calcium signalling, which was restored if the cells were allowed to reconstitute this lipid pool (Fain & Berridge, 1979*b*). The maintenance of this hormone-sensitive pool will depend upon the balance between the rate at which the phosphoinositides are degraded by the receptor mechanism and their rate of synthesis. A high level of free *myo*-inositol may be essential in order for resynthesis to balance receptor-mediated degradation. In nerve endoneurium, a small reduction in the level of *myo*-inositol resulted in a large decline in energy utilization that returned to normal when the level of *myo*-inositol was restored (Simmons *et al.*, 1982). The function of α_1-adrenergic stimuli in regenerating liver is essentially impaired 3 days after hepatectomy, but this loss of sensitivity can be prevented simply by adding free inositol (Huerta-Bahena & García-Sáinz, 1983). The ability of lithium to lower inositol levels in the brain could conceivably alter receptor sensitivity and so account for its therapeutic action in controlling manic-depressive illness.

The action of lithium

The link between lithium and phosphoinositides began when Allison & Stewart (1971) discovered that there was a considerable reduction in the level of *myo*-inositol in the brain of lithium-treated rats. Subsequent studies revealed that lithium not only lowered the level of *myo*-inositol but it simultaneously raised the concentration of Ins1*P* (Allison *et al.*, 1976; Sherman *et al.*, 1981). These effects are brought about by a severe inhibition by lithium of the enzyme *myo*-inositol 1-phosphatase (Fig. 2) that liberates the free *myo*-inositol necessary to resynthesize PtdIns (Hallcher & Sherman, 1980). Lithium thus inhibits the supply of *myo*-inositol originating not only from the inositol phosphate cycle but also from synthesis *de novo* (Fig. 2). The only other supply is plasma inositol, which is freely available to most cells except those in the brain or testis that are closeted behind barriers that are relatively impermeable to *myo*-inositol. When [^{3}H]inositol was infused into plasma, very little label entered the brain (Margolis *et al.*, 1971; Spector & Lorenzo, 1975). The blood–brain barrier possesses a saturable transport mechanism that limits the amount of inositol that can be sequestered from plasma (Spector & Lorenzo, 1975). Even nerves in the periphery seem to be denied access to plasma inositol because of the impermeability of the perineurial membrane (Gillon & Hawthorne, 1983). Since nerve cells are much more reliant on synthesis *de novo* to maintain their supply of *myo*-inositol, they may be much more susceptible to the action of lithium than other tissues that have free access to dietary *myo*-inositol circulating in the plasma.

Disruption of *myo*-inositol metabolism in the brain by lithium may lead to a decline in the level of the phosphoinositides, and this could represent its therapeutic action in the control of manic-depressive illness (Sherman *et al.*, 1981; Berridge *et al.*, 1982). The suggestion is that lithium acts by lowering the level of *myo*-inositol which results in a decline in the synthesis of the PtdIns necessary to maintain the hormone-sensitive pool of phosphoinositide discussed earlier. Prolonged seizures and extensive brain damage occurs if rats on lithium treatment are given a cholinomimetic, such as pilocarpine (Honchar *et al.*, 1983), which will enhance the breakdown and hence the decline in phosphoinositides. Lithium may control manic-depressive illness through a partial inactivation of those receptors that use phosphoinositides as part of their transducing mechanism.

In summary, agonists acting on calcium-mobilizing receptors stimulate the specific hydrolysis of PtdIns4,5P_2 to form diacylglycerol and Ins1,4,5P_3. These two products are then converted back into the PtdIns that functions as a reservoir of lipid that is phosphorylated to replenish the pool of PtdIns4,5P_2. Any process that interferes with the supply of free inositol could lead to changes in receptor sensitivity by altering the size of the hormone-sensitive pool of inositol lipids required by the receptor mechanism. The key feature of this

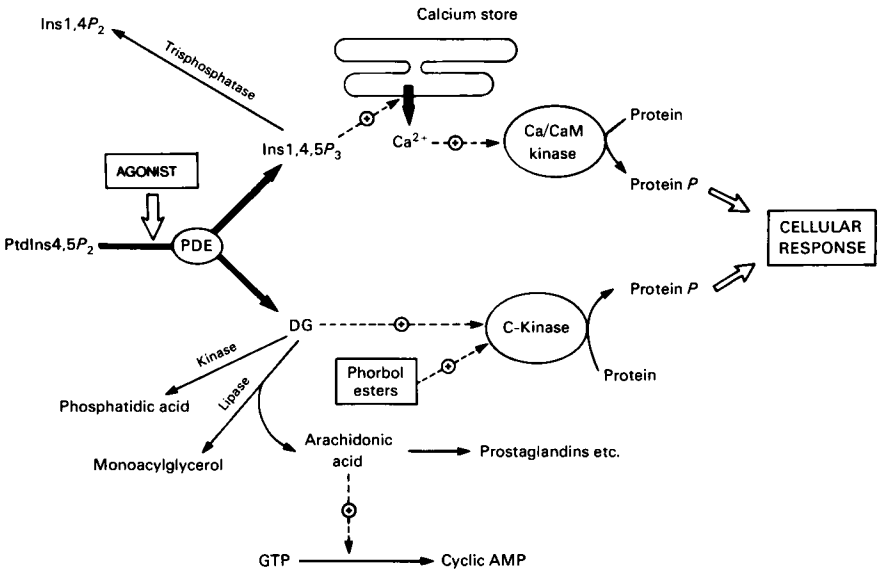

Fig. 3. *Second messenger role of diacylglycerol (DG) and inositol trisphosphate (Ins1,4,5P_3)*
A large number of cellular processes may be switched on through the combined action of these two parallel signal pathways that begin with the agonist-dependent hydrolysis of PtdIns4,5P_2 to give Ins1,4,5P_3 and diacylglycerol. The former is removed by a trisphosphatase, whereas diacylglycerol is converted to either phosphatidic acid or monoacylglycerol. Ins1,4,5P_3 acts to mobilize intracellular calcium, whereas diacylglycerol stimulates the same C-kinase that can be activated by phorbol esters. Abbreviations used: PDE, phosphodiesterase; CaM, calmodulin.

receptor mechanism is the cleavage of PtdIns4,5P_2 into diacylglycerol and Ins1,4,5P_3, which then function as second messengers to trigger subsequent steps in the signal cascade.

The second messenger function of inositol trisphosphate and diacylglycerol

A characteristic feature of calcium-mobilizing receptors is that they initiate a signal cascade leading to the formation of a number of putative second messengers (Berridge, 1981, 1982, 1984; Irvine *et al.*, 1982; Takai *et al.*, 1982). The initial event in this cascade is the hydrolysis of PtdIns4,5P_2 to yield Ins1,4,5P_3 and diacylglycerol. The remaining parts of the signal cascade concern the release of arachidonic acid and the formation of cyclic GMP. The crucial first step of this cascade is the formation of Ins1,4,5P_3 and diacylglycerol, both of which have many of the properties of classical second messengers such as cyclic AMP. They are produced very rapidly, they act at very low concentrations and there are specific mechanisms for removing these messengers once the external signal is withdrawn (Fig. 3). These typical second-messenger properties will be explored further in the following more detailed description of the signal cascade.

Calcium mobilization: the role of inositol trisphosphate

Michell (1975) has proposed that the hydrolysis of phosphoinositides is responsible for mobilizing calcium. A detailed discussion on the relationship between inositol lipid metabolism and calcium is provided by Michell *et al.* (1981) who stress that an important aspect of Michell's hypothesis is that the breakdown of PtdIns4,5P_2 should occur at levels of intracellular calcium that are present in a resting cell. In other words, the receptor-mediated breakdown of the inositol lipids must precede, and thus be independent of, any increase in the intracellular level of calcium. One way of testing this prediction is to establish the calcium-dependency of the PtdIns4,5P_2 phosphodiesterase. When the activity of this enzyme extracted from rat brain was studied using ionic conditions that match the intracellular environment, there was little change in activity when calcium was varied over its physiological range of 10^{-7}–10^{-5}M (Irvine *et al.*, 1984). This study *in vitro* on the calcium sensitivity of the enzyme is entirely consistent with observations on intact cells such as liver (Billah & Michell, 1979; Kirk *et al.*, 1981; Michell *et al.*, 1981), parotid gland (Jones & Michell, 1975; Weiss *et al.*, 1982*a*,*b*), insect salivary gland (Fain & Berridge, 1979*a*), mast cells (Cockcroft & Gomperts, 1979),

blood platelets (Billah & Lapetina, 1982*c*), pancreas (Putney *et al.*, 1983) and leukaemic basophils (Beaven *et al.*, 1984) where the agonist-dependent breakdown of phosphoinositides can occur independently of changes in cytosol calcium. Although there are examples where the stimulation of phosphoinositide metabolism does seem to depend upon an increase in the intracellular level of calcium, as occurs in neutrophils (Cockcroft *et al.*, 1980*a*,*b*; Cockcroft, 1981, 1982) and pancreatic islets (Montague & Parkin, 1980; Axen *et al.*, 1983), by far the majority of the cell systems analysed so far are independent of calcium. This early but small calcium-independent hydrolysis of PtdIns4,5P_2 may sometimes be masked by a massive increase in calcium-dependent lipid metabolism as part of an amplification loop responsible for forming large quantities of arachidonic acid metabolites. Many cells have a calcium-dependent phospholipase A_2 enzyme that is particularly important in cells such as neutrophils and blood platelets that release large amounts of arachidonic acid as part of an amplification loop. Calcium-independent and calcium-dependent changes in phosphoinositide metabolism may thus co-exist in the same cell (Egawa *et al.*, 1981; Farese *et al.*, 1982). With regard to calcium signalling, therefore, we must concentrate on the agonist-dependent hydrolysis of PtdIns4,5P_2 that can occur in many cell types at resting levels of calcium.

What has been missing is the biochemical pathway linking the phosphoinositide response to calcium mobilization. The search for such a link is made more difficult by virtue of the fact that cells use different sources of signal calcium which may either enter from outside across the plasma membrane or can be released from an intracellular reservoir, most likely to be the endoplasmic reticulum. Certain cells, such as the adrenal medulla, mast cells and nerve cells, seem to rely predominantly upon extracellular calcium, whereas others (e.g. liver, pancreas, parotid, blood platelets, anterior pituitary and certain smooth muscle cells) use intracellular calcium, especially during early periods of stimulation, in addition to extracellular calcium. However, the link between the activation of surface receptors and the mobilization of calcium from intracellular reservoirs has been missing. The very rapid formation of inositol phosphates upon activation of the insect salivary gland with 5-hydroxytryptamine suggested that inositol trisphosphate may function as a second messenger to release internal calcium (Berridge, 1983; Berridge & Irvine, 1984).

The finding that inositol trisphosphate induced the release of calcium from the endoplasmic reticulum of permeabilized pancreatic cells (Streb *et al.*, 1983) and hepatocytes (Burgess *et al.*, 1984; Joseph *et al.*, 1984) provides some evidence to support this hypothesis. The rat pancreatic cells were permeabilized by incubation in a low-calcium medium (Streb & Schulz, 1983) and the Ins1,4,5P_3-induced release of calcium was measured by using a calcium electrode. In the case of the hepatocytes, saponin was used to permeabilize the plasma membrane and calcium mobilization was monitored by measuring the release of $^{45}Ca^{2+}$ from prelabelled cells (Burgess *et al.*, 1984) or by measuring extracellular calcium with an electrode or quin2 (Joseph *et al.*, 1984). The release of calcium by Ins1,4,5P_3 was half-maximal at approx. 0.5 μM in pancreas (Streb *et al.*, 1983) and between 0.1 and 0.2 μM for hepatocytes (Burgess *et al.*, 1984; Joseph *et al.*, 1984). After cell fractionation of rat insulinoma cells, Ins1,4,5P_3 released calcium from a microsomal fraction but not from mitochondria or secretory granules (Prentki *et al.*, 1984). The release mechanism was apparently specific for the trisphosphate because there was no release with Ins1,4P_2, Ins1P or inositol 1,2-cyclic phosphate (Streb *et al.*, 1983). The observation that the thrombin-induced loss of PtdIns4,5P_2 in platelets coincides with a decline of membrane-bound calcium as monitored by chlortetracycline fluorescence (Broekman, 1983) is entirely consistent with the idea that Ins1,4,5P_3 mobilizes intracellular calcium in these cells. It has not been established yet just how Ins1,4,5P_3 might release internal calcium. It may either act on an internal receptor to stimulate release or it may inhibit the uptake mechanisms responsible for sequestering intracellular calcium. Irrespective of the mechanism employed, Ins1,4,5P_3 will effectively 'short-circuit' this internal membrane system that functions to maintain low intracellular levels of calcium. Ins1,4,5P_3 may be responsible for controlling this calcium-buffering system even when the cell is at rest. The resting level of calcium may be adjusted up or down through small changes in the slow basal liberation of Ins1,4,5P_3. Hormones and neurotransmitters seem to act by stimulating an increase in the level of Ins1,4,5P_3 that then causes a larger mobilization of the stored calcium.

A more difficult question to answer is whether the hydrolysis of PtdIns4,5P_2 has any role to play in the entry of calcium across the plasma membrane. Some of the ideas that have been advanced to explain how these lipids might function to regulate the permeability of the plasma membrane include the opening of a channel (Michell *et al.*, 1977; Michell, 1982*a*) or the formation of phosphatidic acid that could function as a calcium ionophore (Michell *et al.*, 1977; Salmon & Honeyman, 1980; Putney *et al.*, 1980; Putney, 1981; Harris *et al.*, 1981; Ohsako & Deguchi, 1981; Imai *et al.*,

1982; Lapetina, 1982, 1983; Weiss *et al.*, 1982*b*). While some studies using liposomes have supported an ionophoric role for phosphatidic acid (Serhan *et al.*, 1981), others have obtained negative results (Holmes & Yoss, 1983). Another suggestion is that agonists might alter calcium permeability indirectly through some intracellular messenger that may be either calcium (Petersen & Maruyama, 1983) or Ins1,4,5P_3 (Streb *et al.*, 1983; Burgess *et al.*, 1984). In some instances, agonists may be capable of opening calcium channels in the plasma membrane without any change in phosphoinositide metabolism.

Before further efforts are made to search for a mechanism of regulating calcium entry, we need to obtain unequivocal evidence that agonist-dependent inositol lipid metabolism does indeed induce some change in the permeability of the plasma membrane. Many previous studies purporting to show that increases in intracellular calcium were caused by an enhanced entry of external calcium might be explained by Ins1,4,5P_3 acting primarily to mobilize intracellular calcium. Entry of external calcium is clearly important, especially to maintain calcium signals over long periods. However, it may not be necessary for the permeability of calcium to increase, because a calcium signal could be maintained equally as well by inhibiting the calcium-extrusion mechanism (Lin *et al.*, 1983; Prpić *et al.*, 1984). Indeed, there are indications that Ca^{2+}-ATPase activity might depend upon changes in membrane phosphoinositide levels (Buckley & Hawthorne, 1972; Varsanyi *et al.*, 1983). This possible regulation of calcium pumping through the phosphoinositides has been implicated in short-term memory (Penniston, 1983).

In summary, an important consequence of the agonist-dependent hydrolysis of PtdIns4,5P_2 is the formation of Ins1,4,5P_3 that may function to release calcium from intracellular reservoirs. There may also be an increase in the permeability of the plasma membrane to calcium, but the precise link between phosphoinositide metabolism and calcium entry remains to be established. In addition to controlling calcium mobilization, Ins1,4,5P_3 may also function as a second messenger to regulate enzymic reactions or the permeability of the plasma membrane to monovalent cations.

C-kinase activation: the role of diacylglycerol

The other major product of PtdIns4,5P_2 hydrolysis is diacylglycerol, which also has an important second-messenger function in that it activates C-kinase to phosphorylate specific proteins (Takai *et al.*, 1979; Kishimoto *et al.*, 1980; Kaibuchi *et al.*, 1982; Takai *et al.*, 1982; Nishizuka, 1983). In keeping with its proposed role as a second messenger, very rapid increases in the level of diacylglycerol have been measured upon stimulation of blood platelets (Rittenhouse-Simmons, 1979; Bell *et al.*, 1979; Imai *et al.*, 1982; Prescott & Majerus, 1983), mast cells (Kennerly *et al.*, 1979), thyroid (Igarashi & Kondo, 1980), 3T3 cells (Habenicht *et al.*, 1981), pituitary cells (Martin, 1983; Rebecchi *et al.*, 1983; Drummond *et al.*, 1984) and liver (Thomas *et al.*, 1983). In the case of mast cells, there was a rapid increase in the absolute level of diacylglycerol, which would be consistent with its role as a second messenger to activate C-kinase. The latter is an ubiquitous enzyme present in all organs, particularly in brain, extending through various phyla including the insects (Kuo *et al.*, 1980; Takai *et al.*, 1982). The activation of C-kinase by diacylglycerol is a complex process that requires calcium and phosphatidylserine as cofactors. A particularly fascinating aspect of this C-kinase is its activation by tumour-promoting phorbol esters such as TPA (Castagna *et al.*, 1982). Rohrscheider & Boutwell (1973) predicted that phorbol esters might act by simulating the biological activity of some endogenous substance, which now appears to be the second messenger diacylglycerol. Just as morphine mimicks the action of the natural enkephalins, so these phorbol esters mimick the stimulatory effect of the diacylglycerol normally produced as part of the receptor mechanism (Fig. 3). There is growing evidence that the cellular receptor for the phorbol esters is C-kinase (Niedel *et al.*, 1983; Sando & Young, 1983). A curious feature of the action of TPA is that it causes a rapid decrease in soluble C-kinase with a corresponding increase in the amount of enzyme bound to the plasma membrane (Kraft & Anderson, 1983). Despite its close association with the plasma membrane, C-kinase is capable of phosphorylating cytoplasmic proteins. Using two-dimensional protein separation techniques, Garrison *et al.* (1984) have shown that vasopressin acts on liver to phosphorylate seven cytoplasmic proteins through the calcium-dependent pathway, whereas C-kinase phosphorylates three other separate proteins. Likewise, C-kinase is capable of phosphorylating myosin light chains located in the cytoplasm (Naka *et al.*, 1983). When activated by diacylglycerol (or the phorbol esters) this C-kinase phosphorylates specific cellular proteins that then contribute to various physiological processes (particularly secretion and proliferation), especially those that occur either independently of calcium or at very low levels of calcium. This second messenger action of diacylglycerol is terminated by two mechanisms (Fig. 3). Diacylglycerol can either be phosphorylated to phosphatidic acid by a diacylglycerol kinase or it can be hydrolysed by a diacylglycerol lipase described in the next section.

Release of arachidonic acid

In some cells, the agonist-dependent hydrolysis of PtdIns4,5P_2 may play an important role in

controlling the release of arachidonic acid (Dawson & Irvine, 1978; Bell *et al.*, 1979; Irvine, 1982; Irvine *et al.*, 1982; Rittenhouse, 1982). The fatty acid on the 2-position of the phosphoinositides is usually arachidonic acid, but it can also carry oleate or linoleate. However, during hormonal stimulation there appears to be a preferential degradation of molecules such that the diacylglycerol, and subsequently the phosphatidic acid, produced by the receptor mechanism always carries arachidonic acid on the 2-position (Bell *et al.*, 1979; Mahadevappa & Holub, 1983). Two separate pathways have been proposed for the release of arachidonic acid from these two products of the phosphoinositide response. There are those who consider that a diacylglycerol lipase is responsible for releasing arachidonic acid from diacylglycerol (Bell *et al.*, 1979; Prescott & Majerus, 1983). On the other hand, Lapetina and his colleagues (Billah *et al.*, 1981; Lapetina, 1982) have proposed that arachidonic acid is stripped off phosphatidic acid by a phosphatidic acid-specific phospholipase A_2 enzyme. There is also the suggestion that diacylglycerol may not only function as a substrate for the lipase but it may also play a part in the activation of the enzyme cascade that results in this release of arachidonic acid (Dawson *et al.*, 1984).

This controlled release of arachidonic acid that is initiated by calcium mobilizing receptors must not be confused with a separate pathway that depends upon a release of arachidonic acid from various phospholipids (phosphatidylcholine, phosphatidylethanolamine and PtdIns) by a less-specific phospholipase A_2. The latter pathways seem to require much higher levels of calcium (Billah *et al.*, 1980) and may only occur when cells are subjected to supramaximal stimulation.

Activation of guanylate cyclase

Finally we come to the possible relationship that exists between phosphoinositide metabolism and the formation of cyclic GMP. Many of the calcium-mobilizing receptors that trigger an increase in the hydrolysis of phosphoinositides stimulate guanylate cyclase to elevate the intracellular level of cyclic GMP (Michell, 1975; Berridge, 1981). The way in which such receptors are linked to guanylate cyclase is still very much a mystery but it does seem to depend in some way upon an increase in the intracellular level of calcium. The fact that guanylate cyclase can be activated by a variety of fatty acids has led to the suggestion that this enzyme might be regulated normally by either arachidonic acid or one of its metabolites (Peach, 1981; Takai *et al.*, 1982; Gerzer *et al.*, 1983). The calcium-mobilizing receptors may thus activate guanylate cyclase indirectly by first of all releasing arachidonic acid, as described above, which is then converted into a metabolite that stimulates the formation of cyclic GMP. The calcium-dependency of cyclic GMP formation is accommodated in this hypothesis because calcium is necessary first of all to release arachidonic acid (Berridge, 1982, 1984).

Synergistic interactions between the diacylglycerol and calcium signal pathways

The hydrolysis of PtdIns4,5P_2 by agonists represents a bifurcation in the signal pathway in that it results in the formation of two separate second messengers (Fig. 3). One limb of the pathway depends upon diacylglycerol activating C-kinase to phosphorylate specific cellular proteins, whereas the other pathway depends on calcium which acts through calmodulin to phosphorylate a separate group of proteins. An intriguing consequence of having this bifurcating signal pathway is that it provides the versatility necessary to introduce subtle variations in the control mechanisms. The two pathways may contribute to the final response by acting either co-operatively or synergistically. The relative importance of each pathway may also vary with time, in that calcium may be responsible for initiation whereas diacylglycerol may be more important in maintaining the response (Kojima *et al.*, 1983; Zawalich *et al.*, 1983). The liver provides an interesting example of how this receptor mechanism can function to regulate separate processes because the calcium pathway stimulates phosphorylase whereas the C-kinase pathway acts to inhibit glycogen synthase (Roach & Goldman, 1983). It is also conceivable that some agonists might be capable of activating one limb of the signal pathway without the other. For example, if an agonist stimulated the hydrolysis of PtdIns4*P* instead of PtdIns4,5P_2, the Ins1,4P_2 would not release calcium but the diacylglycerol could activate the C-kinase pathway in the usual way. The following description of platelet aggregation and cellular proliferation will serve to illustrate the versatility of this ubiquitous signalling system.

Blood platelets

The first indication of a synergistic interaction between these two signals emerged from studies on blood platelets. Low concentrations of calcium ionophore or 1-oleoyl-2-acetylglycerol (a diacylglycerol derivative that activates C-kinase) that alone induce very little secretion can produce a full secretory response when added in combination with each other (Kaibuchi *et al.*, 1982, 1983). Synergistic effects obtained by combining the ionophore A23187 and the phorbol ester TPA have also been described in lymphocytes (Mastro & Smith, 1983; Kaibuchi *et al.*, 1984) and in adrenal glomerulosa cells (Kojima *et al.*, 1983). The ability of TPA to potentiate the secretory effect of the sulphonylurea

gliclazide on insulin-secreting islet cells (Malaisse *et al.*, 1983) could also be explained through such synergistic interactions between diacylglycerol and calcium.

The synergism between the diacylglycerol and calcium pathways has been explored further by using the fluorescent dye quin2 to monitor the intracellular level of calcium. When blood platelets were treated with a low dose of ionomycin (50nM) in the absence of external calcium, there was a small increase in intracellular calcium but no aggregation or secretion (Rink *et al.*, 1982). However, subsequent addition of thrombin caused aggregation and secretion without any further change in the level of calcium. Rink *et al.* (1982) suggested that thrombin was capable of activating these cellular events through a calcium-independent pathway. Subsequent studies revealed that this calcium-independent pathway was the C-kinase pathway (Rink *et al.*, 1983). Activation of the C-kinase pathway with either TPA or 1-oleoyl-2-acetylglycerol was able to stimulate secretion and aggregation without any change in the resting level of calcium. However, the onset of secretion is somewhat delayed and has a slow time course that can be greatly improved if the resting level of calcium is elevated (Rink *et al.*, 1983). Similar observations have been performed on neutrophils, where phorbol esters can stimulate degranulation without any change in the level of calcium, again suggesting that diacylglycerol may function as a second messenger in addition to calcium (Sha'afi *et al.*, 1983).

Some clues as to the molecular basis for this synergistic interaction between the two signal pathways are beginning to emerge. In the case of thymic lymphoblasts, Whitfield *et al.* (1973) have suggested that the phorbol esters may sensitize the effector system, in this case DNA synthesis, to the stimulatory action of calcium. A similar idea has been put forward by Knight & Baker (1983), who consider that diacylglycerol may act in adrenal cells to alter the affinity of exocytosis for calcium. A similar conclusion was reached from studies on permeabilized blood platelets where both thrombin and 1-oleoyl-2-acetylglycerol were capable of increasing the sensitivity of the secretory process to calcium (Knight & Scrutton, 1983). The importance of calcium was apparent from the observation that there was no enhancement of secretion if calcium was reduced to very low levels.

Another way of tackling this problem is to identify the proteins that are phosphorylated by the two pathways (Fig. 3). Thrombin is known to increase the phosphorylation of a 40kDa and a 20kDa protein (Lyons & Atherton, 1979). The former appears to be a specific substrate for C-kinase (Kaibuchi *et al.*, 1982, 1983) while the 20kDa protein, which appears to be the light chain of myosin, can be phosphorylated by both C-kinase and the Ca^{2+}/calmodulin-sensitive myosin light chain kinase (Naka *et al.*, 1983). This myosin light chain, which thus represents the focal point for both signal pathways, is phosphorylated on separate sites by the two converging pathways. It remains to be seen how these myosin light chains contribute to platelet function and how this function is altered by phosphorylation of these separate sites.

Cell proliferation

Cell growth can be triggered by a large number of mitogenic signals that include fertilization, conventional neurotransmitters and hormones, and a large number of growth factors. The fact that phorbol esters display remarkable synergistic interactions with many growth factors (Dicker & Rozengurt, 1980) suggests that the C-kinase pathway, and by inference the inositol lipids, may play a fundamental role in regulating cellular proliferation.

The two major ionic events that contribute to this onset of proliferation are changes in the level of calcium (Boynton *et al.*, 1974; Berridge, 1975; Whitfield *et al.*, 1976, 1981; Metcalfe *et al.*, 1980; Durham & Walton, 1982) and the activation of a neutral Na^+/H^+ exchange carrier (Rozengurt & Heppel, 1975; Smith & Rozengurt, 1978; Koch & Leffert, 1979; Mendoza *et al.*, 1980; Moolenaar *et al.*, 1981, 1982, 1983). Of particular significance is the extrusion of protons that causes a marked alkalinization of the cytoplasm when sea urchin eggs are fertilized (Lopo & Vacquier, 1977; Shen & Steinhardt, 1978), or when fibroblasts are stimulated with mitogens (Cassel *et al.*, 1983; Moolenaar *et al.*, 1983). It has been suggested that the Na^+/H^+ exchanger may function as a signal transducer mediating the action of growth factors (Moolenaar *et al.*, 1983). As protons are extruded, the cytoplasm will load up with sodium, which has also been implicated in the control of growth (Cone & Tongier, 1973; Cone & Cone, 1976). In addition to these ionic events, cyclic AMP may also be an important second messenger for initiating proliferation (Rozengurt, 1981).

Multifunctional calcium-mobilizing receptors may be responsible for initiating both the ionic events as well as the increase in cyclic AMP that contribute to the onset of proliferation. Changes in phosphoinositide metabolism have been described upon activation of cultured cells (Ristow *et al.*, 1973, 1980; Hoffman *et al.*, 1974; Diringer & Friis, 1977; Sawyer & Cohen, 1981; Habenicht *et al.*, 1981) and lymphocytes (Fisher & Mueller, 1968; Hui & Harmony, 1980; Hasegawa-Sasaki & Sasaki, 1982) by a variety of mitogens. Much of

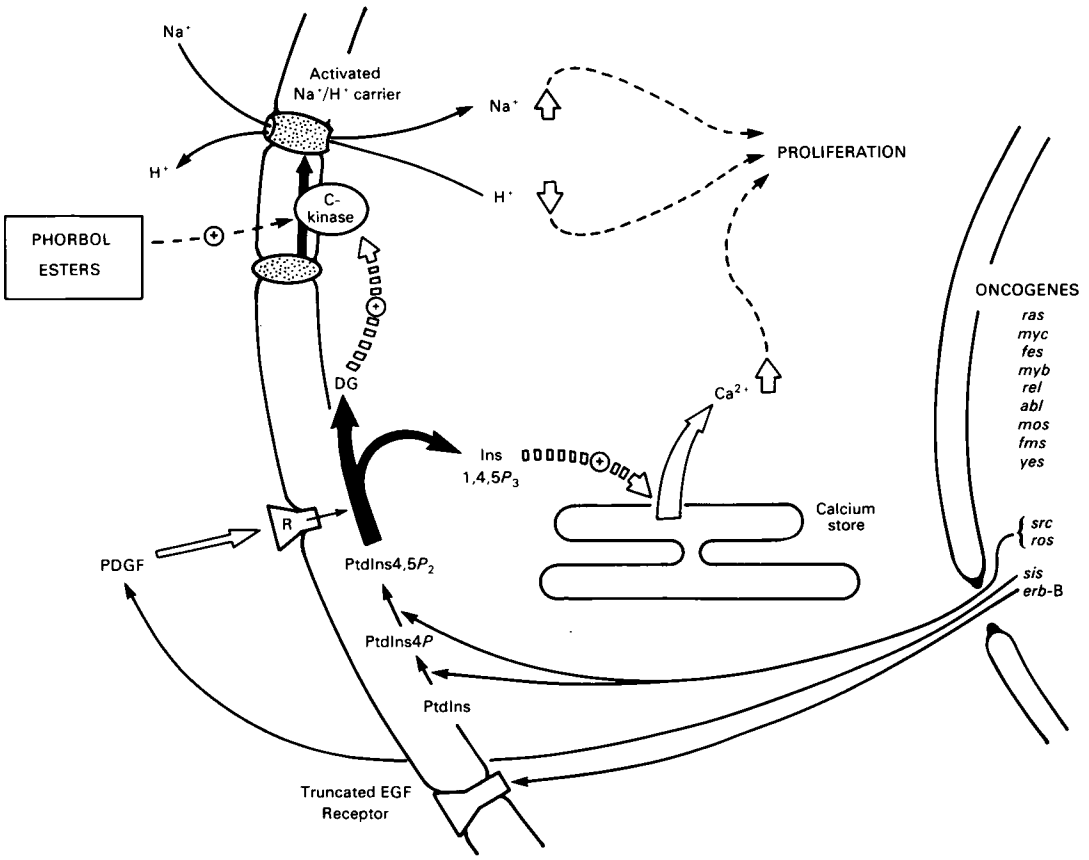

Fig. 4. *Model to illustrate the proposed role of phosphoinositides in the action of mitogenic signals*
Included on this Figure is the contribution of various oncogene products to this inositol lipid signalling system. Abbreviations used: DG, diacylglycerol; EGF, epidermal growth factor; PDGF, platelet-derived growth factor.

this literature on the possible relationship between phosphoinositide metabolism and cell growth has been reviewed by Michell (1982*b*). A model illustrating how the phosphoinositide response might contribute to the onset of proliferation is shown in Fig. 4. Mitogenic signals such as platelet-derived growth factor may initiate the breakdown of PtdIns4,5P_2 to give the two second messengers diacylglycerol and Ins1,4,5P_3 (M. J. Berridge, J. P. Heslop, R. F. Irvine & K. D. Brown, unpublished work). The latter may induce a calcium signal by mobilizing internal calcium. In this respect, it is interesting to note that one of the earliest events during stimulation of quiescent cultures of Swiss 3T3 cells with fresh serum is a large mobilization of calcium from intracellular stores (Lopez-Rivas & Rozengurt, 1983). Direct measurements with the fluorescent dye quin2 have revealed that platelet-derived growth factor induces a rapid increase in the intracellular level of calcium (Moolenaar *et al.*, 1984). Removing external calcium did not prevent the rise in intracellular calcium, thus providing further evidence that growth factors can act on internal reservoirs. This mobilization of intracellular calcium in Swiss 3T3 cells may thus be mediated by Ins1,4,5P_3 in the same way as this messenger is thought to release calcium in other cells (Streb *et al.*, 1983; Burgess *et al.*, 1984).

The diacylglycerol limb of the signal pathway may be responsible for regulating the other major ionic event of sodium entry and hydrogen extrusion (Fig. 4). The observation that the phorbol esters, which are thought to act through C-kinase, can increase pH in 3T3 cells (Burns & Rozengurt, 1983) suggests that diacylglycerol may be responsible for activating the amiloride-sensitive Na^+/H^+ carrier system. The fact that the two major ionic events are controlled by the two separate limbs of the signal pathway would help to explain the synergistic interactions that have been described between calcium and the phorbol esters in BALB/c3T3 cells (Boynton *et al.*, 1976) and in lymphocytes (Mastro & Smith, 1983; Kaibuchi *et al.*, 1984).

The cyclic AMP that contributes to the mitogenic action of growth factors seems to depend

upon the formation of an E-type prostaglandin (Rozengurt *et al.*, 1983) that functions in an autocrine manner to activate adenylate cyclase. When 3T3 cells are stimulated by purified platelet-derived growth factor, the normal increase in cyclic AMP level is abolished by adding indomethacin, which inhibits the conversion of arachidonic acid to prostaglandin E (Rozengurt *et al.*, 1983). The precise source of this arachidonic acid has not been established but it may come from the action of a phospholipase A_2 enzyme acting either on phosphatidylcholine and phosphatidylethanolamine (Shier, 1980) or on PtdIns (Hong & Deykin, 1981). Another possibility is that it is derived from the diacylglycerol produced during the breakdown of the phosphoinositides (Habenicht *et al.*, 1981) as outlined in Fig. 4. Diacylglycerol may thus be important not only as a second messenger but also as a precursor for the release of arachidonic acid.

The hydrolysis of PtdIns4,5P_2 may thus be a key event in the action of mitogenic agents because it initiates a signal cascade (Fig. 4) that results in elevated levels of calcium, cyclic AMP and sodium as well as a fall in the level of protons.

Oncogenes and phosphoinositides

The transforming genes of retroviruses display very close homologies with a class of cellular genes that are normally concerned with regulating cell growth (Cooper, 1982; Bishop, 1983). Alterations in these cellular growth-control genes will lead to transformation identical with that induced by their viral counterparts. The current view is that this small set of oncogenes is responsible for regulating all aspects of cellular growth. There is reason to believe that some of these oncogenes may code for proteins that function in those receptors that employ the inositol lipids for signal transduction (Fig. 4).

The first hint of a link between oncogenes and the inositol lipids was the discovery that the v-*sis* gene of simian sarcoma virus encodes a protein that is almost identical with platelet-derived growth factor (Doolittle *et al.*, 1983; Waterfield *et al.*, 1983). Another clue emerged from the finding that the *erb*-B gene produces a protein that is very similar to a part of the receptor for epidermal growth factor (Downward *et al.*, 1984) which is also thought to function through changes in inositol lipid metabolism (Sawyer & Cohen, 1981). An even closer link between oncogenes and the inositol lipids emerges from the finding that the v-*ros* gene from avian sarcoma virus UR2 and the v-*src* gene from Rous sarcoma virus encode proteins that can phosphorylate PtdIns to both PtdIns4*P* and PtdIns4,5P_2 (Macara *et al.*, 1984; Sugimoto *et al.*, 1984). The corresponding cellular counterparts (c-*ros* and c-*src*) may thus code for the kinases (steps *a* and *b* in Fig. 1) which function to channel PtdIns to the PtdIns4,5P_2 used by the receptor (Fig. 4). There also are suggestions that the *src* gene product may function to phosphorylate diacylglycerol to phosphatidic acid (Sugimoto *et al.*, 1984). So far, therefore, oncogenes have been found to code for at least three key aspects of the signal-processing pathway that employs the inositol lipids. They code for one of the growth factors (*sis*), a growth factor receptor (*erb*-B) and also for enzymes (*ros* and *src*) that provide the lipid substrate required by these receptors. There is every reason to suspect that some of the other oncogenes, whose functions are still unknown, may also code for proteins that participate in the receptor mechanism that uses inositol trisphosphate and diacylglycerol as intracellular second messengers. The possible link between oncogenes and inositol lipids is explored in greater detail elsewhere (Berridge, 1984).

Conclusion

The action of many hormones and neurotransmitters depends upon the hydrolysis of membrane phosphoinositides. Agonists induce the cleavage of PtdIns4,5P_2 resulting in the formation of diacylglycerol and inositol trisphosphate. In addition, there are degradative pathways that rapidly remove these internal signals when the external signal is withdrawn. An inositol trisphosphatase converts Ins1,4,5P_3 into Ins1,4P_2, whereas diacylglycerol is either phosphorylated to phosphatidic acid by a diacylglycerol kinase or dephosphorylated to monoacylglycerol by a diacylglycerol lipase. The level of these signals will thus depend upon the balance between their rates of formation and degradation. Agonists act by increasing the rate of formation by stimulating the hydrolysis of PtdIns4,5P_2 to diacylglycerol and Ins1,4,5P_3. These two products then function as second messengers to activate two independent but parallel signal pathways that may also be responsible for releasing arachidonic acid and for activating guanylate cyclase. Diacylglycerol functions within the plane of the membrane to increase protein phosphorylation by activating C-kinase. Inositol trisphosphate is released to the cytosol to function as a second messenger to mobilize calcium from intracellular stores. These two signal pathways appear to function in a synergistic manner to stimulate a wide variety of cellular processes. This bifurcating signalling system generates a diverse repertoire of intracellular second messengers that provide these receptors with the versatility necessary to control a wide range of cellular processes, including cellular proliferation.

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