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Inositol phospholipid and phosphatidic acid metabolism in response to membrane receptor activation

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The status of *myo*-inositol (Fig. 1(*a*)) as a vitamin is uncertain since some human tissues can synthesize it from glucose-6-phosphate. Eagle *et al.* (1957), however, showed that it was an essential growth factor for many human cells in tissue culture. The concentration required for optimum growth was higher than would be expected of a typical vitamin, suggesting a metabolic role for inositol. It now seems clear that this role involves the phospholipids which contain inositol, phosphatidylinositol (PtdIns), phosphatidylinositol-4-phosphate (PtdIns4P; Fig. 1(*b*)) and phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P$_2$; Fig. 1(*c*)).

For the nutritionist these phosphoinositides are of interest in at least three ways. First, their metabolism is linked to activation of many cell-surface receptors, most of which mobilize calcium (Michell, 1975). Second, very recent studies connect the phosphoinositides with control of cell proliferation through epidermal growth factor and with malignant transformation of cells. Third, these lipids are rich in arachidonic acid and in some cells this particular pool of the acid is used as a source of prostanoids. The present paper concentrates on the first two of these topics since they are attracting a good deal of attention and some controversy.

Phosphoinositides and Ca mobilization

Michell (1975) suggested that phosphoinositide-linked receptors were those which utilized Ca^{2+} as a 'second messenger' and he developed a theory linking
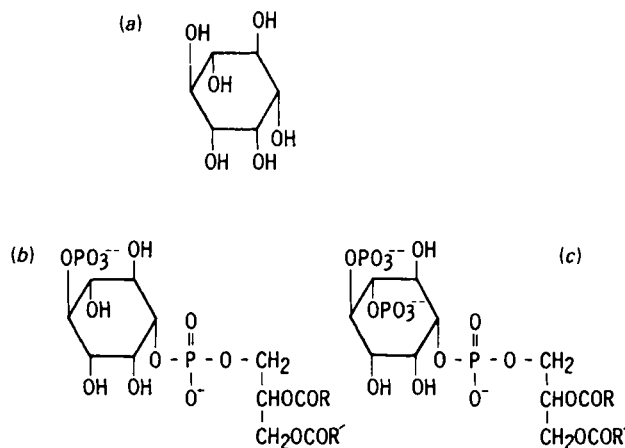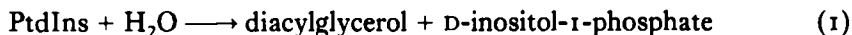

Fig. 1(*a*), *myo*-Inositol; (*b*), phosphatidylinositol-4-phosphate; (*c*), phosphatidylinositol-4,5-bisphosphate.

hydrolysis of PtdIns with Ca gating. Occupation of the receptor was considered to activate the phospholipase C (EC 3.1.4.3) catalysing reaction (1):
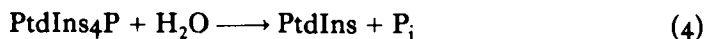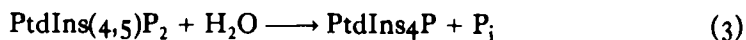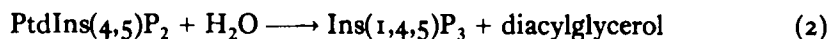
$$\text{PtdIns} + H_2O \longrightarrow \text{diacylglycerol} + \text{D-inositol-1-phosphate} \qquad (1)$$

An enzyme of this type was first recorded from my laboratory (Kemp $et\ al.$ 1959). It attacked both PtdIns and brain diphosphoinositide (now known to be a mixture of PtdIns4P and PtdIns(4,5)P$_2$) and it had an absolute requirement for Ca^{2+}. Subsequently, Dawson $et\ al.$ (1971) showed that the initial product of the reaction was D-inositol-1,2-cyclic phosphate.

Evidence that loss of PtdIns occurs in response to muscarinic cholinergic stimulation was provided by Jones & Michell (1974). An apparently more convincing demonstration of reaction (1), since inositol-1,2-cyclic phosphate was detected, came from a study of 5-hydroxytryptamine action on blowfly salivary gland (Fain & Berridge, 1979). The cyclic phosphate result has now been shown to be incorrect (Berridge $et\ al.$ 1983).

The Michell (1975) PtdIns Ca-gating hypothesis has been criticized on several grounds (Hawthorne, 1982, 1983) but only two questions need concern us here: (a) Is PtdIns the correct phosphoinositide? (b) Is Ca-mobilization always associated with the phosphoinositide changes? The wider term Ca-mobilization is used to cover both entry of Ca from extracellular sources and its release from intracellular organelles.

Receptor-linked metabolism of PtdIns or polyphosphoinositides?

Polyphosphoinositide is the rather unsuitable generic name for PtdIns4P or PtdIns(4,5)P$_2$. The polyphosphoinositides are most abundant in myelin and are usually found in plasma membranes or related structures such as the chromaffin-granule membrane. For this reason they are better candidates for a response linked to cell-surface receptors than PtdIns, which occurs in most of the membranes of the cell. They are also particularly labile, a point that was made when the kinase-forming PtdIns(4,5)P$_2$ was first described (Kai $et\ al.$ 1968), the relative rates of synthesis and hydrolysis being compared with those for acetylcholine. Because of this it was suggested by Kai $et\ al.$ (1968) that PtdIns(4,5)P$_2$ had properties reminiscent of a chemical transmitter which was rapidly destroyed after receptor activation. Polyphosphoinositides may be hydrolysed by two active enzyme systems, a diesterase (reaction 2) which has been given much attention because of its link with surface receptors, and a monoesterase (reactions 3 and 4) which is largely ignored at present. It is not yet clear whether the same enzyme catalyses reactions 1 and 2:

$$\text{PtdIns(4,5)P}_2 + H_2O \longrightarrow \text{Ins(1,4,5)P}_3 + \text{diacylglycerol} \qquad (2)$$

$$\text{PtdIns(4,5)P}_2 + H_2O \longrightarrow \text{PtdIns4P} + P_i \qquad (3)$$

$$\text{PtdIns4P} + H_2O \longrightarrow \text{PtdIns} + P_i \qquad (4)$$

where $Ins(1,4,5)P_3$ is inositol-1,4,5-trisphosphate and P_i is inorganic phosphate. Chemical analysis of inositol lipids in tissues is difficult because of rapid breakdown post-mortem. Brain, nerve and adrenal medulla contain most polyphosphoinositides, but even there the three phosphoinositides account for only around 6% of total lipid-phosphorus. Other tissues contain much less polyphosphoinositide; for example, Downes & Michell (1982) quote a total concentration of 20–40 nmol/g rat liver.

Some indication of polyphosphoinositide hydrolysis in response to acetylcholine was obtained first in brain synaptosomes (Durell *et al.* 1968; Schacht & Agranoff, 1972). More direct evidence of receptor-linked polyphosphoinositide breakdown was provided by Abdel-Latif *et al.* (1977) for iris muscle and by Kirk *et al.* (1981) for hepatocytes. Until quite recently, however, most workers considered that PtdIns was the lipid which responded. The early evidence was based on increased labelling of this lipid with ^{32}P in response to an agonist such as acetylcholine. Better evidence is provided by loss of prelabelled phosphoinositide, since this is a more direct measurement of reactions 1 or 2. More recently, many workers have used the ion-exchange columns first described by Ellis *et al.* (1963) to characterize the inositol phosphates produced in these reactions. The most recent theory of Berridge & Irvine (reviewed by Berridge, 1984) is that $Ins(1,4,5)P_3$ released by reaction 2 is a second messenger mobilizing Ca from internal stores in cells such as hepatocytes, and pancreatic acinar cells. A cycle of reactions would need to be operating (Fig. 2).

According to this theory, the loss of PtdIns seen in response to agonists occurs only because it is required in the resynthesis of $PtdIns(4,5)P_2$. The evidence for this is that loss of labelled $PtdIns(4,5)P_2$ is more rapid that loss of labelled PtdIns. At the present time, however, it is not easy to exclude the possibility that receptor activation causes PtdIns breakdown directly by reaction 1.
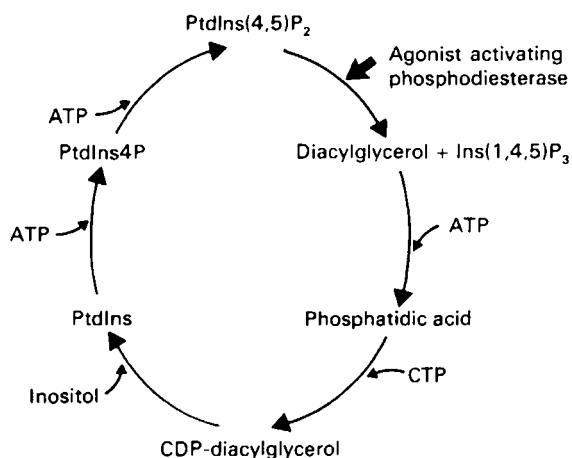

Fig. 2. Cycle of reactions involved in receptor-activated hydrolysis of phosphatidylinositol-4,5-bisphosphate $PtdIns(4,5)P_2$. PtdIns, phosphatidylinositol; $PtdIns4P$, phosphatidylinositol-4-phosphate; $Ins(1,4,5)P_3$, inositol-1,4,5-trisphosphate.

Is Ca mobilization always associated with receptor-activated phosphoinositide breakdown?

Some acetylcholine receptors of the muscarinic type are inhibitory and their activation does not cause a rise in the intracellular concentration of Ca. Nevertheless, activation of such receptors leads to the typical phosphoinositide changes. Examples are certain muscarinic receptors of heart (Brown & Brown, 1983), those of the adrenal chromaffin cells (Swilem & Hawthorne, 1984) and probably those of the cerebral cortex (Kendall & Nahorski, 1984).

The most detailed information is available for bovine adrenal chromaffin cells in culture (Swilem & Hawthorne, 1984). Stimulation of these cells by muscarinic drugs leads to breakdown of about 25% of the labelled pool of $PtdIns(4,5)P_2$ in 30 s, yet there is no change in intracellular free Ca^{2+} as measured by the fluorescent indicator Quin-2. Activation of nicotinic receptors on the chromaffin cells causes entry of Ca^{2+} and promotes catecholamine secretion but there is no associated phosphoinositide effect. The muscarinic receptors appear to modulate catecholamine secretion (Swilem *et al.* 1983) but their mechanism of action is not understood.

Another important point arises from the adrenal chromaffin-cell work and probably applies to most other systems in which receptors cause loss of $PtdIns(4,5)P_2$. At the same time there is an equally-rapid loss of $PtdIns4P$ suggesting that the phosphodiesterase is also hydrolysing this lipid. The water-soluable product, inositol-1,4-bisphosphate ($Ins(1,4)P_2$), is not able to mobilize Ca^{2+} (Streb *et al.* 1983). It is thus difficult to see why this phosphate should be released. Another explanation is that $PtdIns4P$ is rapidly lost by phosphorylation to $PtdIns(4,5)P_2$ but this is unlikely in chromaffin cells, where the loss of $PtdIns4P$ is actually more rapid than that of $PtdIns(4,5)P_2$ with muscarinic agonists (Swilem & Hawthorne, 1984).

At present therefore, Berridge's (1984) concept that $Ins(1,4,5)P_3$ is a Ca-mobilizing second messenger cannot explain all receptor-mediated phospho-inositide changes. Furthermore, if it is proved that $Ins(1,4)P_2$ release accompanies $Ins(1,4,5)P_3$ release, some role for the bisphosphate will be required before the theory is satisfactory.

Phosphoinositides and the control of cell division

Erwin Chargaff is best known for his work on DNA base-pairing, which laid the foundations of the double helix structure, but he also made a pioneering observation on *myo*-inositol and cell division (Chargaff *et al.* 1948) which is ignored in most reviews of this subject. Inositol was shown to block the anti-mitotic action of colchicine. The work has been confirmed for rat fibroblasts in culture and extended to rat intestinal mucosa (Lymberopoulos & Hawthorne, 1980). Inositol is almost certainly involved here as a constituent of the phosphoinositides since mitogens which activate T-lymphocytes produce early effects on the metabolism of these lipids (Fisher & Mueller, 1968). Changes in

phosphoinositide metabolism was also seen in Rous sarcoma virus-transformed cells (Diringer & Friis, 1977).

Nishizuka (1984) and his co-workers have described a protein kinase C which can be activated by the diacylglycerol released when phosphoinositides are hydrolysed in response to signals from surface receptors. Tumour-promoting phorbol esters can also activate this protein kinase C, although the substrates for the kinase are not yet identified.

The peptide hormone epidermal growth factor (EGF) causes increased labelling of PtdIns (Sawyer & Cohen, 1981), and certain viral oncogenes produce a kinase similar to that of EGF receptor. This kinase was first thought to be a protein kinase but has now been shown to phosphorylate PtdIns (Macara *et al.* 1984; Sugimoto *et al.* 1984).

Cell proliferation is considered to be controlled at least partly by entry of Ca^{2+} into the cytoplasm (Durham & Walton, 1982) so there may be a link between this process and the phosphoinositide changes. It is too soon to say exactly how the inositol lipids act. Berridge (1984) has suggested that the breakdown of $PtdIns(4,5)P_2$ is a key event, providing (a) inositol trisphosphate to mobilize Ca^{2+} from an intracellular store and (b) diacylglycerol to activate protein kinase C and in turn an Na^+-H^+ exchanger which plays a role in growth control.

Whether or not these speculations prove correct, there is no doubt that the phosphoinositides play an important part in controlling the growth and division of cells and that this involves both protein and lipid phosphorylations.

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