Review Letter

PHOSPHATIDYLINOSITOL METABOLISM IN CELLS RECEIVING EXTRACELLULAR STIMULATION

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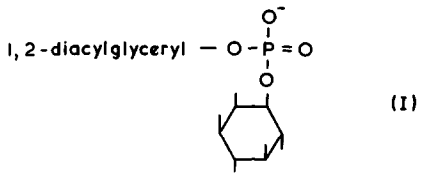
Received 16 January 1973

1. Introduction

In the early nineteen-fifties Hokin and Hokin found that pancreatic exocrine tissue, when induced to secrete digestive enzymes by the addition of acetylcholine or pancreozymin, showed a greatly increased rate of uptake of ^{32}P into its phospholipids [1, 2]. Acetylcholine also provoked increases in phospholipid metabolism in cerebral cortex slices, avian salt gland and in secretory tissues other than pancreas [3–5]. They later showed that these changes were largely confined to the metabolism of phosphatidylinositol (PI-structure I) and of a precursor, phosphatidic acid (PA-structure II). There was much less change in the labelling of the other phospholipids, even though these constituted the bulk of the lipids of the tissues. These and subsequent studies of nervous and secretory tissues have been reviewed by Hokin [5–8] and by Hawthorne [9].



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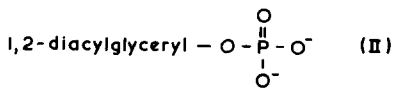

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It has become apparent in the last few years that a rapid enhancement of phosphatidylinositol turnover occurs in a wide range of different types of stimulated tissue and that this response may be evoked by a variety of stimuli (table 1). It now seems appropriate to broaden the discussions to include the available information from all types of tissues, in the hope that this may help in the explanation of this rather widespread response. Throughout this review we shall therefore make the assumption, unproven but supported by the similarity of the metabolic events seen in many systems, that the mechanism underlying enhanced PI turnover is the same in all tissues. The experimental observations which are discussed are derived mainly from studies of nervous tissues (cerebral cortex, sympathetic ganglia), secretory tissues (adrenal medulla, parotid, pancreas, pineal), lymphocytes and polymorphonuclear leucocytes.

Previous discussions of enhanced PI turnover have usually emphasized the possible significance of the interconversion of small quantities of particular lipids in the affected cells and the consequences of such changes in terms of the functioning of the membranes modified thereby [5–13]. It is equally valid, however, to consider the possible significance of the rapid removal and replacement (i.e. turnover) of the water-soluble phosphorylinositol portion of PI and one recent suggestion has been based on a mechanism of the latter type [14]. We will attempt to consider the relative merits of these two types of interpretation. Our conclusion is that such an analysis strongly favours mechanisms of the latter type and a model is proposed for the intracellular events which appears to be

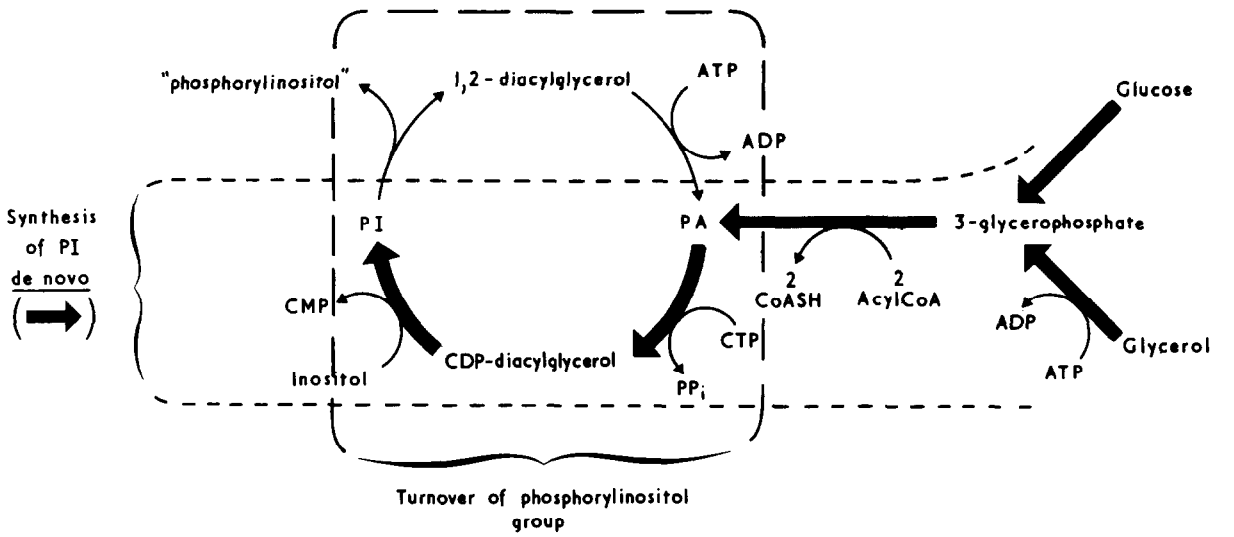


Fig. 1. Biosynthesis of phosphatidylinositol and turnover of its phosphorylinositol group. PI, phosphatidylinositol: PA, phosphatidic acid.

consistent with considerably more of the experimental evidence than were the previous suggestions.

2. The pattern of reactions involved

Synthesis of PI de novo follows the pathway shown in fig. 1 and in some tissues enhanced labelling is due to synthesis of complete PI molecules via this route (pancreas [5], thyroid [15]). In other systems, however, the pattern of incorporation of labelled precursors indicates that during stimulated PI turnover only the phosphorylinositol portion of the molecule shows enhanced turnover (cerebral cortex [6, 16], lymphocytes [17, 18], polymorphonuclear leucocytes [19, 20], adrenal medulla [21]). This is thought to occur by a cycle of reactions which is also shown in fig. 1 [4, 10, 13, 22]. Any general model for these events must therefore include a mechanism whereby the 1,2-diacylglycerol portion of the molecule can be conserved and utilised for resynthesis of PI.

3. The tissues showing enhanced PI turnover and the stimuli which provoke the effect

These are summarised in table 1. Many of the tissues listed are nervous or secretory and often the stimuli in successful experiments have been cholinergic or electrical (parasympathetic, sympathetic or non-specific). This seems, though, to have been largely a reflection of the interests of individual investigators and the hypotheses available for investigation; clearly many combinations of tissues and stimuli have given positive results. Many of the stimuli listed are known to interact with cells via cell surface receptors (insulin, acetylcholine, phytohaemagglutinin, adrenaline, thyrotrophin). There are, however, some situations where activation of cell-surface receptors has not provided a specific enhancement of PI turnover (eg. effects of glucagon [43]), so it does not seem that this is merely a generalised response to any extracellular stimulus mediated through cell-surface receptors.

The observed change in PI labelling on stimulation is usually at least a doubling and in some systems may be as much as tenfold.

The effect of cinchocaine, a local anaesthetic [25, 30, 36] and of phentolamine and propranolol, which are agents used for producing blockade, respectively, of adrenergic α- and β-receptors, clearly does

Table 1
Tissues which show enhanced PI turnover and the stimuli evoking this response*.

Tissue	Stimulus	References
Cerebral cortex	Acetylcholine, electrical	[6, 13, 23]
Sympathetic ganglia	Acetylcholine, electrical	[6, 13, 24]
Vagus nerve	Cinchocaine	[25]
Pancreas	Acetylcholine, pancreozymin, caerulein	[5, 7, 8, 26, 27]
Submaxillary gland	Acetylcholine, adrenaline, electrical (sympathetic or parasympathetic)	[5, 7, 28]
Parotid gland	Acetylcholine, adrenaline, cinchocaine, propranolol, phentolamine	[5, 7, 29, 30]
Adrenal medulla	Acetylcholine	[5, 7, 21]
Thyroid	Thyroid stimulating hormone, long-acting thyroid stimulator, propranolol	[5, 7, 15, 31–35, 46]
Gastric mucosa	Histamine, acetylcholine	[5, 7]
Anterior pituitary	Corticotrophin-releasing factor	[5, 7]
Pineal	Noradrenaline, cinchocaine, propranolol, phentolamine	[36]
Sweat glands	Acetylcholine	[5, 7]
Salt gland (avian)	Acetylcholine	[4]
Polymorphonuclear leucocytes (and other phagocytes)	Phagocytizable particles, digitonin, deoxycholate, bacterial endotoxin	[19, 20, 37]
Heart	Adrenalin	[38]
Adipose tissue	Insulin	[39]
Lymphocytes	Phytohaemagglutin, anti-lymphocyte antiserum	[17, 18, 40, 41]
Fibroblasts grown to confluence**	Addition of fresh serum	[42]

* Where possible recent references involving some discussion are given. Original references may be found therein.
** Turnover was measured as loss of label from PI on stimulation.

not fit into this picture. It does seem, however, that cinchocaine and propranolol probably share a common mode of action based on local anaesthetic properties common to both, and that phentolamine acts via some route other than that adrenergic α-blockade [36].

4. How are the changes in PI metabolism linked to interaction of ligands with extracellular receptors?

In principle this might be either via the mediation of cAMP (or of another, as yet unidentified, second messenger) or through a direct functional link at the cell surface membrane between one of the enzymes involved in the modified PI metabolism and the receptor. Mediation by cAMP has been ruled out for parotid [29, 44], pancreas [26, 27, 45] thyroid [31–35, 46] and pineal [36]. In these tissues studies with extracellular stimuli, with cAMP and its dibutyryl derivative, and with inhibitors of cAMP phosphodiesterase have all shown that, whereas secretion and certain other cellular events are mediated via cAMP, the changes in PI metabolism are not correlated in any consistent way with the levels of tissue cAMP. It thus appears likely that mediation of this effect is via either direct effects on a cell-surface receptor or through control by a second messenger other than cAMP.

5. Which step in the cycle is controlled?

The stimulation, in some tissues, of the turnover of only the phosphorylinositol group suggests that the stimulus occurs at either the conversion of phosphatidylinositol to 1,2-diacylglycerol or of the latter to phosphatidic acid, as these are the steps not involved in synthesis of phosphatidylinositol de novo (fig. 1).

This conclusion is supported by the ability of gammexane (a hexachlorocyclohexane with a conformation somewhat related to that of myoinositol) to block the stimulated labelling without affecting the normal rate of phosphatidylinositol biosynthesis de novo [40, 47]. Both reactions in the conversion of PI to phosphatidic acid are now known to be localised at least partially in the cell surface membrane in cerebral cortex [23, 48], suggesting that one or other could be directly linked to the cell surface receptors; both have been suggested in the past as the site of action of extracellular stimuli [10, 18, 22, 49].

Although we have not ourselves observed any direct effect of stimulation on either the cleavage of phosphatidylinositol (unpublished results) or the phosphorylation of diglyceride [23] we tentatively ascribe the site of action of the stimulus to the former reaction for several reasons. a) There is a limited amount of direct evidence for such a link in cerebral cortex [10, 50]. b) If ^{32}P-labelled lymphocytes are stimulated in the presence of ^{31}P then radioactivity is rapidly "chased" from phosphatidylinositol [17]. Stimulation of 1,2-diacylglycerol phosphokinase would not have this effect. c) An effect cited as evidence for stimulation by phytohaemagglutinin of 1,2-diacylglycerol kinase, assayed in lymphocyte plasma membranes using endogenous substrate and [^{32}P]ATP [18], can equally be interpreted as evidence for a raised 1,2-diacylglycerol concentration in the membranes. This would be a consequence of increased phosphatidylinositol breakdown. d) Control would be expected at the first, not the second, step of a pathway. e) Finally, the evidence which has been presented relating to control of diacylglycerol kinase has been either open to alternative interpretations [5–7, 18, 51] or negative [23, 52]; it is not clear how the whole cycle of reactions could be stimulated, with reutilisation of the diglyceride, if this were the controlled step.

6. What are the events in lipid metabolism which follow stimulation and where in the cell do the labelled lipids appear?

After cleavage of PI it seems that two alternative routes are taken by different tissues. In some, the released 1,2-diacylglycerol is specifically utilised for resynthesis of PI, and in others it is dismantled and the cells renew their PI complement by synthesis of new PI molecules de novo. As would be expected, the kinetics of PA and PI labelling in stimulated cells are consistent with a precursor–product relationship between these two lipids [5, 21, 53].

Autoradiographic studies by Hokin indicated that newly-labelled PI was distributed in all of the membranes of stimulated cells [7]. This important observation has been directly confirmed by studies of the subcellular distribution of the newly synthesised PI in lymphocytes, thyroid, pineal, cerebral cortex and pancreas which had been stimulated for various periods [5, 8, 12, 13, 18, 36]. In pancreas there is some evidence for localisation of the newly-synthesised PI in endoplasmic reticular membranes [5, 8] but there is no evidence from any of these systems for an expected localisation of this lipid at the cell surface. In contrast, in the lymphocyte the newly-labelled phosphatidic acid was at least partially localised in surface membranes [18].

7. Where are the enzymes of the cycle located?

An enzyme cleaving PI is a constituent of the cytosol of many tissues, but its acid pH optimum and cytoplasmic localisation do not make it a suitable candidate for a role in the effects being discussed [14, 48]. A role for this enzyme in microtubule function has recently been suggested [54].

A membrane-bound enzyme catalysing PI cleavage was recently found in cerebral cortex [14, 48, 50]. Both this and 1,2-diacylglycerol phosphokinase are present in the cell surface in cerebral cortex to an appreciable extent [23, 48]. The enzymes which catalyse conversion of PA to PI are, however, located in intracellular membranes [55–62].

8. General considerations of cellular metabolism

There is a considerable body of evidence which indicates that the enhancements of PI and PA labelling are not trivial consequences of changes in sizes or specific activities of the cellular pools of P_i

[21, 28, 38, 53, 63–65], ATP [20, 21, 28, 38, 53, 63–65], or 3-glycerophosphate [20, 64].

9. General considerations of membrane dynamics

Recent evidence has indicated that the constituents of cell membranes are in a highly fluid and dynamic state in at least three different senses:

i) Lipid molecules are not able to rapidly move from one face to the other in a lipid bilayer, but do diffuse laterally in the plane of a lipid layer very rapidly [66].

ii) Proteins on the cell surface move laterally relatively rapidly [67–69].

iii) Phospholipids move rapidly between different intracellular membranes by a process catalysed by specific soluble cytoplasmic proteins [70–72].

10. Models based on local changes in the lipids of membranes: an assessment

Proposed explanations for modified PI metabolism in various systems have usually relied on the idea that the key change which occurs is an interconversion of PI and PA, or of PI and 1,2-diacylglycerol, at highly localised sites in membranes. The hypotheses included suggestions for either (a) a direct role of such interconversions in neurotransmission [10, 73] or other processes linked to cell-surface receptors [11, 15], (b) a role in controlling a cell-surface phenomenon such as salt-pumping [4], (c) participation in the membrane flux accompanying secretion [5, 7], or (d) a longer-term role in the changes provoked by stimuli which produce adaptive responses [14, 18, 34, 74].

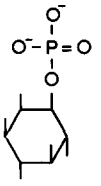
The former two alternatives are relatively short-term and have been very difficult to sustain on kinetic grounds [5, 6, 10, 11, 75]. The last alternative has been little investigated, but the relationship of enhanced PI turnover to secretion has been thoroughly analysed and many factors now rule out a direct relationship between the two processes. These include: i) Blockage of secretion, but not the lipid effect, by Ca^{2+} omission [76, 77]. ii) Control of secretion and other metabolic effects, but not PI turnover, through cAMP (see above), iii) Differences in the dose-response relationships for the two processes, with PI turnover requiring either a greater [74] or lesser [32] level of stimulation, and iv) a lack of correlation between the time-courses of the two processes [8].

Thus there is little experimental support for the previous hypotheses involving local modifications to membranes. In addition, there are general arguments which suggest that such hypotheses will not provide a satisfactory explanation of such phenomena. The nature of these hypotheses is such that the turnover of a restricted pool of PI would usually have to occur in and be confined to specific cellular sites, often the surface membrane [10, 11, 13, 15]. The material summarised above suggests, however, that within stimulated cells the "responsive" fraction of the PI is not localised, rather it is in equilibrium with the bulk of the PI in all membranes of the cells. There is no evidence for the presence of all the enzymes involved in PI turnover at any single site in the cell. Further, it is difficult to see how any such models could be easily reconciled with the fluidity of membranes and the rapidity with which phospholipids originating at single sites reach equilibrium throughout the membranes of a cell.

11. Models based on a function for a water-soluble product resulting from PI turnover

The water-soluble products of the reactions involved in PI turnover appeared, until recently, to be ADP, PP_i, CMP and myoinositol 1-phosphate (III), none of which attracted much interest in discussion of the effects of stimulation. The recent identification of myoinositol 1,2-cyclic phosphate (IV) as a product of PI cleavage [78], and the subsequent discovery of a surface membrane enzyme catalysing its production [48] and a phosphodiesterase catalysing its breakdown [79] have changed this situation. As a result we suggested that a primary response of cells to extracellular stimuli may be production, by cleavage of PI, of intracellular myoinositol 1,2-cyclic phosphate, and that the observed changes in PA and PI metabolism may reflect renewal of the PI pool of the cell [14].

This suggestion attaches special significance to the cleavage of PI, which is the enzymic step which we have concluded from other evidence to be the most



(III)

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likely site of action of stimuli on PI metabolism (see above). We have therefore attempted to put together a likely model of intracellular events in lipid metabolism subsequent to cleavage of PI to yield 1,2-diacylglycerol at the cell surface, and to assess its compatibility with the evidence discussed earlier. The result is shown in fig. 2.

We suggest that the 1,2-diacylglycerol released in the cell surface membrane is either destroyed by diacylglycerol lipase [80] or rapidly converted to PA by diacylglycerol kinase. The latter mechanism would provide a method whereby in certain tissues the diacylglycerol backbone of cleaved PI could be conserved. The surface membrane enzymes catalysing PI cleavage and phosphorylation of 1,2-diacylglycerol are both activated by deoxycholate at similar concentrations [23, 48]. This may reflect an "uncoupling" of two tightly bound enzymes, the detergent solubilising the enzymes and substrates and also breaking a tight functional link whereby diacylglycerol released from PI is passed in an enclosed hydrophobic environment to 1,2-diacylglycerol phosphokinase.

The enzymes which catalyse conversion of phosphatidic acid to phosphatidylinositol are not located in the cell surface; therefore the phosphatidic acid which has been synthesised there must be moved elsewhere before completion of its conversion back to phosphatidylinositol. Similarly, the final step of

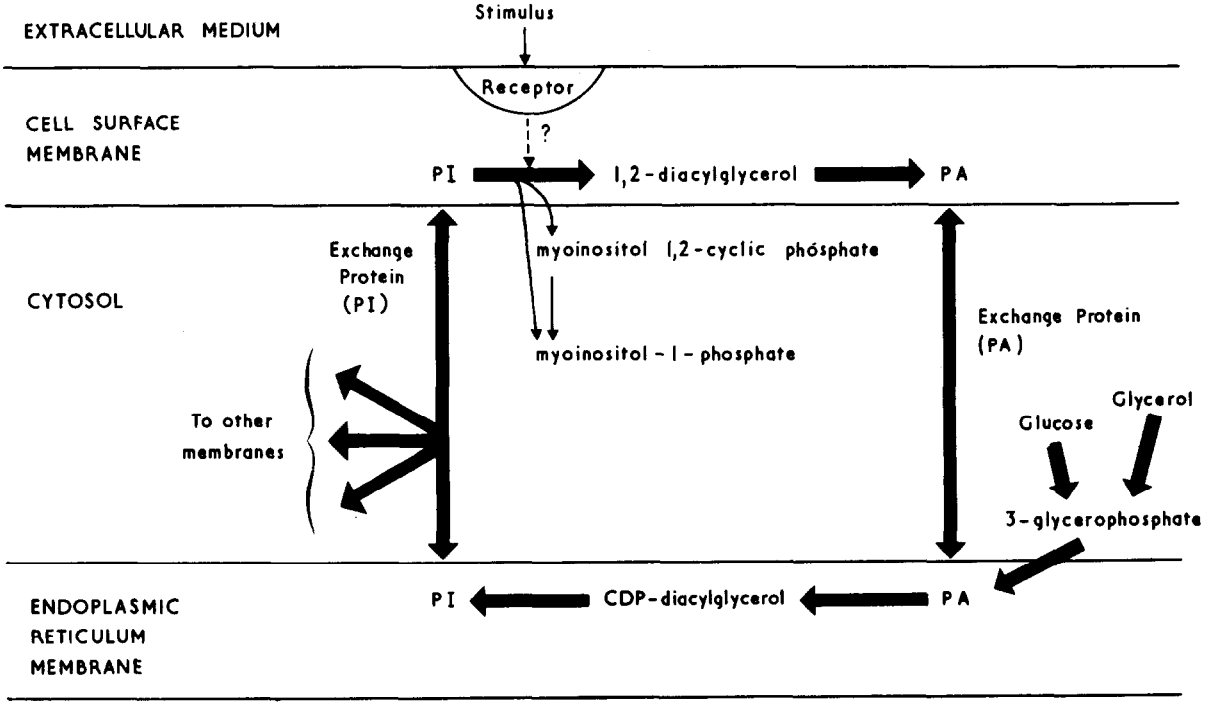

Fig. 2. A suggested model for the events which occur in a stimulated cell which shows enhanced phosphatidylinositol turnover.

phosphatidylinositol biosynthesis occurs in the endoplasmic reticulum, and there must be some mechanism for distribution of the synthesised lipid rapidly from here to other membranes. It is likely that these movements of phosphatidylinositol and phosphatidic acid are achieved by exchange proteins. These cytoplasmic proteins catalyse exchange of phospholipids between different membranes within the cell [72] and between isolated subcellular fractions in suspension [70, 71, 81–84]. Differences have been observed in the relative rates of exchange of several phospholipids between labelled liver microsomes and unlabelled mitochondria in the presence of soluble fractions from different tissues. This suggests that several proteins are present in the cytoplasm of cells, each one catalysing exchange of a different phospholipid [81]. This conclusion is supported by the isolation, from a liver soluble fraction which catalyses exchange of several lipids, of a protein which catalyses exchange only of phosphatidylcholine [83]. In such experiments with isolated subcellular fractions, the exchange of phosphatidylinositol was rapid in comparison to that of other phospholipids [81] and phosphatidic acid exchange was comparable to that of other lipids [84].

Experiments with acetylcholine-treated cerebral cortex slices [13] and phytohaemagglutinin-stimulated lymphocytes [18] suggest that in these systems the phosphatidylinositol exchange within the cells is so rapid that equilibration of newly-synthesised lipid with the pre-existing lipid is complete in very few minutes. The phosphatidic acid exchange may be somewhat slower, as accumulation of labelled phosphatidic acid can be detected in the plasma membranes of lymphocytes after brief stimulation [18] and in the salt gland much of the lipid that is "cycled" during stimulation may accumulate as phosphatidic acid [4]. The rapid exchange of PI would not only explain the distribution of newly-labelled lipids throughout the cells, but could also account for the speed with which ^{32}P can be chased out of PI, presumably by its breakdown at the cell surface, when labelled lymphocytes are stimulated with phytohaemagglutinin [17].

In fig. 2 the site for both reactions involved in the conversion of PA to PI is given as endoplasmic reticulum membranes. This is based on the known localisation of CDP-diglyceride:inositol phosphatidyltransferase in microsomal fractions from brain [57–59] and on the ability of microsomal preparations from liver and brain to convert labelled 3-glycerophosphate to phosphatidylinositol [55, 61, 62], with evidence for a close link between the two enzymes involved in the conversion of PA to PI [62]. There is, however, some conflict over the intracellular localisation of CTP-phosphatidate cytidylyltransferase in different studies. It has been reported to be microsomal in liver [60] but mitochondrial in brain [57]. This apparent conflict may occur because CDP-diglyceride is a precursor for both cardiolipin and phosphatidylinositol. Cardiolipin synthesis seems to occur in the inner mitochondrial membrane [85] and CDP-diglyceride synthesised for cardiolipin synthesis would, by virtue of the required fatty acid composition [86], be unsuitable for phosphatidylinositol synthesis. CDP-diglyceride destined for phosphatidylinositol would be equally unsuitable for biosynthesis of cardiolipin [87]. Possibly CDP-diglyceride synthesis occurs both in endoplasmic reticulum and mitochondria, the former product being the precursor for phosphatidylinositol. If not, there must be a mechanism for transfering PA from plasma membranes to mitochondria and CDP-diglyceride from mitochondria to the endoplasmic reticulum.

12. Concluding remarks

Enhanced PI turnover is a more widespread, and thus potentially more interesting, characteristic of stimulated cells than has previously been generally acknowledged. From the above discussion it seems that a search for a function of a water-soluble product of this lipid turnover – possibly myoinositol 1,2-cyclic phosphate – may be a fruitful approach to elucidating the role in cells of this phenomenon. The working model presented here accomodates in a single framework three key points which have not been reconciled by any previous proposal: localisation of the primary response at the cell surface, mediation of the PI response independently of cAMP, and distribution of the newly-labelled PI throughout the cell very rapidly. It also provides possible explanations for several previously puzzling findings, including the localisation of newly-synthesised PA in the cell surface membrane of stimulated lymphocytes [18] and the high activity of

tissue soluble fractions in catalysing PI exchange between membranes [81]. The fact that compounds which are effective metabolic antagonists of inositol bear substituents at the 2-position [88], rather than the 1-position, may be related to the function of this hydroxyl group in forming the cyclic ester.

It is now of primary interest to study the metabolism and function of myoinositol 1,2-cyclic phosphate in cells. A specific phosphodiesterase which causes its breakdown is fairly evenly distributed in tissues, except for disproportionate activity in brush borders of the epithelial cells in renal proximal tubules [79, 89]. This exception may indicate a special function for the cyclic ester in these cells, but might also be an expression of biological economy as inositol is not rapidly synthesised by most tissues. Sequential action in kidney tubules of this phosphodiesterase, alkaline phosphatase [89] and an inositol-accumulating system [90] would prevent a loss into the urine of myoinositol.

At present enhanced PI turnover, which probably reflects raised tissue levels of cyclic inositol phosphate, has no known functions. Control of neurotransmission and the secretion of macromolecules, the two processes with which a link was suggested by earlier work on PI turnover, are probably not directly related to the lipid turnover. An approach to this problem might emerge from one or both of the following clues. First, changed PI turnover appears to be somehow linked to adaptive changes in cells [11, 18, 34, 42, 74]. Secondly, enhanced PI turnover is not mediated by a mechanism involving cAMP and often, though not always, stimuli which evoke PI turnover either depress or do not change the tissue levels of cAMP (α-adrenergic [91], insulin [92], serum stimulation [93], acetylcholine [94]): insight into these systems might come from experiments which seek for controls on metabolism which produce effects opposite to those produced by cAMP.

Acknowledgements

We are grateful for financial support from the Wellcome Trust and the Medical Research Council and for the critical comments of our colleagues, particularly Dr. J.B. Finean, Dr. R. Coleman, Dr. D. Allan, Dr. H. Dixon, Dr. G.G. Lunt and Dr. J.G. Salway.

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