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Review Articles

Phospholipids and Islet Function

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There is an increasing number of examples of cells which, upon activation, display enhanced turnover of phospholipids, particularly the inositol-containing lipids and phosphatidic acid. Such an effect, commonly referred to as the phosphatidylinositol or PI effect, was first demonstrated in pancreactic exocrine tissue by Hokin and Hokin [1]. Subsequently, the list of examples of this effect has been extended to include a wide spectrum of agonist-receptor interactions and an even more diverse range of biological responses, including propagation of nerve impulses, cell motility, cell division and differentiation, and exocytosis. This topic has been extensively surveyed in recent years, notably by Michell and co-workers [2–4], Hawthorne et al. [5, 6], Berridge [7] and Putney [8]. The aim of this review is to examine the evidence for enhanced phospholipid metabolism in pancreatic islets during stimulation and to speculate upon the possible role(s) that phospholipids may play in stimulus-secretion coupling.

Nature of the Phosphatidylinositol Effect

Figure 1 shows a simplified scheme for phospholipid interconversions in mammalian tissues, diacylglycerol forming an important intermediate both in the metabolism of phosphatidylcholine and phosphatidylethanolamine, and in the phosphatidylinositol cycle. Enhanced phospholipid metabolism during stimulation is generally confined to phosphatidic acid and the inositol-containing lipids. The key step in triggering turnover of the phosphatidylinositol cycle may be the breakdown of this lipid to diacylglycerol [2, 3, 6, 8] or as suggested by recent studies [7, 9, 10], the breakdown of one or both of the polyphosphoinositides (phosphatidylinositol-4-phosphate and phosphatidylinositol-4,5-bisphosphate) via specific phospholipases C.

Studies of enhanced phospholipid turnover in cells are often based on the use of radioactive precursors (e.g. $^{32}PO_4^{3-}$, ^{3}H-glycerol, ^{3}H-inositol, labelled fatty acids). The rate of incorporation of precursor into lipid can be measured in the presence or absence of stimulus, providing an index of phospholipid synthesis (or resynthesis following breakdown). Alternatively, to assess phospholipid breakdown, the phospholipids may be prelabelled close to isotopic equilibrium, the unincorporated label removed and the tissue subsequently stimulated. These techniques should be combined ideally with measurements of phospholipid concentrations by chemical analysis. However, the limited amounts of tissue available using islets of Langerhans present considerable technical difficulties in measuring individual phospholipids, particularly those (such as the phosphoinositides) which constitute only a small proportion of the total cellular lipid.

Enhanced Phospholipid Metabolism in Pancreatic Islets

In a study of ^{32}P-labelling of phospholipids in obese mouse islets, Fex and Lernmark [11] first showed that glucose caused increased labelling, most markedly in a fraction containing phosphatidylinositol and phosphatidylserine. Freinkel et al. [12, 13] also noted that glucose stimulated ^{32}P-labelling of phosphatidylinositol, phosphatidic acid and phosphatidylethanolamine in rat islets. Tanigawa et al. [14] have shown recently that glucose results in a specific stimulation of phosphatidylinositol labelling when islet nucleotide pools are pre-labelled with ^{32}P before stimulation. We have confirmed this finding and also demonstrated enhanced labelling in response to glucose of phosphatidic acid and the polyphosphoinositides [15], additional components of the phosphatidylinositol cycle.

Montague and Parkin [16] demonstrated that glucose caused a depletion in radioactivity associated with

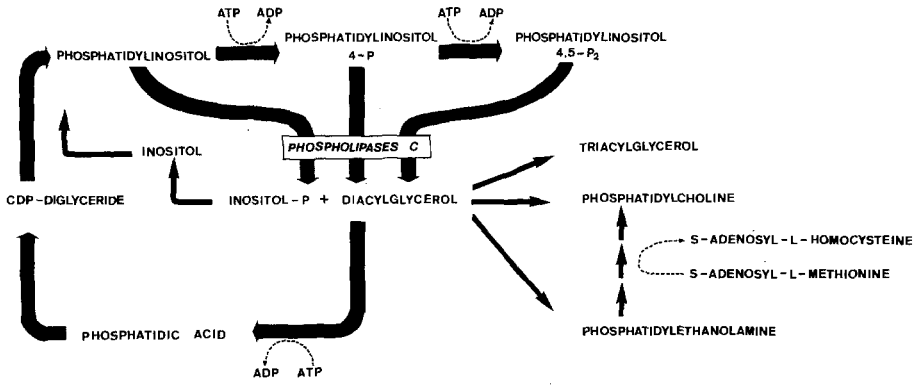

Fig. 1. Schematic diagram of metabolic interconversions of phospholipids in mammalian tissues

phospholipids in islets which had been prelabelled with ^{3}H-glycerol. This fall in phospholipid-bound radioactivity was apparent in all phospholipid fractions and therefore may not correspond to the specific stimulation of turnover of the phosphatidylinositol cycle.

Using islets pre-labelled with ^{3}H-inositol, which is incorporated solely into the inositol-containing lipids, Clements and Rhoten [17] observed a fall in lipid-bound radioactivity upon subsequent exposure of rat islets to glucose, together with an increase in the release of water-soluble ^{3}H-inositol-derived products. These authors suggested that glucose-induced insulin secretion was associated with accelerated phosphatidylinositol metabolism. Since the polyphosphoinositides were apparently poorly labelled in this study, the contribution of these lipids to such an effect could not be assessed.

Secretagogues which Stimulate Islet Phospholipid Metabolism

In certain tissues, enhanced phospholipid metabolism is only observed upon stimulation with certain classes of stimuli (e.g. cholinergic or α-adrenergic agonists), suggesting that the phenomenon is not simply a secondary, non-specific response to cell activation, but may play an important role in the cellular response to those stimuli. The evidence summarised in the previous section indicates that glucose causes a marked stimulation of islet phospholipid turnover, particularly that of the phosphatidylinositol cycle. The list of secretagogues which also exert such an effect has been extended by Clements et al. [18] to include a number of stimulatory carbohydrates and also tolbutamide, ouabain and isoproterenol. No stimulatory effects were seen, however, with insulinotropic amino acids. In contrast, Fex and Lernmark [19] reported that leucine and arginine, though not glibenclamide, caused increased ^{32}P-labelling of phosphatidylinositol. We have recently obtained evidence [20] that enhanced ^{32}P-labelling of phosphatidic acid and the phosphoinositides occurs in response to nutrient secretagogues, such as glucose and α-ketoisocaproate, and also those substances (e.g. 3-phenylpyruvate and 2-amino-2-carboxyl-bicycloheptane) which promote the metabolism of endogenous nutrients in islets [21–24]. The concentration-response curve for phosphatidylinositol labelling in response to glucose was found to closely resemble that previously reported for islet NAD(P)H levels [25]. In addition, enhanced phospholipid labelling was found to be markedly impaired by menadione, a drug previously shown to reduce islet NAD(P)H levels without affecting those of ATP [26]. These observations suggest that stimulated turnover of the phosphatidylinositol cycle by nutrient secretagogues may result from generation of reduced nicotinamide nucleotides following metabolism of these nutrients.

We have also observed stimulated ^{32}P-labelling of islet phospholipids in response to carbamylcholine and to cholecystokinin-pancreozymin, whereas no significant effects were found with arginine, glibenclamide, glucagon or thyrotropin [27]. Thus, in common with several other tissues including exocrine pancreas [28], stimulated turnover of the phosphatidylinositol cycle in islets can be triggered by hormone-neurotransmitter stimuli. In addition, pancreatic islets provide one, if not the only example of a tissue in which accelerated phospholipid metabolism occurs in response to a metabolic signal.

Time Course, Initial Event and Intracellular Location of Stimulated Phospholipid Turnover

If stimulated phospholipid metabolism is involved in stimulus-secretion coupling in islets, altered phospholipid labelling and/or levels upon stimulation should be detectable before, or at least in parallel to, the secretory response. Fex and Lernmark [11] failed to detect a significant increase in ^{32}P-labelling of phospholipids within 10 min of exposure to high glucose concentrations, and concluded that the effect was probably related to a later phase of insulin secretion. However, as pointed out by Clements and Rhoten [17], the measurement of ^{32}P-incorporation into lipids essentially monitors resynthesis of lipids, as opposed to breakdown which may be the initial event in enhanced phospholipid turnover. Using islets pre-labelled with ^{3}H-inositol, Clements and Rhoten [17] were able to demonstrate a loss of lipid-bound

radioactivity (presumably reflecting phosphoinositide breakdown) 2 min after stimulation with glucose. We have observed that islets pre-labelled with ^{32}P show a rapid, marked accumulation of ^{32}P-phosphatidic acid upon stimulation with carbamylcholine [27]. Furthermore, islets pre-labelled with ^{3}H-inositol also show an equally rapid production of ^{3}H-inositol phosphates upon treatment with carbamylcholine [29], suggesting that phosphoinositide breakdown to diacylglycerol may be an early, possibly the initial event, in cholinergic-mediated phospholipid turnover in islets. Which of the phosphoinositides is primarily broken down remains to be established. In the case of nutrient stimuli (e.g. glucose), there may be an additional effect on phospholipid biosynthesis [30]. Different mechanisms of activation would perhaps not be surprising when one considers the nature of these two types of stimulus. Neurotransmitters (and possibly some hormones), acting through plasma-membrane-bound receptors, presumably trigger an event at, or close to the cell surface. On the other hand, nutrient secretagogues undergo intracellular metabolism, and may thus influence phospholipid turnover at an intracellular location. In an attempt to localise altered islet phosphoinositide metabolism upon glucose stimulation, Clements et al. [31] subjected ^{3}H-inositol-prelabelled rat islets to subcellular fractionation following a 30 min incubation in the presence or absence of glucose. It was found that glucose produced a significant fall in radioactivity associated with the secretory granule fraction, and these authors suggested that phosphatidylinositol breakdown in the B-granule membrane in response to glucose could play a role in the migration and/or fusion of the granule with the plasma membrane during exocytosis. However, this study did not report whether any changes occurred in label associated with the plasma membrane fraction. In addition, it is possible that considerable redistribution of label could have occurred between various cellular lipid pools during a 30-min period of incubation with glucose. Thus, the most heavily labelled fraction (in this study, the secretory granules) might be expected to show the greatest change in radioactivity, though this would not necessarily prove that the initial step in stimulated phospholipid metabolism occurred in, or was limited to the secretory granule membrane. A further difficulty in this type of study is the lability of phospholipids – particularly the polyphosphoinositides – following cell homogenisation [9]. These factors pose serious obstacles to the subcellular location of enhanced phospholipid turnover.

Interrelationships between Stimulated Phosphatidylinositol Metabolism and Calcium Mobilisation in Islets

A considerable amount of effort into the investigation of phosphatidylinositol metabolism has been motivated by the hypothesis forwarded by Michell in 1975 [2] that enhanced turnover of this phospholipid may be involved in some way in the regulation of calcium mobilisation during cell activation. If such a relationship exists, it would be expected that altered phospholipid metabolism would not itself be dependent upon calcium mobilisation. A large number of studies offer evidence both in support [7, 8, 10] and otherwise [32, 33] of Michell's hypothesis.

In pancreatic islets, Freinkel and Cohanim [12] first reported that enhanced ^{32}P-labelling of phospholipids in response to glucose persisted in the absence of added Ca^{2+}, whereas insulin secretion was impaired. These authors concluded that altered phospholipid metabolism occurred prior to the calcium-dependent aspects of secretion. Berne [30] also noted that the biosynthesis of phospholipids from ^{14}C-glucose in mouse islets was unimpaired by the omission of Ca^{2+}. However, Clements et al. [18] later reported that the fall in lipid-bound radioactivity in ^{3}H-inositol-labelled rat islets following glucose stimulation was prevented by EGTA, Ca^{2+} or by Ni^{2+}, which were believed to inhibit calcium fluxes. It was suggested from these observations that glucose-induced phosphatidylinositol hydrolysis resulted from transmembrane movement of Ca^{2+}, or from increased cytosolic Ca^{2+} concentrations. However, it is paradoxical that in the same study, these authors failed to demonstrate effects comparable to glucose on phosphatidylinositol metabolism of secretagogues such as α-ketoisocaproate, leucine and arginine which have also been shown to trigger calcium uptake in islets [34–36]. We have recently shown that enhanced ^{32}P-labelling of phosphatidylinositol in rat islets in response to either neurotransmitter [27] or nutrient [15, 20] secretagogues persists in the absence of added Ca^{2+}; conditions known to impair insulin secretion [37, 38]. In contrast, increased phospholipid labelling by either type of stimulus was markedly reduced by the addition of excess EDTA. We suggested consequently that at least one step in the phosphatidylinositol cycle in islets is Ca^{2+}-dependent, but that initiation of turnover of the cycle does not itself result from increased calcium uptake into the islet. In support of this conclusion, we have been unable to detect enhanced phosphatidylinositol labelling in response to either ionophore A23187 or Ba^{2+}, both of which are thought to trigger insulin secretion via a rise in cytosolic Ca^{2+} levels [39, 40].

The distinction between calcium-dependent and calcium-activated processes is an important one, and may to some extent explain the apparent inconsistencies which appear in the literature relating to this topic. Thus, it is still conceivable that stimulated phospholipid metabolism in islets is linked in some way to the regulation of calcium movements, and a number of mechanisms have been proposed as to how such a control might be achieved. It is well-known that phospholipids, particularly the phosphoinositides which contain a polar, acidic head-group, are powerful chelators of divalent cations. It is conceivable, therefore, that the cleav-

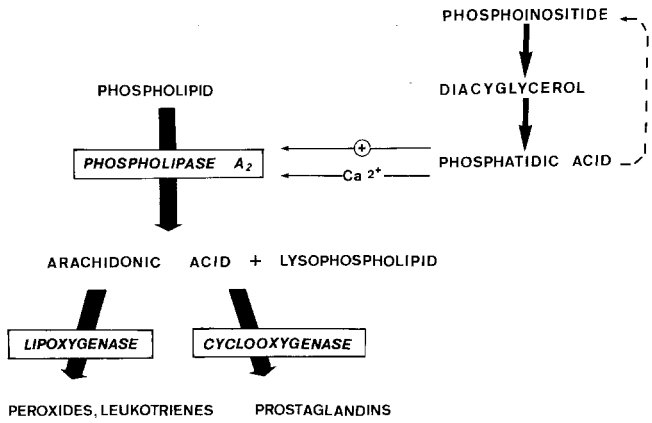

Fig. 2. Possible mechanisms of arachidonic acid release from membrane phospholipids

age of this head-group by a phospholipase C may release a proportion of divalent cations bound in the membrane, and might also result in a marked change in permeability of the membrane to cations [7, 10]. An additional, or alternative mechanism whereby phospholipid breakdown may trigger calcium mobilisation is through the formation of endogenous ionophoretic substances [7, 8]. One potential candidate for such a compound is phosphatidic acid which has been shown to possess potent ionophoretic activity in artificial systems [41]. It is interesting to note that Dunlop et al. [42] have recently demonstrated a correlation between phosphatidic acid levels, ionophoretic activity and glucose-induced insulin secretion in rat islets during the post-natal period. Phosphatidic acid may thus be an important component of the ionophoretic activity demonstrated in organic extracts of rat islets [43–46].

Whilst a link between accelerated phospholipid turnover and calcium mobilisation is an attractive possibility, there is no shortage of alternative suggestions concerning the role of the former in cell regulation. For instance, the hydrolysis of phospholipid to diacylglycerol may have an effect on membrane fluidity [32] and facilitate membrane fusion at the exocytotic site [47]. Indeed, glucose has been shown to increase membrane fluidity in pancreatic islet cells [48]. Another possible link between the formation of diacylglycerol and the stimulation of hormone secretion arises from the discovery of Ca^{2+}-activated, phospholipid-dependent protein kinase (C-kinase) which can be activated by diacylglycerol [49]. This enzyme was recently identified in pancreatic islets, and its activation by diacylglycerol derived from phosphatidylinositol proposed to play a role in glucose-induced insulin secretion [14].

The discovery that a large proportion of the inositol-phosphates derived from phosphoinositide breakdown was in the form of inositol-1,2-cyclic monophosphate [50] lead to the suggestion that this nucleotide might play a second messenger role in a manner analogous to cyclic AMP [51]. However, investigations to date have failed to provide conclusive evidence in favour of this hypothesis [52].

The Release and Metabolism of Arachidonic Acid in Pancreatic Islets

The liberation of arachidonic acid for the synthesis of prostaglandins and lipoxygenation products is a process which is entirely dependent upon phospholipid breakdown. In mammalian cells, virtually all of the arachidonate is esterified in the 2-position of phospholipids and must be enzymatically cleaved before subsequent metabolism of the fatty acid can occur. It is generally believed that arachidonate release results from activation of a phospholipase A_2 (Fig. 2).

In a recent study, Laychock [53] has described a phospholipase A_2 activity in islets which is stimulated upon prior exposure of islets to glucose. This author has also reported that glucose stimulates the incorporation of ^{14}C-arachidonic acid into certain islet phospholipids and suggested that enhanced exchange of the fatty acid may result from phospholipase A_2 activation by the sugar [54]. Yamamoto et al. [55] have shown that two purported inhibitors of phospholipase A_2 – mepacrine and bromophenacylbromide – also reduce insulin secretion in response to the tumour promoter 12-0-tetradecanoylphorbol-13-acetate. However, the production of arachidonic acid or its metabolites was not assessed in this study, and the specificity of these drugs as inhibitors of phospholipase A_2 also remains to be established.

In recent years, Lapetina and his colleagues have elaborated a scheme whereby the regulation of arachidonate release is intimately associated with turnover of the phosphatidylinositol cycle (Fig. 2; [56]). These workers have suggested that the formation of phosphatidic acid subsequent to phosphoinositide hydrolysis may activate phospholipase A_2, either directly or as a result of calcium mobilisation. This enzyme would then be responsible for cleaving arachidonate from phospholipids. It is not known whether this scheme represents a general mechanism for arachidonate liberation in mammalian tissues, but it might in part explain why the stimulation of islets by secretagogues such as glucose, which promote enhanced turnover of the phosphatidylinositol cycle, is often associated with the production of metabolites of arachidonic acid [57, 58].

The role of these arachidonic acid metabolites in islet function has also been the subject of a large number of investigations, and several reviews have dealt with this topic [57–60]. Initially, the literature relating to arachidonic acid metabolites and islet function can be confusing and appear contradictory. For example, the majority of in vivo studies indicate that prostaglandins may act as inhibitors of insulin secretion [59, 61–63]. In contrast, several reports have shown that in vitro, prostaglandins can exert a positive effect on insulin secretion [58–60]. One possible explanation for these discrepan-

cies may be that prostaglandins can induce both stimulatory or inhibitory effects on insulin secretion depending on the concentration of prostaglandin to which the islet is exposed. This phenomenon of bell-shaped dose-response curves is well-documented in prostaglandin pharmacology [64]. Other factors which may be important include type and concentration of secretagogue used [57], secondary effects of prostaglandins on the islet via changes in vascular supply or the release of other hormones or neurotransmitters [59, 60] and the unproven specificity of drugs used in several studies. The latter is particularly important since there is evidence that inhibition at different levels in the arachidonic acid metabolic cascade may result in different effects on insulin secretion [58]. For example, in a recent study, Yamamoto et al. [65] have provided evidence that the metabolism of arachidonic acid via the lipoxygenase pathway, in addition to the cyclo-oxygenase pathway, may be an important determinant in the regulation of glucose-induced insulin secretion. Thus, it is possible that cyclo-oxygenase products inhibit whereas lipoxygenase products stimulate insulin secretion [66–68]. In any case, the accumulating evidence suggests strongly that metabolites of arachidonic acid do play a role in islet function although they now appear more likely to act as modulators than as mediators [58].

One interesting proposal which has arisen from such considerations is that certain types of diabetes mellitus in which glucose-induced insulin secretion is impaired, might benefit from pharmacological or dietary suppression of prostaglandin production [57–60].

Fuel Function of Fatty Acids in Pancreatic Islets

Whilst the liberation of arachidonic acid from phospholipids for the subsequent formation of biologically active metabolites is an important step in the generation of intracellular (and possibly extracellular) signals or messengers, it should be kept in mind that the release of esterified fatty acids from glycerolipids may contribute towards supplying endogenous fuel in islets. In the absence of exogenous nutrient, the uptake of O_2 and secretion of insulin can persist for a considerable time, utilising endogenous nutrients. Berne [30] has shown that islets pre-labelled with ^{14}C-palmitate show a progressive decline in labelled triacylglycerol when subsequently incubated in the absence of exogenous nutrients. Further work has provided evidence that a major proportion of the respiratory rate in islets is accounted for by the oxidation of endogenous fuels (such as fatty acids and amino acids) even when the islet is exposed to physiological concentrations of glucose [69]. The regulation of triacylglycerol breakdown in pancreatic islets is therefore likely to be an important process in regulating the supply of free fatty acids for subsequent oxidation.

Phospholipid Methylation and Islet Activation

During the past few years, evidence has been accumulating, particularly from the laboratory of Axelrod and co-workers, that phospholipid methylation may be actively involved in the recognition of certain receptor-mediated stimuli [70, 71]. In a number of cell-types [72–74], stimulation is accompanied by a rapid, transient methylation of phosphatidylethanolamine to phosphatidylcholine. This conversion involves three sequential methylations by two methyltransferases [75] and is accompanied by the translocation of the methylated phospholipid from the cytoplasmic to the outer surface of the plasma membrane [70]. Such a process is thought to result in decreased membrane viscosity and a subsequent alteration in ion permeability and facilitation of receptor-enzyme coupling in the membrane [71]. The use of inhibitors of methyltransferase reactions suggests that a close relationship may exist between phospholipid methylation and the cellular response [70]. Glucose-induced insulin secretion in rat pancreatic islets is inhibited by the methyltransferase inhibitors 3-deaza-adenosine and homocysteine [76]. The incorporation of label from ^{3}H-methyl-methionine into phospholipids and proteins in islets was also inhibited by these drugs, although glucose oxidation and ^{45}Ca uptake were not altered, suggesting that transmethylation reactions may be involved in a late stage of the insulin secretory process. Campillo and Ashcroft [77] also presented evidence that protein carboxymethylation may play a role in the regulation of islet function. Clearly, further work is necessary to fully elucidate the role of phospholipid and protein methylation in stimulus-secretion coupling in pancreatic islets.

Conclusions

The work reviewed above provides evidence that enhanced phospholipid turnover in islets may be a determinant of the secretory response to nutrient secretagogues and certain neurotransmitter and hormonal stimuli. The available data are compatible with the hypothesis that stimulated phospholipid turnover may be involved in the control of calcium mobilisation in islets, although additional possibilities clearly exist, such as the facilitation of membrane fusion during exocytosis and the liberation of arachidonic acid for subsequent metabolism via the cyclo-oxygenase and lipoxygenase pathways. Phospholipid and protein methylation may also be involved at some stage in stimulus-secretion coupling in pancreatic islets. Continued investigation of these topics is likely to contribute to a better understanding of the control of secretory activity in pancreatic islets, which in turn may throw light on pathophysiological aspects of islet function and perhaps suggest novel therapeutic approaches.

References

1. Hokin MR, Hokin LE (1954) Effects of acetylcholine on phospholipids of the pancreas. J Biol Chem 209: 549–558
2. Michell RH (1975) Inositol phospholipids and cell surface receptor function. Biochim Biophys Acta 415: 81–147
3. Lapetina EG, Michell RH (1973) Phosphatidylinositol metabolism in cells receiving extracellular stimulation. FEBS Lett 31: 1–10
4. Michell RH (1982) Stimulated inositol lipid metabolism: an introduction. Cell Calcium 3: 285–294
5. Hawthorne JN, Kai M (1970) Metabolism of phosphoinositides. In: Lajtha A (ed) Handbook of neurochemistry. Plenum Press, New York, pp 491–508
6. Hawthorne JN, Pickard MR (1979) Phospholipids in synaptic function. J Neurochem 32: 5–14
7. Berridge MJ (1981) Phosphatidylinositol hydrolysis: a multifunctional transducing mechanism. Mol Cell Endocrinol 24: 141–163
8. Putney JW Jr (1981) Recent hypotheses regarding the phosphatidylinositol effect. Life Sci 29: 1183–1194
9. Downes P, Michell RH (1982) Phosphatidylinositol-4-phosphate and phosphatidylinositol-4,5-bisphosphate: lipids in search of a function. Cell Calcium 3: 467–502
10. Michell RH (1982) Is phosphatidylinositol really out of the calcium gate? Nature 296: 492–493
11. Fex G, Lernmark A (1972) Effect of D-glucose on the incorporation of ^{32}P into phospholipids of mouse pancreatic islets. FEBS Letters 25: 287–291
12. Freinkel N, Cohanim N (1972) Islet phospholipogenesis and glucose-stimulated insulin secretion. J Clin Invest 51: 33a (Abstract)
13. Freinkel N, El Younsi C, Dawson RMC (1975) Interrelations between the phospholipids of rat pancreatic islets during glucose stimulation and their response to medium inositol and tetracaine. Eur J Biochem 59: 245–252
14. Tanigawa K, Kuzuya H, Imura H, Taniguchi H, Baba S, Takai Y, Nishizuka Y (1982) Calcium-activated, phospholipid-dependent protein kinase in rat pancreas islets of Langerhans. FEBS Letters 138: 183–186
15. Best L, Malaisse WJ (1982) Study of phospholipid metabolism in rat islets using different types of labelled precursors. Diabetologia 23: 155 (Abstract)
16. Montague W, Parkin EN (1980) Changes in membrane lipids of the B-cell during insulin secretion. Horm Metab Res (Suppl) 10: 153–157
17. Clements RS, Rhoten WB (1976) Phosphoinositide metabolism and insulin secretion from isolated rat pancreatic islets. J Clin Invest 57: 694–691
18. Clements RS, Evans MH, Pace CS (1981) Substrate requirements for the phosphoinositide response in rat pancreatic islets. Biochim Biophys Acta 647: 1–9
19. Fex G, Lernmark A (1975) Effects of insulin secretagogues on phospholipid metabolism in pancreatic B-cells. Biochim Biophys Acta 388: 1–4
20. Best L, Malaisse WJ (1983) Effects of nutrient secretagogues on phospholipid metabolism in rat pancreatic islets. Mol Cell Endocrinol (in press)
21. Hellerström C, Andersson A, Welsh M (1980) Respiration of the pancreatic B-cell: effects of glucose and 2-aminonorbornane-2-carboxylic acid. Horm Metab Res (Suppl) 10: 37–43
22. Sener A, Malaisse-Lagae F, Malaisse WJ (1981) Stimulation of pancreatic islet metabolism and insulin release by a nonmetabolizable amino acid. Proc Natl Acad Sci (USA) 78: 5460–5464
23. Malaisse WJ, Sener A, Welsh M, Malaisse-Lagae F, Hellerström C, Christophe J (1983) Mechanism of 3-phenylpyruvate induced insulin release. Metabolic aspects. Biochem J 210: 921–927
24. Sener A, Welsh M, Lebrun P, Garcia-Morales P, Saceda M, Malaisse-Lagae F, Herchuelz A, Valverde I, Hellerström C, Malaisse WJ (1983) Mechanism of 3-phenylpyruvate induced insulin release. Secretory, ionic and oxidative aspects. Biochem J 210: 913–919
25. Malaisse WJ, Sener A, Herchuelz A, Hutton JC (1979) Insulin release: the fuel hypothesis. Metabolism 28: 373–386
26. Malaisse WJ, Hutton JC, Kawazu S, Sener A (1978) The stimulus-secretion coupling of glucose-induced insulin release. Metabolic effects of menadione in isolated islets. Eur J Biochem 87: 121–130
27. Best L, Malaisse WJ (1983) Phosphatidylinositol and phosphatidic acid metabolism in rat pancreatic islets in response to neurotransmitter and hormonal stimuli. Biochim Biophys Acta 750: 157–163
28. Farese RV, Larson RE, Sabir MA (1982) Ca^{2+}-dependent and Ca^{2+}-independent effects of pancreatic secretagogues on phosphatidylinositol metabolism. Biochim Biophys Acta 710: 391–399
29. Best L, Malaisse WJ (1983) Stimulation of phosphoinositide breakdown in rat pancreatic islets by glucose and carbamylcholine. Biochem Biophys Res Commun (in press)
30. Berne C (1975) The metabolism of lipids in mouse pancreatic islets. The biosynthesis of triacylglycerols and phospholipids. Biochem J 152: 667–673
31. Clements RS, Rhoten WB, Starnes WR (1977) Subcellular localisation of the alterations in phosphatidylinositol metabolism following glucose-induced insulin release from rat pancreatic islets. Diabetes 26: 1109–1116
32. Hawthorne JN (1982) Is phosphatidylinositol now out of the calcium gate? Nature 295: 281–282
33. Cockcroft S (1981) Does phosphatidylinositol breakdown control the Ca^{2+}-gating mechanism? Trends Pharmacol Sci 2: 340–342
34. Malaisse WJ, Hutton JC, Carpinelli AR, Herchuelz A, Sener A (1980) The stimulus-secretion coupling of amino acid-induced insulin release. Metabolism and cationic effects of leucine. Diabetes 29: 431–437
35. Hutton JC, Sener A, Herchuelz A, Atwater I, Kawazu S, Boschero AC, Somers G, Devis G, Malaisse WJ (1980) Similarities in the stimulus-secretion coupling mechanisms of glucose- and 2-keto acid-induced insulin release. Endocrinology 106: 203–219
36. Malaisse WJ, Carpinelli AR, Lebrun P, Herchuelz A, Sener A (1981) The stimulus-secretion coupling of amino acid-induced insulin release. IV. Ionic response to L-leucine and L-glutamine. Pflügers Arch 391: 112–118
37. Burr IM, Slonim AE, Burke V, Fletcher T (1976) Extracellular calcium and adrenergic and cholinergic effects on islet B-cell function. Am J Physiol 231: 1246–1249
38. Grodsky GM, Bennett LL (1966) Cation requirements for insulin secretion in the isolated perfused pancreas. Diabetes 15: 910–913
39. Ashby SP, Speake RN (1975) Insulin and glucagon secretion from isolated islets of Langerhans. The effects of calcium ionophores. Biochem J 150: 89–96
40. Hales CN, Milner RDG (1968) Cations and the secretion of insulin from rabbit pancreas in vitro. J Physiol (London) 199: 177–187
41. Tyson CA, Vande Zande H, Green DE (1976) Phospholipids as ionophores. J Biol Chem 251: 1326–1332
42. Dunlop M, Larkins RG, Court JM (1982) Endogenous ionophoretic activity in the neonatal rat pancreatic islet. FEBS Lett 144: 259–263
43. Valverde I, Malaisse WJ (1979) Ionophoretic activity in pancreatic islets. Biochem Biophys Res Commun 89: 386–395
44. Anjaneyulu K, Anjaneyulu R, Malaisse WJ (1980) The stimulus-secretion coupling of glucose-induced insulin release. XLIII. Na-Ca countertransport mediated by pancreatic islet native ionophores. J Inorg Biochem 13: 178–188
45. Dieryck P, Winand J, Malaisse WJ (1981) Ionophoretic activity of lipids extracted from pancreatic islets. IRCS Med Sci 9: 190
46. Deleers M, Mahy M, Malaisse WJ (1982) Calcium ionophoresis by pancreatic islet extracts in model membranes. Biochem Int 4: 47–57
47. Allan D, Billah MM, Fineau JB, Michell RH (1976) Release of diacylglycerol-enriched vesicles from erythrocytes with increased intracellular Ca^{2+}. Nature 261: 58–60
48. Deleers M, Ruysschaert JM, Malaisse WJ (1981) Glucose induces membrane changes detected by fluorescence polarisation in endocrine pancreatic cells. Biochem Biophys Res Commun 98: 255–260

49. Takai Y, Kishimoto A, Iwasa Y, Kawahara Y, Mori T, Nishizuka Y (1979) Calcium-dependent activation of a multifunctional protein kinase by membrane phospholipids. J Biol Chem 254: 3692–3695
50. Dawson RMC, Freinkel N, Jungalwala FB, Clarke N (1971) The enzymic formation of myoinositol 1,2-cyclic phosphate from phosphatidylinositol. Biochem J 122: 605–607
51. Michell RH, Lapetina EG (1972) Production of cyclic inositol phosphate in stimulated tissues. Nature New Biol Lond 240: 258–260
52. Freinkel N, Dawson RMC (1973) The role of inositol cyclic phosphate in stimulated tissues. Nature Lond 243: 535–537
53. Laychock SG (1982) Phospholipase A_2 activity in pancreatic islets is calcium-dependent and stimulated by glucose. Cell Calcium 3: 43–54
54. Laychock SG (1983) Fatty acid incorporation into phospholipids of isolated pancreatic islets of the rat. Relationship to insulin release. Diabetes 32: 6–13
55. Yamamoto S, Nakadata T, Nakaki T, Ishii K, Kato R (1982) Tumor promoter 12-0-tetradecanoylphorbol-13-acetate-induced insulin secretion: inhibition by phospholipase A_2- and lipoxygenase-inhibitors. Biochem Biophys Res Commun 105: 759–765
56. Lapetina EG (1982) Regulation of arachidonic acid production: role of phospholipases C and A_2. Trends Pharmacol Sci 3: 115–118
57. Metz SA (1981) Feedback modulation of glucose-induced insulin secretion by arachidonic acid metabolites: possible molecular mechanisms and relevance to diabetes mellitus. Prostaglandins Med 7: 581–589
58. Pek SB, MacAdams MR (1982) Modulatory role of prostaglandins in regulation of secretion of insulin and glucagon. In: McConn (ed) Role of chemical mediators in the pathophysiology of acute illness and injury. Raven Press, New York, pp 291–295
59. Robertson RP (1979) Prostaglandins as modulators of pancreatic islet function. Diabetes 28: 943–948
60. Robertson RP (1981) Interrelationships between prostaglandins and biogenic amines during modulation of B-cell function. In: Cooperstein SJ, Watkins D (eds) The islets of Langerhans. Academic Press, New York, pp 173–188
61. Sacca L, Perez G, Rengo F, Pascucci I, Condorelli M (1975) Reduction of circulating insulin levels during the infusion of different prostaglandins in the rat. Acta Endocrinol 79: 266–274
62. Giugliano D, Torella R, Sgambato S, D'Onofrio F (1979) Effects of α- and β-adrenergic inhibition and somatostatin on plasma glucose, free fatty acids, insulin, glucagon and growth hormone responses to prostaglandin E_1 in man. J Clin Endocrinol Metab 48: 302–308
63. Robertson RP, Gavareski DJ, Porte D Jr, Bierman EL (1974) Inhibition of in vivo insulin secretion by prostaglandin E_1. J Clin Invest 54: 310–315
64. Horrobin DF (1977) Interrelationship between prostaglandins and calcium. The importance of bell-shaped dose-response curves. Prostaglandins 14: 667–677
65. Yamamoto S, Nakadate T, Nakaki T, Ishii K, Kato R (1982) Prevention of glucose-induced insulin secretion by lipoxygenase inhibition. Eur J Pharmacol 78: 225–227
66. Morgan RO, Pek SB (1982) Divergent effects of cyclooxygenase- and lipoxygenase-activated arachidonate metabolites on pancreatic hormone secretion; glucose as an important determinant. Endocrinology 110 (Suppl 330) (Abstract)
67. Metz SA, Fujimoto WY, Robertson RP (1982) Lipoxygenation of arachidonic acid: a pivotal step in stimulus-secretion coupling in the pancreatic beta cell. Endocrinology 111: 2141–2143
68. Metz SA, Fujimoto WY, Robertson RP (1983) A role for the lipoxygenase pathway of arachidonic acid metabolism in glucose- and glucagon-induced insulin secretion. Life Sci 32: 903–910
69. Malaisse WJ, Best L, Kawazu S, Malaisse-Lagae F, Sener A (1983) The stimulus-secretion coupling of glucose-induced insulin release. Fuel metabolism in islets deprived of exogenous nutrient. Arch Biochem Biophys 224: 102-110
70. Axelrod J, Hirata F (1982) Phospholipid methylation and the receptor-induced release of histamine from cells. Trends Pharmacol Sci 3: 156–158
71. Hirata F, Axelrod J (1980) Phospholipid methylation and biological signal transmission. Science 209: 1082–1090
72. Hirata F, Axelrod J, Crews FT (1979) Concanavalin A stimulates phospholipid methylation and phosphatidylserine decarboxylation in rat mast cells. Proc Natl Acad Sci (USA) 76: 4813–4816
73. Bareis DL, Hirata F, Schiffmann E, Axelrod J (1982) Phospholipid metabolism, calcium flux and the receptor-mediated induction of chemotaxis in rabbit neutrophils. J Cell Biol 93: 690–697
74. Castano JG, Alemany S, Nieto A, Mato JM (1980) Activation of phospholipid methyltransferase by glucagon in rat hepatocytes. J Biol Chem 255: 9041–9043
75. Hirata F, Viveros OH, Diliberto EJ, Axelrod J (1978) Identification and properties of two methyltransferases in conversion of phosphatidylethanolamine to phosphatidylcholine. Proc Natl Acad Sci (USA) 75: 1718–1721
76. Saceda M, Garcia-Morales P, Valverde I, Malaisse WJ (1983) Glucose-induced stimulation of lipid methylation in pancreatic islets. Diabetes 32 (Suppl 1): 39a (Abstract)
77. Campillo JE, Ashcroft SJH (1982) Protein carboxymethylation in rat islets of Langerhans. FEBS Lett 138: 71–75

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