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Phospholipid metabolism in pancreatic islets

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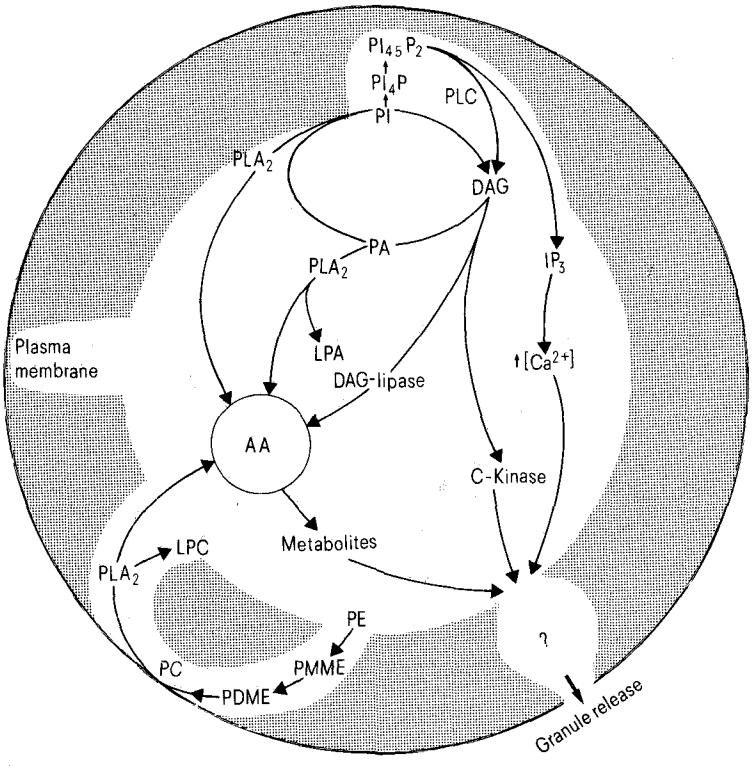
In recent years, considerable advances have been made in understanding how insulin is synthesized and secreted, and the mechanisms by which these processes can be triggered upon exposure of the islet to different types of secretagogue. Thus, the stimulus-secretion coupling of insulin release is thought to involve an interaction of numerous processes, including nutrient metabolism, agonist-receptor binding, cyclic nucleotide production, calcium mobilization (and other ionic fluxes) and, through activation of protein kinases, the phosphorylation of a number of islet proteins. The purpose of this section is to review a topic which until recently has received relatively little attention; that of enhanced metabolism of islet phospholipids during stimulation. In particular, the nature of stimulated phospholipid turnover will be considered together with some speculation as to the role of this process and possible interactions with other biochemical events which occur during islet activation.

A) Nature of stimulated phospholipid metabolism

In 1953, Hokin and Hokin[40] noted that cholinergic stimulation of exocrine pancreas resulted in a marked increase of ^{32}P incorporation into phospholipids, suggesting enhanced phospholipid turnover. Subsequent work by these and other workers has identified the phosphoinositides and phosphatidic acid as the principal lipid components involved in accelerated metabolic turnover, and a generally accepted scheme for the interconversion of these lipids is shown in the figure. For more detailed descriptions, the reader is referred to a number of excellent reviews on the subject[6, 28, 36, 60, 61, 65]. In pancreatic islets Fex and Lernmark[29] first demonstrated enhanced ^{32}P-labelling of phospholipids, predominantly a fraction containing phosphatidylinositol and phosphatidylserine, upon exposure to high glucose concentrations. Similarly, Freinkel and colleagues[31] showed that glucose stimulated ^{32}P-labelling of phosphatidylinositol, phosphatidic acid and phosphatidylethanolamine in rat islets. In subsequent studies where islet nucleotide pools were pre-labelled with ^{32}P, a specific stimulation of labelling of phosphatidylinositol[11, 78], phosphatidic acid and the polyphosphoinositides[11] has been demonstrated. It is generally assumed in such studies that the measurement of enhanced labelling of these phospholipids with ^{32}P reflects turnover of the polar head group, or more specifically, resynthesis following breakdown. However, it should be emphasised that a stimulation of de novo phospholipid synthesis, or even breakdown of a non-inositol-containing phospholipid would also be expected to result in enhanced ^{32}P-labelling of phosphatidic acid and thereby the phosphoinositides. Thus, ^{32}P-labelling studies provide at best an indirect measurement of phospholipid breakdown and should be interpreted with caution. An alternative approach has been adopted by Montague and Parkin[62] who pre-labelled guinea pig islets with ^{3}H-glycerol and noted a subsequent fall in the levels of all major labelled phospholipids upon glucose stimulation. These authors also detected increased production of diacylglycerol and suggested that glucose caused phospholipid breakdown via removal of the polar head group. In a study designed specifically to investigate the breakdown of inositol-containing lipids, Clements et al.[18, 19] prelabelled islet phospholipids with ^{3}H-inositol and observed a subsequent fall in labelled lipids upon exposure to glucose. This effect was accompanied by increased production of water-soluble derivatives of ^{3}H-inositol and suggested that glucose provoked a breakdown of inositol phospholipids in islets. Using a similar approach, we have also demonstrated a loss of ^{3}H-inositol-labelled lipids in rat islets in response to stimulatory glucose concentrations, together with a marked stimulation of ^{3}H-inositol phosphate production[12], suggesting that phosphoinositide breakdown occurs via phospholipase C activation. It was orginally supposed that phosphatidylinositol was the lipid primarily hydrolyzed by such a mechanism, but a series of elegant studies from the laboratory of Berridge and coworkers[7, 8] have indicated that breakdown of the polyphosphoinositides (phosphatidylinositol 4,5-bisphosphate and possibly phosphatidylinositol 4-phosphate) is the initial event which occurs upon stimulation. The previously observed loss of phosphatidylinositol probably reflects replenishment of the polyphosphoinositide pool following hydrolysis of the latter. In rat islets, analysis of the water-soluble ^{3}H-labelled products of stimulated inositol lipid hydrolysis reveals detectable quantities of ^{3}H-inositol 1,4,5-trisphosphate and ^{3}H-inositol 1,4-bisphosphate together with ^{3}H-inositol 1-phosphate[13a], again suggesting that polyphosphoinositide hydrolysis at least contributes inositol lipid hydrolysis in islets. Furthermore, labelling of islet polyphosphoinositides to isotopic equilibrium with ^{32}P and subsequent exposure to either glucose[13a, 50] or carbamylcholine[13a] results in a significant loss of labelled phosphatidylinositol 4,5-bisphosphate (and in some cases, phosphatidylinositol 4-phosphate) suggesting an initial breakdown of these lipids in response to the above stimuli.

Schrey and Montague[72] have recently described a cytosolic enzyme in guinea pig islets which hydrolyses phosphatidylinositol yielding diacylglycerol and inositol phosphate. However, these authors did not examine enzymic activity using polyphosphoinositides as substrate and so no firm conclusion can be drawn as to which of the inositol phospholipids are broken down in the intact islet under physiological conditions.



Schematic representation of cellular phospholipid transformations: possible involvement in calcium mobilisation, protein kinase C activation, arachidonic acid (AA) release and insulin secretion in pancreatic islets. The following abbreviations were used: AA: arachidonic acid; DAG: 1,2-diacylglycerol; DAG-lipase: diacylglycerol lipase; IP_3: inositol 1,4,5-trisphosphate; LPA: lysophosphatidic acid; LPC: lysophosphatidylcholine; PA: phosphatidic acid; PC: phosphatidylcholine; PDME: phosphatidyl-N,N-dimethylethanolamine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PI_4P: phosphatidylinositol 4-phosphate; $PI_{4,5}P_2$: phosphatidylinositol 4,5-bisphosphate; PLA_2: phospholipase A_2; PLC: phospholipase C; PMME: phosphatidyl-N-methylethanolamine.

Whilst there is strong evidence that the stimulation of pancreatic islets with certain secretagogues results in hydrolysis of inositol-containing phospholipids, it seems likely that such secretagogues can also stimulate the de novo biosynthesis of phospholipids. Thus, Berne[5] has shown that exposure of rat islets to high concentrations of glucose enhances the incorporation of ^{14}C-glucose or ^{14}C-palmitate into phospholipids, whilst Laychock[49] observed increased incorporation of labelled palmitate and arachidonate into islet phospholipids in response to glucose or carbamylcholine. We have recently shown that stimuli which induce inositol lipid hydrolysis in rat islets also promote de novo synthesis of these acidic phospholipids using ^{3}H-glycerol as precursor[13b]. The importance of this enhanced de novo synthesis of phospholipids in islets is unknown, although it is possible that this process may be initiated either directly or indirectly during stimulation in order to replenish and maintain the size of the inositol lipid pools in islets during prolonged exposure to secretagogues. It is also of interest to note that secretagogues such as glucose can provide not only a stimulus to islet phospholipid biosynthesis but also, via the formation of glycerol-3-phosphate, supply one of the metabolic precursors for phospholipid (and glyceride) biosynthesis.

Stimuli which provoke increased phospholipid metabolism in pancreatic islets

A number of authors have reported that glucose, physiologically the most important stimulus for insulin release, causes either breakdown[4, 12, 18, 19, 50, 62] or otherwise accelerated turnover[11, 29–31, 78] of the inositol phospholipids. It also appears that several other nutrient secretagogues such as mannose[17], leucine[30] and α-ketoisocaproate[11], and in addition substances such as 3-phenylpyruvate and 2-amino-2-carboxyl-bicycloheptane[11] which promote insulin release via the mobilization of endogenous islet nutrients[38, 54, 73] produce similar effects on phospholipid turnover. Thus the pancreatic islet provides an example of a tissue in which accelerated phospholipid metabolism can be induced by the generation of a metabolic signal. The nature of this signal is not known although glucose-induced ^{32}P-labelling of phospholipids is markedly reduced by menadione[11], a drug which reduces islet NAD(P)H levels without affecting those of ATP[51]. Furthermore, the concentration-response curves for NAD(P)H levels in response to glucose closely parallel phosphatidylinositol labelling[11, 53], again suggesting a possible connection between generation of reduced nicotinamide nucleotides and enhanced phospholipid turnover.

Other insulinotropic agents which have been shown to stimulate phosphoinositide metabolism include the neurotransmitter secretagogues carbamylcholine and cholecystokinin-pancreozymin[10]. Clements et al.[17] have also reported that tolbutamide, ouabain and isoprenaline induce inositol lipid breakdown, although Fex and Lernmark[30] found glibenclamide to be ineffective in promoting ^{32}P-labelling of islet phospholipids. In a number of studies[10, 12] we have been unable to demonstrate any stimulatory effect using arginine, glibenclamide, glucagon, TSH, high K^+, ionophore A23187 or Ba^{2+}, suggesting that enhanced phospholipid metabolism in rat islets is specific to nutrient and neurotransmitter stimuli and

is not the result of increased Ca^{2+} mobilization upon stimulation.

Stimulated phospholipid methylation in pancreatic islets

In a number of cells and tissues, stimulation through certain types of receptor is accompanied by phospholipid N-methylation. This reaction involves the rapid sequential methylation of phosphatidyl-ethanolamine by two methyltransferases to form phosphatidylcholine (for reviews, see Hirata and Axelrod[39] and Mato and Alemany[55]). The discovery of this effect led to the suggestion by Hirata and Axelrod[39], that phospholipid methylation may play an important role in the transduction of biochemical signals across cell membranes during activation. In particular, these authors have proposed a link between phospholipid methylation, phospholipase A_2 activation, the liberation of arachidonic acid and subsequent formation of metabolites, and changes in membrane viscosity and permeability to cations. Such an hypothesis has been derived largely from work with cells of the immune system and little information so far exists concerning the possible role of lipid methylation in hormone secretion. Saceda et al.[71] have reported that pancreatic islets exposed to glucose show a transient increase in incorporation of ^{3}H-methyl from [^{3}H-methyl]methionine into phospholipids. In addition, the treatment of islets with DL-homocysteine and 3-deazaadenosine, which inhibit cellular transmethylation reactions, results in the inhibition of insulin secretion in response to glucose, glucose plus gliclazide, leucine plus glutamine, and Ba^{2+} plus theophylline[9]. However, these drugs exerted no significant influence on glucose oxidation, K^+ conductance, or Ca^{2+} fluxes in islets under the same conditions, making it more likely that phospholipid methylation is involved at a more distal site in the secretory sequence. It should be emphasized that the methylation reaction inhibitors prevented not only phospholipid N-methylation[9,71] but also methylation of proteins[9], raising the possibility that the latter may also play a role in the regulation of hormone secretion. Indeed, Campillo and Ashcroft[16] have identified methyl acceptor proteins in islets and suggested that the methylation of such proteins may play a regulatory role in the secretion of insulin. Further investigations are clearly necessary to elucidate the nature and role of phospholipid and protein methylation in pancreatic islets.

B) Consequences of enhanced phospholipid metabolism

An important factor in considering the possible role(s) that stimulated phospholipid turnover may play in the regulation of pancreatic islet function is the time course of these events in relation to hormone secretion. It is therefore of interest to note that inositol lipid breakdown[12,18,25,50], inositol phosphate production[12] and phosphatidate accumulation[10,11,25] all occur within minutes of stimulation. These observations are compatible with a regulatory role for increased phospholipid metabolism in insulin release, particularly inositol lipid breakdown which is generally considered to be the initial step in enhanced phospholipid turnover. A number of possibilities exist as to how the latter may be involved in the control of islet function.

Inositol lipid breakdown and calcium mobilization

In 1975, Michell[60] put forward the hypothesis that enhanced inositol lipid turnover might be related in some way to the regulation of calcium mobilization during cell stimulation through certain classes of receptor. It is known that polar, acidic phospholipids such as the phosphoinositides can bind divalent cations. Hydrolysis of these lipids upon cell stimulation could thus result in the release of divalent cations from the membrane together with a change in ion permeability of that membrane. Furthermore, it has recently been shown that inositol 1,4,5-trisphosphate, the water-soluble product of phosphatidylinositol 4,5-bisphosphate hydrolysis, can mobilize calcium from an intracellular source (probably non-mitochondrial) in permeabilized pancreatic acinar cells[75]. If inositol lipid breakdown plays a role in the regulation of calcium fluxes in islets, one would expect that the former would not itself occur as a result of increased calcium mobilization. The majority of studies in islets suggest that stimulated phospholipid metabolism persists in the absence of added extracellular calcium[5,10-12,25,31], conditions known to impair insulin secretion[15,33]. In contrast, Clements[17], Laychock[50] and Axen et al.[4] have reported that inositol lipid breakdown in islets in response to glucose was inhibited by EGTA, suggesting that this response is secondary to calcium mobilization. A possible explanation for these apparent discrepancies is that inositol lipid hydrolysis in islets is a calcium-dependent process but it does not itself occur as a result of increased calcium mobilization. This suggestion is borne out by the failure of a number of secretagogues, including ionophore A23187 and K^+ which promote calcium fluxes in islets, to exert any appreciable effect upon inositol lipid metabolism in islets[10,12] (also L. Best and W.J. Malaisse, unpublished observations). In fact, it appears that A23187 does exert a marked effect on the metabolism of phosphatidic acid in islets, causing an accumulation of this phospholipid[13], but this effect was distinct from nutrient- or neurotransmitter-induced breakdown of inositol lipids.

Consequences of diacylglycerol formation

The hydrolysis of inositol phospholipids by a phospholipase C mechanism occurs by cleavage of the polar inositol phosphate headgroup to form 1,2-diacylglycerol. It has been suggested that the formation of this latter compound upon cell activation may play a key role in a number of subsequent cellular reactions. For example, increased concentrations of diacylglycerol in a cellular membrane could have profound effects on membrane fluidity[35] and fusion[2], and it is interesting to note that glucose increases membrane fluidity in pancreatic islet cells[22]. In addition, the identification of a protein kinase (C-kinase) which is calcium-dependent and activated by diacylglycerol[63] suggests another mechanism by which inositol lipid breakdown may regulate cell function. C-Kinase activity has been demonstrated in pancreatic islets[41,78], and it appears that this enzyme can be activated

by unsaturated diacylglycerol and also by the tumour promoting agent 12-O-tetradecynoyl-phorbol-13-acetate (TPA[41]). This substance is also a potent insulinotropic agent[52, 82, 85], suggesting a possible connection between C-kinase activation and insulin release. However, it is as yet unknown whether diacylglycerol formed from inositol lipid breakdown actually plays a physiological role in the activation of this enzyme in islets, and it should be borne in mind that diacylglycerol can be rapidly phosphorylated to form phosphatidic acid. An alternative metabolic fate of diacylglycerol may be its further hydrolysis by a lipase to release free arachidonic acid and monoacylglycerol. Schrey and Montague[72] have recently provided evidence that guinea-pig islets contain such a diacylglycerol lipase activity, and this pathway could contribute towards the liberation from phospholipids of arachidonate and its subsequent metabolism to prostaglandins and related compounds. The latter subject will be considered more fully later.

Consequences of phosphatidic acid formation

The finding that phosphatidic acid can mobilize calcium in a number of cell types[64, 66] and artificial systems[74, 79] has led to the suggestion that this acidic phospholipid may function as an endogenous cellular ionophore under external control[66]. Such ionophoretic activity has been identified in organic extracts of islets from adult[3, 21, 80] and neonatal[26] rats. In the latter case, stimulation of islets with glucose[24] or islet activating protein[23] results in increased levels of endogenous calcium ionophoretic activity. The further demonstration that levels of phosphatidic acid in islets correlate with ionophoretic activity and glucose-induced insulin secretion[26] suggests that this phospholipid might be at least a component of the endogenous ionophoretic activity.

An alternative, or additional role that phosphatidic acid might play in the regulation of islet function is as a source of arachidonate. Lapetina[47] has proposed a general mechanism whereby phosphatidic acid formed as a result of inositol lipid breakdown is subsequently hydrolyzed by a phospholipase A_2 to liberate free arachidonate. The observation that homogenates of neonatal islets release arachidonate from arachidonyl-labelled phosphatidic acid (M. E. Dunlop and R. G. Larkins, unpublished observations) suggests that such a pathway might exist in pancreatic islets. Under similar conditions, arachidonate release has been observed using labelled phosphatidyl-ethanolamine[48] or phosphatidylcholine as substrate[14a] (M. E. Dunlop and R. G. Larkins, unpublished observation), suggesting that these lipids might also be susceptible to the action of phospholipase A_2 in stimulated islets. It should be noted that phospholipid hydrolysis by a phospholipase A_2 mechanism will yield, in addition to a free fatty acid, lysophospholipids which themselves possess potent biological activity[32].

Possible role of arachidonic acid and its metabolites in islet function

In mammalian tissues, arachidonic acid is esterified almost exclusively in the '2' position of glycerolipids, and its liberation, by breakdown of these lipids is a prerequisite for the formation of prostaglandins, leukotrienes and other metabolites of arachidonic acid. As described in previous sections, there are at least two possible mechanisms whereby arachidonic acid could be cleaved from phospholipids, namely phospholipase C (in conjunction with diacylglycerol lipase) and phospholipase A_2 action.

Direct measurements of the metabolites of arachidonic acid or the prostaglandin endoperoxide PGH_2 in islets or perfused pancreas have detected prostaglandin E_2 (PGE_2)[27, 44, 58], prostacyclin (PGI_2[83]), prostaglandin D_2 (PGD_2), prostaglandin $F_2\alpha$ ($PGF_2\alpha$) and thromboxanes[1]. In addition, it has been shown that arachidonic acid may be metabolized via the lipoxygenase pathway to a series of hydroxyeicosatetraenoic (HETE) derivatives including 5-HETE, 12-HETE and 15-HETE[85]. 5-HETE is an intermediate in the biosynthesis of leukotrienes, about which little is documented in pancreatic islets. It is generally believed that the metabolic transformation of arachidonate to the above substances is a prerequisite for biological activity. However, it should be borne in mind that unsaturated fatty acids can themselves influence a number of aspects of cell function including the metabolism of inositol phospholipids[76] and membrane fusion[20].

Investigations of the role(s) of arachidonic acid metabolites in islets have, in general, followed two major themes: the effects of exogenous prostaglandins and lipoxygenase products on islet function and the effects resulting from inhibition of synthesis of these compounds in the islet. The majority of in vivo studies indicate that prostaglandins inhibit insulin release[34, 67–70], an effect which could possibly reflect effects on vascular supply to the islet, release of other hormones or neurotransmitters[67, 68] or the actions of prostaglandin metabolites[57, 67, 68]. These findings contrast with several reports of stimulatory effects of PGE_2, PGD_2[56], $PGF2\alpha$[46] and PGH_2[1] on insulin release in the perfused rat pancreas. The observations that adenylate cyclase activity in islets[37, 42, 43, 45] and human insulinoma[81] is increased by some exogenous prostaglandins may explain their stimulatory effects on insulin release. Of the lipoxygenase products, 5-HETE appears to stimulate insulin secretion, whilst 12- and 15-HETE inhibit glucose-induced insulin secretion[84]. In a recent study, Metz and coworkers[59] also demonstrated a potentiation of glucose-induced insulin release by the unstable intermediate 12-hydroperoxyeicosatetraenoic acid (12-HPETE). The mechanism by which these substances stimulate insulin release remains, however, unknown.

A considerable amount of information has arisen from studies of the effects on islet function of pharmacological inhibitors of arachidonate release and metabolism. Yamamoto and colleagues[85] have reported that mepacrine and p-bromophenacylbromide, two purported inhibitors of phospholipase A_2, inhibit insulin secretion in response to the tumor-promoter TPA. Similarly, Laychock[49] has reported that p-bromophenacylbromide suppresses glucose-induced insulin secretion. In contrast, Tanaka and coworkers[77] reported that the inhibition of arachidonate release by low concentrations of mepacrine actually enhances insulin release in cultured

islet cells. We have found that both mepacrine and p-bromophenacylbromide can inhibit inositol lipid breakdown in islets by a phospholipase C route and also impair several other parameters of islet function including ^{45}Ca uptake, glucose oxidation and insulin release[14]. Thus, the effects of these drugs on insulin secretion need not be related solely to reduced availability of arachidonate, and clearly drug specificity and the concentrations employed are important considerations when interpreting data arising from their use. Despite these limitations, the impairment of glucose-induced insulin secretion by inhibitors of the lipoxygenase enzyme system, such as nordihydroguaiaretic acid[58, 84, 86], 3-amino-1-(3-trifluoromethyl-phenyl)-2-pyrazoline[58, 84], 1-phenyl-3-pyrazolidinone[84, 86] and 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadinylyl)-1,4-benzoquinone[84] suggests that the products of the lipoxygenase pathway may enhance the secretion of insulin. On the other hand, inhibition of the cyclooxygenase pathway by acetylsalicylic acid[27, 46, 84] or indomethacin[83, 84, 86] results in enhanced insulin release, implying that the production of prostaglandins in islets may be responsible for a negative modulation of insulin secretion.

The pharmacological or dietary modification of arachidonic acid metabolism in islets thus provides an additional approach to the manipulation of insulin secretion which may be of potential value in the treatment of certain types of diabetes mellitus.

Conclusions

The secretion of insulin can be elicited by a wide spectrum of stimuli including nutrients, hormones and neurotransmitters as well as a large number of pharmacological agents such as tumor-promoters and sulphonylureas. The diversity of these secretagogues suggests that islets may be activated through a number of distinct biochemical mechanisms. The work discussed in this review suggests that certain of the above-mentioned secretagogues, especially nutrient and neurotransmitter stimuli, may induce insulin secretion by a mechanism involving enhanced metabolism of inositol-containing lipids. The way in which this process is coupled to secretion is not known, although several possibilities exist. The hydrolysis of phosphoinositides and release of inositol phosphates may result, respectively in altered calcium permeability of the plasma membrane and mobilization of calcium from intracellular sources. The accompanying production of diacylglycerol might also influence membrane permeability and fluidity and also lead to activation of protein kinase C. Diacylglycerol can be phosphorylated to form phosphatidic acid which may play a role as an endogenous ionophore. Finally, inositol lipid breakdown could lead, through diacylglycerol and/or phosphatidic intermediates, to the liberation of arachidonic acid and subsequent conversion to active metabolites of the cyclo-oxygenase and lipoxygenase pathways. Thus, enhanced phospholipid metabolism in islets could, theoretically, result in the generation of a range of intracellular signals which mediate or modulate insulin secretion during stimulation by certain types of secretagogues. Continued investigation is clearly neccessary in order to elucidate the mechanisms by which such secretagogues provoke increased phospholipid metabolism and to understand the role(s) of this process in the regulation of islet function.

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Secretory granules

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Introduction

The pancreatic B-cell in common with many exocrine and endocrine cells stores its secretory product in membrane-limited intracellular vesicles and releases it in a graded response through regulation of membrane fusion events. By releasing the hormone from preformed stores a more rapid and sustained response may be achieved than could be attained by control exerted at the translational or transcriptional level. Such a process, however, does require the coordinated synthesis and precise segregation of a large number of proteins and lipids which are specifically required for the vesicle formation and function.

The secreted product is itself of heterogeneous composition. The granule matrix of the insulin secretory granule not only contains insulin and the related peptides, proinsulin and C-peptide, but also a number of other peptides among which are found the proteases required to process the prohormone.

The movement of the granule within the cell, its interaction with other cytoskeletal elements and the fusion reactions of its membranes are likely to be mediated by proteins on the external surface of the granule membrane. These might, either directly or indirectly, respond to changes in the concentrations of second messengers generated by secretagogue stimulation of the cell. A further level of complexity which will be reflected in the granule protein composition is that the granule itself participates in regulation of the concentrations of these same second messengers.

The objective of this review is to consider current knowledge of the nature and functions of the proteins found in isolated insulin secretory granules. To a large extent the physical properties of the organelle and its interaction with other elements of the cell are not considered. The reader is referred to other articles in this series for these aspects and to previously published reviews on this subject[9, 27, 30, 43].

Experimental models

A major obstacle to the chemical analysis and investigation of the biochemical properties of the secretory granule of the pancreatic B-cell is the limited amount of material provided by mammalian islets and the cellular heterogeneity of the material so obtained. A number of recent studies including our own have therefore been performed with B-cell tumors and derived permanent cell lines. The transplantable rat insulinoma used in this laboratory[12] retains a high insulin content and a relatively normal morphology and has been useful for the preparation of subcellular organelles and purification of cellular proteins. The tissue yield per animal is approxi-