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LIPID ASSOCIATED CALCIUM IONOPHORES IN ISLET CELL PLASMA MEMBRANE FOLLOWING GLUCOSE STIMULATION

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SUMMARY: The effect of glucose exposure on lipid associated calcium ionophoretic activity was measured in cultured neonatal rat pancreatic islet cells using two model systems. The first measured the ability of a lipid extract of islet cells to facilitate calcium transfer from an aqueous to organic phase and thus detected lipids which transfer calcium in the manner of authentic ionophores or which chelate the ion. In this system glucose stimulation was followed by an increase in total cell ionophoretic activity and a decrease in the activity associated with the plasma membrane. The second system measured the transfer of calcium across an artificial phospholipid membrane and detected authentic ionophoretic activity. In this model an increase in total ionophoretic activity was again seen following glucose but there was no change in the ionophoretic activity of a plasma membrane extract. The results indicate that the lipid modifications which accompany glucose-induced insulin release may alter cellular calcium stores by decreasing lipid bound calcium at the plasma membrane and increasing the capacity for calcium ionophoresis at intracellular sites.

The ability of the pancreatic β cell to regulate the transposition of calcium in response to glucose is an obligatory step in the coupling of the stimulus to insulin release (1,2). Stimulation of the secretory process may include alterations in membrane phospholipids (3,4). We have demonstrated that the impaired insulin secretory response to glucose in neonatal islets is associated with deficient lipid associated native calcium ionophoretic activity measured by calcium translocation in model systems (5). The chemical nature of the ionophoretic species has not been determined. Michell (6) has proposed that hydrolysis of phosphatidylinositol may play a role in the control of cell calcium permeability. We have shown that phosphatidic acid (PA),

Abbreviations: IBMX, 3-isobutyl-1-methylzanthine; PA, phosphatidic acid; PI, phosphatidylinositol; $PI_{4,5}P_2$ phosphatidylinositol 4,5 bisphosphate.

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which acts as a physiological calcium ionophore in cell systems and an authentic ionophore in model systems, enhances the endogenous calcium ionophoretic ability of neonatal islets (5). A number of other candidates for physiologic lipid ionophores exist. These include the products of the cyclooxygenase and lipoxygenase enzyme systems and lysophospholipids (7,8). In addition phospholipids with ion-chelating properties may contribute to ion flux. In particular a role for the phosphorylated derivatives of phosphatidylinositol (PI), phosphatidylinositol 4 phosphate (PI_4P) and phosphatidylinositol 4,5 bisphosphate ($PI_{4,5}P_2$), as calcium binding ligands at the cytoplasmic surface of the cell has been proposed (9,10). It remains unclear from many studies what proportion of the calcium required for insulin release is supplied by displacement of previously bound calcium from membrane or other intracellular stores and what proportion is supplied from the extracellular medium.

The following study was designed to investigate the changes in endogenous cell calcium ionophoretic activity following glucose exposure which could allow enhanced calcium translocation. calcium ionophoresis was determined by methods which allowed distinction between lipids with authentic ionophoretic properties and those with ion-chelating ability.

MATERIALS AND METHODS

Preparation of cultured neonatal islet cells.

Pancreas from neonatal rats, less than 24 h old, were dispersed and cultured by the technique described by Hellerstrom et al (11). modified for neonatal islets as described previously (12). After 50 h of culture aliquots of islets were transferred to medium RPMI 1640 containing 3-isobutyl-1-methylxanthine (IBMX, 0.1 mmol/l). After 4 days medium was removed and replaced with IBMX-free medium containing glucose (1.67 mmol/l). The cultures were harvested and dispersed by gentle shaking to give a single cell dispersion when viewed using phase contrast microscopy. The use of IBMX to promote formation of monolayers from cultured islets has been described by Dobersen et al (13) and Kohnert et al (14) and shown to produce a cell-enriched cell population using immunofluorimetric staining. This characterization has not been made in the present study. Thus the preparation has been termed an islet cell suspension.

Glucose stimulation of neonatal islet cells and plasma membrane isolation using microcarrier culture beads.

Islet cells were resuspended in sucrose-acetate buffer (pH 5.2) with hydrated Cytodex 1 beads at a cell to bead ratio of 100:1 as described by Gotlib (15).

Cells (10^4/ ml) were incubated in Krebs Ringer bicarbonate buffer containing bovine serum albumin (12). Following 20 minutes incubation at glucose concentration 1.6 mmol/l glucose was added to a final concentration of 1.6, 5.6, 8.3, 11.1 or 16.7 mmol/l. After 5 minutes beads and cells were pelleted by centrifugation. The insulin content of the supernatant medium was determined by radioimmunoassay using rat insulin standards.

Aliquots of beads with attached cells were taken for determination of 5'-nucleotidase (16), DNA (17) and protein (18). Buffer was decanted from the beads and attached cells, and phospholipids were extracted from the attached cells with addition of chloroform:methanol:conc. HCl (200:100:0.1 v/v), two phases generated with the addition of KCl and chloroform as described by Schacht (19) and the extracted phospholipids dried under N_2 (Total fraction). In separate aliquots, buffer was decanted from beads with attached cells and cells lysed with hypotonic Tris buffer (10 mM Tris-HCl, pH 8.0). The mixture was vortexed and centrifuged (300 rpm). Aliquots of beads with attached membranes were resuspended in buffer for determination of 5'-nucleotidase, DNA and protein. Phospholipids were extracted from beads with attached membranes as indicated above (Membrane fraction).

In this manner a total cell or membrane phospholipid extract was obtained within 10 min of glucose removal from incubated cells. Total phospholipid phosphorus was determined by colorimetric estimation of phosphorus with malachite green (20).

Lipid associated Ca^{2+} ionophoretic activity of total cell and cell membrane extracts.

Ca^{2+} ionophoresis was determined in two model systems described previously (5). The first was a bulk phase system which measured $^{45}Ca^{2+}$ translocation from an aqueous buffer phase to an organic phase containing lipid extracted from total cell or plasma membrane fraction of 2.5×10^4 islet cells. The ionophoresis was quantified as pmol Ca^{2+} translocated/2.5×10^4cells/3min.

The second method determined the translocation of Ca^{2+} between two aqueous compartments across intact lipid multilayers adsorbed to Millipore filters. Net transfer of $^{45}CaCl_2$ through the filter in the presence of islet or membrane lipid extract was taken to indicate ionophoretic activity and quantified as pmol Ca^{2+} translocated/2.5×10^4/15 minutes. In addition the ionophoretic capacity of phosphatidic acid and phosphatidylinositol 4,5 bisphosphate was determined in these systems.

RESULTS

Glucose-induced insulin release in neonatal islet cell suspension is shown in Figure 1. Over a 5 minute period the cells respond to increasing glucose concentrations with insulin release with a Km of 7 mmol/l.

The characteristics of the total cell and membrane fraction after attachment of Cytodex beads is shown in Table 1. Purification of the membrane fraction is indicated by an absence of DNA in the membrane preparation, a 4.8 fold increase in 5' nucleotidase activity and enhanced total phospholipid content (3.8 fold) in the membrane fraction attached to beads. Whilst the

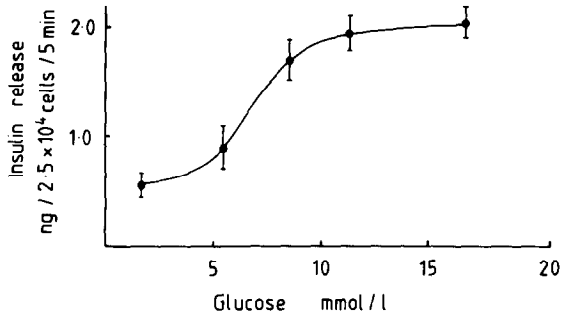

Figure 1. Glucose induced insulin release from neonatal islet cells attached to Cytodex 1 beads. Values shown are mean ± SEM for 6 observations at each experimental point.

values for 5'-nucleotidase represent a recovery of 95.5%, phospholipid recovery was 76%. This would indicate a purification of plasma membrane with a loss of phospholipid from internal membranes.

The calcium ionophoresis measured after incorporation of lipid fractions into the two model systems is shown in Table 2.

In both systems lipid associated calcium ionophoresis was significantly enhanced following glucose stimulation. There was however a divergence in the findings of the membrane fraction. Using the bulk phase system there was significant loss of ionophoretic capacity after glucose stimulation. This loss was not seen when ionophoresis was assessed by the ability of the extract to facilitate calcium translocation across phospholipid adsorbed filters. A partial explanation may be found in the

Table 1. Analysis of islet cells and islet cell plasma membranes isolated on Cytodex 1.

	Cytodex + cells	Cytodex + membranes
DNA (μg/10^6 cells)	18.1	<0.25
Protein (μg/10^6 cells)	78.9	15.7
5'-nucleotidase		
(μmol/h/10^6 cells)	0.068	0.056
(μmol/h/mg protein)	0.86	4.14
Total phospholipid		
(nmol Phosphorus/10^6 cells)	408	309
(nmol Phosphorus/mg protein)	5171	19649

The values shown are the mean of triplicate preparations of islet cells taken from culture (glucose 11.1 mmol/l) and were not different from preparation with glucose exposure of 1.67 or 16.7 mmol/l

Table 2. Lipid associated calcium ionophoretic activity following glucose stimulation of islet cells.

	Bulk Phase System		Phospholipid Adsorbed Filter	
Glucose, mmol/l	1.67	16.7	1.67	16.7
	pmol calcium/2.5.10^4 cells			
Total Fraction	400 ± 24	567.5 ± 27[c]	167.5 ± 10	230 ± 16[b]
Membrane Fraction	340 ± 16	270 ± 21[a]	107.5 ± 7	110 ± 14

a, b, c; $p < 0.05$, 0.01 and 0.005, respectively compared to the value in the absence of stimulatory glucose concentration. Values shown are mean ± SEM for 6 observations at each experimental condition.

behaviour of phosphatidic acid and phosphatidylinositol bisphosphate in these systems as shown in Figure 2.

The ionophoretic activity of PA and $PI_{(4,5)}P_2$ was identical in the bulk phase system. In the phospholipid adsorbed filter, significant ionophoretic activity was seen only with the inclusion of PA and not seen following $PI_{(4,5)}P_2$.

DISCUSSION

The results indicate that there is an increase in the ionophoretic capacity of neonatal islet cells following glucose exposure, as assessed by two independent methods. The increase would appear to be associated with the cytosol or internal membranes of the cell rather than the plasma membrane, as no increase in ionophoretic activity could be demonstrated in a plasma membrane enriched fraction of islet cells. Rather, the bulk phase system, which detects the presence of both authentic ionophoretic species and calcium ion chelators, indicated a loss of a lipid species with calcium binding ability from the plasma membrane following glucose stimulation. As this loss could not be detected with a system which preferentially detects phosphatidic acid which is also a calcium ionophore in other model systems, it may indicate that the loss in membrane lipid is related to the loss of a lipid species capable of binding calcium but not of effecting ionophoresis in the phospholipid adsorbed filter system. A polyphosphoinositide was shown to fulfill this description in the two model systems used.

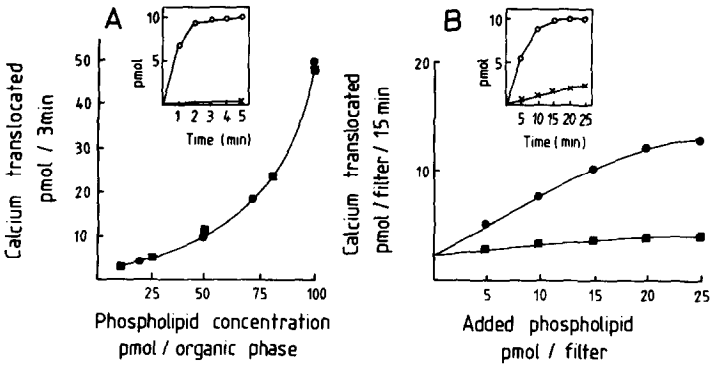

Figure 2. Effect of inclusion of lipid preparations on calcium translocation in a bulk phase system (A) or through multilamellar membranes in a phospholipid adsorbed filter (B). Calcium translocation with increasing concentrations of phosphatidic acid (●) or phosphatidylinositol 4,5, bisphosphate (■) included in the system is shown together with the time course of calcium translocation (inset) in the absence (x) of additional lipid and the presence of 50 and 15 pmol phosphatidic acid (o) in the bulk phase and phospholipid adsorbed filter system, respectively. Values shown are the mean of triplicate determination.

Although the identities of particular lipid associated ionophores have not been determined in this study, it is possible to make some analogy with the lipid changes in stimulated platelet, liver and parotid tissue. Michell (6) and others (21,22) have described a role for phospholipase C dependent hydrolysis of phosphatidylinositol in receptor agonist induced calcium gating. Phospholipase C is responsible for the cleavage of the inositol head group of phosphatidylinositol. Treatment of islets with this enzyme has been shown to increase insulin secretion (23). More recently Michell has proposed that phospholipase C may also act on the polyphosphoinositides (24).

Initial hydrolysis of phosphatidylinositol or the polyphosphoinositides is followed by phosphatidic acid formation in many tissues indicating the transient formation of diacylglycerol which has been shown in thrombin treated platelets following the action of calcium-dependent phospholipase C (25). Glucose induced insulin secretion in the cell has also been shown to result in a calcium dependent increase in 1,2 diacylglycerol in glycerol labelled islets (26). The plasma membrane associated events are rapid and the synthesis of phosphatidic acid is followed by transfer to the endoplasmic reticulum if phosphatidylinositol resynthesis is to occur (6). The finding of an intracellular increase in ionophoretic activity following glucose stimulation may be consistent with the synthesis of phosphatidic acid and its

intracellular relocation. Alternatively, additional ionophoretic species may be formed within the cell due to lipase activity leading to the release of arachidonic acid from diacylglycerol or phosphatidic acid by the action of diacylglycerol lipase or phospholipase A_2, respectively (25). Of the products formed by enzymic oxygenation of arachidonic acid by cylooxygenase and lipoxygenases, thromboxane A_2 (25) and leukotriene B_4 (5) have been described as ionophores. Both these arachidonic acid products have been detected in the pancreatic islet (27,28). A contribution of the lipoxygenase derivative to enhanced glucose-induced insulin release has been suggested (29).

Thus lipid modification, in particular phospholipid turnover may contribute to glucose-induced insulin release through the production of ionophoretic species detectable at intracellular sites and perhaps responsible for the release of calcium from intracellular stores. In addition, following glucose neonatal cultured islet cell plasma membrane lipids are modified in a manner that may lead to the liberation of bound calcium from membrane stores.

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