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Time-course of altered islet phospholipids and of calcium binding and ionophoretic properties of islet lipids following glucose stimulation

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The time-course of alteration in islet cell phospholipid content following D-glucose exposure in islet cells and in islet cell membranes was related to the ability of lipids extracted from both cultured pancreatic islet cells and from plasma membranes isolated from the islet cells to translocate calcium in two model membrane systems. The first model system (bulk-phase system) detected lipid species with the ability to bind calcium, irrespective of their ability to enhance calcium transport across cell membranes. The second system (multilamellar membrane system) detected lipid species with the ability to both bind calcium and to enhance calcium transport across cell membranes (true ionophores). Pre-exposure to high D-glucose concentration led to a rapid (within 1 min) fall in membrane phosphoinositides. This was partially blocked by mannoheptulose. A concurrent fall in calcium binding activity of lipids from the plasma membrane was observed. In the whole islet cell fraction, D-glucose induced a marked increase in Ca^{2+} ionophoretic activity. Unlike the fall in membrane polyphosphoinositides and membrane Ca^{2+} binding activity, these changes were dependent on the presence of added extracellular calcium. L-Glucose was without effect on membrane phosphoinositide content. It is concluded that altered membrane and intracellular phospholipids may contribute to the increased availability of intracellular Ca^{2+} following D-glucose stimulation by virtue of their Ca^{2+} binding and ionophoretic properties.

Introduction

Considerable evidence exists for the interaction of membrane phospholipids with mono- and divalent cations, resulting in altered membrane characteristics. The acidic phospholipids phosphatidylserine (PS), phosphatidylinositol (PI) and its polyphosphorylated derivatives phosphatidylinositol 4-phosphate (PI4*P*) and phosphatidylinositol 4,5-bisphosphate (PI4,5P_2) exhibit very strong affinities for calcium (Ca^{2+}) in model systems (this may also extend to the divalent cation Mg^{2+}) [1–3]. Phosphatidic acid (PA) [4] and its lyso derivative (lysoPA) can bind Ca^{2+} by virtue of the phosphate moiety and in the latter case the hydrophobic conformation of the single acyl chain renders the Ca^{2+}-lipid complex lipid soluble and confers the ability for transport across membranes [5]. The ionophoretic peroperty of the other acidic phospholipids remains to be established for upon interaction with Ca^{2+}, the lipid-Ca^{2+} complex appears to remain at a membrane location [6]. Similarly within the plasma membrane there is a strong interaction of Ca^{2+} with phosphatidylethanol-

Abbreviations: PS, phosphatidylserine; PI, phosphatidylinositol; PI4*P*, phosphatidylinositol 4-phosphate; PI4,5P_2, phosphatidylinositol 4,5-bisphosphate; PA, phosphatidic acid; lysoPA, lysophosphatidic acid; PE, phosphatidylethanolamine; PDE, phosphatidyl-*N*,*N*-dimethylethanolamine; PC, phosphatidylcholine; lysoPC, lysophosphatidylcholine.

amine (PE) and its N-methylated derivative, phosphatidyl-*N*,*N*-dimethylethanolamine (PDE) which is most apparent at alkaline pH [7].

Investigations by Valverde and Malaisse [8] have shown that lipid material associated with membrane-enriched subcellular fractions of adult pancreatic islets was able to translocate Ca^{2+} in a solvent system of toluene/butanol, which provides a model for the lipid environment of natural biological membranes [9]. This ion transfer was enhanced at elevated pH. Using this system and a further model system which allows the estimation of facilitation of Ca^{2+} transport across a multilamellar phospholipid membrane we have shown previously that glucose * induced insulin release from the B cell of the neonatal rat islet is accompanied by an increase in the ability of the lipids of the total cell to both bind Ca^{2+} and translocate it in an ionophoretic manner [10]. When the lipids of the plasma membrane alone were studied, it was found that they were modified in a way which reduced Ca^{2+} binding capacity. The present study extends this work to present the time course of glucose induced alteration in lipid Ca^{2+} binding and ionophore-mediated Ca^{2+} exchange in the presence of monovalent (Na^+, K^+) and other divalent (Mg^{2+}) cations and indicates that changes occur sufficiently rapidly to contribute to Ca^{2+} redistribution within the B cell. A concurrent study of phospholipid species with Ca^{2+} binding and/or ionophoretic properties in the cell membrane and in the whole cell was performed.

Materials and Methods

Preparation and extraction of islet cells

Neonatal islet cells were cultured and stimulated with glucose (16.7 mmol/l) for a period of 1–15 min by a method described previously [10]. Briefly aliquots of 400 islets were dispersed to give a single-cell suspension which was attached to hydrated Cytodex beads prior to stimulation with D-glucose, 1.7 or 16.7 mM in the presence of Ca^{2+}, 40 μM or 2.5 mM in the presence and absence of mannoheptulose, 20 mM or with L-glucose, 20 mM. This allowed a membrane fraction to be isolated rapidly by lysis of the cells attached to the Cytodex beads at 4°C using Tris-HCl (10 mmol/l, pH 8.0). Lipids were extracted from beads and attached cells or lysed membranes to give a total cell or membrane lipid extract, respectively. Total phospholipid phosphorus was determined colorimetrically using malachite green [11].

Labelling of islet cells with [^{14}C]arachidonic acid

Islet cells were attached to Cytodex beads as described above and incubated in RPMI-1640 with 10% fetal bovine serum containing [^{14}C]-arachidonic acid (400 000 dpm/ml) in glass tubes for 3 h at 37°C in an atmosphere of CO_2 (5% in air), with agitation each 15 min. After the incubation the islet cells attached to Cytodex were washed twice with RPM1-1640, stimulated as above and lipid extracts prepared. For the separation of lipids and phospholipids in the extracts silicic acid column partitioning and thin-layer chromatography was as described previously [12].

$^{45}Ca^{2+}$ binding and translocation by islet cell lipids

The capacity of lipid extracts to bind or translocate Ca^{2+} was determined following incorporation into two model systems also described previously [10]. The first was a bulk phase system which measured $^{45}Ca^{2+}$ translocation over 3 min from an aqueous phase buffered with Hepes (25 mM, pH 7.4 which contained mono- and divalent cations in the following concentrations, Na^+ 115 mM; K^+ 5 mM; Mg^{2+} 1.25 mM and Ca^{2+} 1.25 mM) to an organic phase containing the lipid under study. Ca^{2+} translocation was quantified as nmol/400 islets (10^6 isolated cells).

The second model system determined the translocation of Ca^{2+} between two aqueous compartments across intact multilamellar planar membranes into which islet lipid extracts were incorporated. Although these planar membranes bind calcium they maintain conformation and present an effective barrier to the ion in the absence of added islet extract [13]. Enhanced transfer of $^{45}Ca^{2+}$ across the membrane was quantitated and taken to indicate Ca^{2+} permeation due to the presence of extract. Results were expressed as nmol Ca^{2+} translocated/400 islets (10^6 isolated cells).

* Reference to glucose is for the D-glucose isomer unless specifically indicated.

Results

The distribution of [^{14}C]arachidonic acid in phospholipids of the membrane and whole cell fraction prepared after 3 h incubation is shown in Table I. Islet membrane fraction prepared on Cytodex contained 36.9 ± 0.20% ($n = 7$) of the total cell labelled phospholipids. Glucose stimulation is accompanied by a number of phospholipid changes the most prominent being almost complete loss of membrane and whole cell polyphosphoinositides within 1 min of stimulation. The loss was partially blocked in the presence of mannoheptulose, 59% and48% of polyphosphoinositides remaining in the membrane and whole cell fraction, respectively, up to 15 min after glucose stimulation. Other effects of glucose stimulation which are blocked by mannoheptulose are a decrease in PE and PI (8.2% and 24.8%, respectively) and an increase in PA in the membrane fraction following 15 min of stimulation. The major differences between the membrane preparation and the whole cell fractions are a rapid 3-fold increase in PA, following 1 min glucose stimulation and a 27-fold increase in lysoPA seen after 15 min glucose stimulation in the whole cell but not the membrane fraction. Both responses are attenuated by mannoheptulose. There was no effect on phospholipid content of either the whole cell or membrane preparations following the inclusion of L-glucose in the incubation for 1 or 15 min (results not shown).

The characteristics of lipid extracts prepared in exactly the same way except for the absence of [^{14}C]arachidonate were then investigated in the model systems.

First, the results in the bulk phase system, where Ca^{2+} translocation can be taken to represent Ca^{2+} binding in the organic phase, will be considered. The effect of pH on the translocation of Ca^{2+} in this system, in the presence of a lipid extract of plasma membranes of nonstimulated

TABLE I

DISTRIBUTION OF ISLET [^{14}C]ARACHIDONIC ACID-LABELLED PHOSPHOLIPIDS IN FRACTIONS PREPARED AFTER INCUBATION OF ISLET CELLS WITH GLUCOSE WITHOUT OR WITH MANNOHEPTULOSE

Values are the mean ± S.E. for seven or eight determinations at each experimental condition. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	10^3 dpm/10^6 cells					
	Gluose 1.7 mM	Glucose 1.7 mM mannoheptulose, 20 mM		Glucose 16.7 mM	Glucose, 16.7 mM mannoheptulose, 20 mM	
	0	0	1 min	15 min	1 min	15 min
Membrane associated						
PC	8.23 ± 0.91	8.28 ± 0.92	8.27 ± 0.91	8.56 ± 0.90	8.28 ± 0.90	8.29 ± 0.91
LysoPC	0.78 ± 0.10	0.77 ± 0.12	0.77 ± 0.11	0.77 ± 0.11	0.77 ± 0.10	0.77 ± 0.11
PE	4.36 ± 0.32	4.42 ± 0.30	4.26 ± 0.34	4.00 ± 0.31	4.36 ± 0.27	4.29 ± 0.37
PS	0.90 ± 0.08	0.90 ± 0.10	0.90 ± 0.07	0.90 ± 0.07	0.90 ± 0.07	0.90 ± 0.07
PI	3.54 ± 0.35	3.74 ± 0.41	2.75 ± 0.35	2.66 ± 0.31 *[a]	3.34 ± 0.31	3.24 ± 0.30
PA	1.01 ± 0.09	1.00 ± 0.09	0.94 ± 0.09	1.80 ± 0.09 ***[a]	0.92 ± 0.09	0.92 ± 0.10
LysoPA	< 0.005	< 0.005	< 0.005	0.01 ± 0.001	< 0.005	< 0.005
PI4P/PI4,5P_2	0.35 ± 0.04	0.37 ± 0.04	0.01 ± 0.001 ***[a]	0.01 ± 0.001 ***[a]	0.17 ± 0.01 ***[b]	0.17 ± 0.01 ***[b]
Whole cell fraction						
PC	26.90 ± 1.88	26.92 ± 1.90	26.94 ± 2.00	26.27 ± 2.10	26.94 ± 2.21	28.90 ± 1.90
LysoPC	1.10 ± 0.09	0.92 ± 0.09	1.10 ± 0.09	1.72 ± 0.10	1.10 ± 0.09	0.94 ± 0.07 ***[b]
PE	12.10 ± 0.99	12.12 ± 0.98	12.14 ± 0.98	11.74 ± 0.99	12.14 ± 0.99	12.09 ± 1.01
PS	1.65 ± 0.10	1.65 ± 0.10	1.68 ± 0.09	1.67 ± 0.10	1.65 ± 0.09	1.69 ± 0.14
PI	9.90 ± 0.91	9.91 ± 0.84	8.61 ± 0.91	7.38 ± 0.90 *[a]	9.82 ± 0.75	9.75 ± 1.20
PA	1.43 ± 0.09	1.42 ± 0.09	4.70 ± 0.36 ***[a]	1.02 ± 0.09	2.40 ± 0.35 **[b]	1.28 ± 0.24
LysoPA	0.02 ± 0.001	0.01 ± 0.001	0.01 ± 0.001	0.54 ± 0.04 ***[a]	0.01 ± 0.001	0.01 ± 0.001 ***[b]
PI4P/PI4,5P_2	0.43 ± 0.04	0.41 ± 0.04	0.01 ± 0.001 ***[a]	0.01 ± 0.001 ***[a]	0.21 ± 0.02 ***[b]	0.19 ± 0.02***[b]

[a] Significant difference from glucose, 1.7 mM at time 0.

[b] Significant difference from corresponding condition in the absence of mannoheptulose.

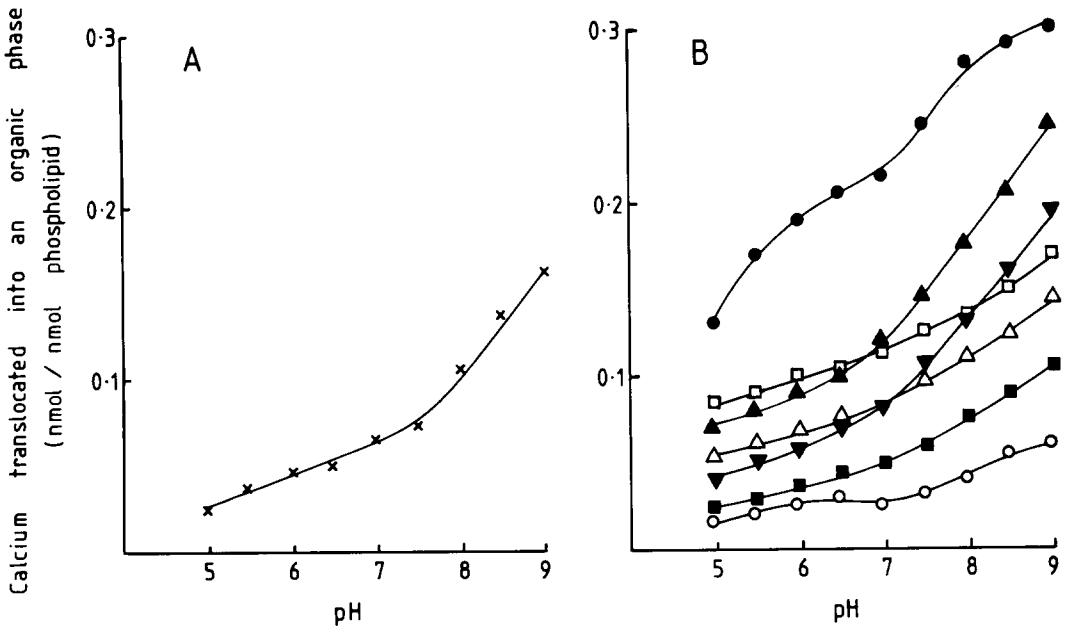

Fig. 1. Calcium ion translocated into the organic phase of a bulk phase model by the inclusion of islet extract (panel A) or phospholipids (panel B): phosphatidylserine, ●, phosphatidylinositol 4,5-bisphosphate, ▲; phosphatidyldimethylethanolamine, ▼; phosphatidic acid; □, phosphatidylcholine, ○. The values shown are the mean of multiple replicate analyses (10–20 observations) and the S.E. is contained within the dimensions of the mean point in all cases.

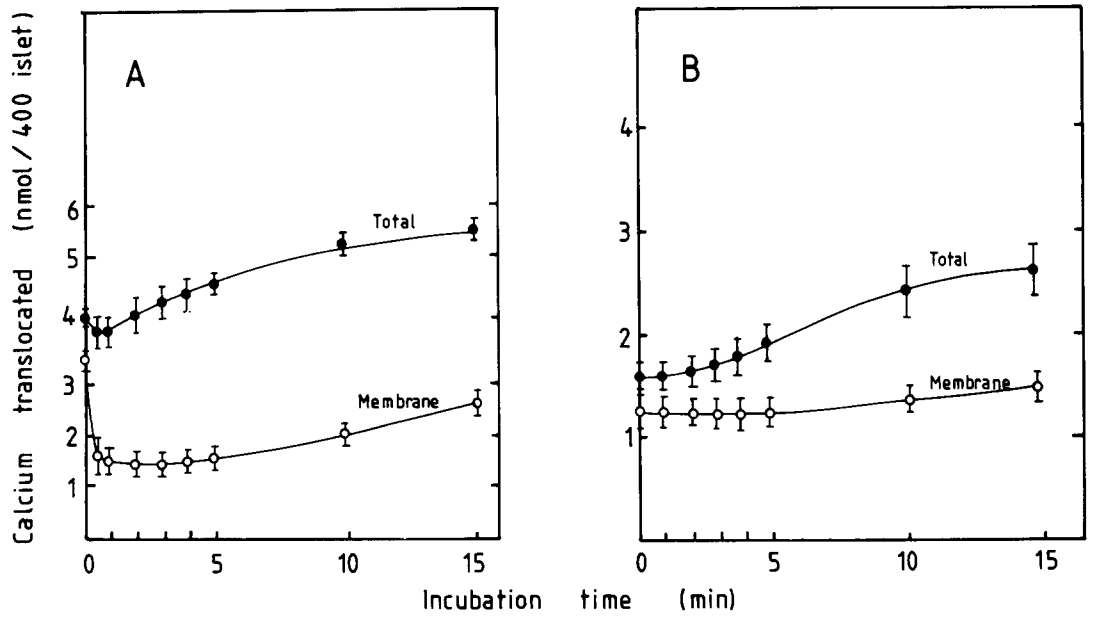

Fig. 2. Calcium translocation in a bulk phase system (panel A) and a multilamellar model system (panel B) by extracts of glucose-stimulated islets. The horizontal axis indicates the time after stimulation with glucose 16.7 mM at which total cell or membrane fractions were extracted for inclusion into the model systems. The values shown are mean ± S.E. for eight determinations at each experimental point.

islets and a number of intrinsic membrane phospholipids is shown in Fig. 1. It can be seen that a pH dependent increase in Ca^{2+} entry to the organic phase was caused by islet plasma membrane extract, and all the tested phospholipids, with PS, PI4,5P_2 and PDE being the most potent at alkaline pH. The effect of islet plasma membrane extract was enhanced at alkaline pH. pH 7.5 was chosen for subsequent studies of Ca^{2+} translocation in the bulk phase system.

Exposure of intact islet cells to high glucose concentration (16.7 mM) caused a prompt (within 30 s) fall in the Ca^{2+} binding activity of lipid extracts of the plasma membranes, as reflected by Ca^{2+} translocation in the bulk phase system shown in Fig. 2. The Ca^{2+} binding activity remained depressed to less than half its initial value for the first 5 min of exposure to high glucose concentrations, with a slow rise back towards the initial value in the next 10 min. Even 15 min after the exposure to high glucose was commenced, the Ca^{2+} binding capacity of the lipid extract of the plasma membranes was significantly depressed below the initial value. These changes did not reflect the changes in the whole islet, as lipid extracts of whole islets exposed to high glucose concentration for periods between 30 s and 15 min showed an initial minor fall, followed by a protracted rise in calcium binding activity reflected by the bulk phase system. The fall in the Ca^{2+} binding activity of the plasma membrane component was seen in islets in which the exposure to high glucose was carried out in the absence of added extracellular Ca^{2+} (where the Ca^{2+} concentration was 40 μmol/l) (Table II). The fall in Ca^{2+} binding activity of the plasma membrane on exposure to high glucose concentration was reduced in the presence of mannoheptulose.

Using the multilamellar membrane system to measure the ability of lipid extracts to facilitate

TABLE II

GLUCOSE-STIMULATED LIPID ASSOCIATED Ca^{2+} TRANSLOCATION IN NEONATAL ISLETS

Values are the mean ± S.E. for eight determinations at each experimental condition. * $p < 0.05$, ** $p < 0.001$ when compared with time 0.

Stimulating condition	nmol Ca^{2+} translocated/400 islets		
	0	1 min	15 min
Bulk phase system			
Membrane associated			
Glucose 16.7 mM; Ca^{2+} 2.5 mM	3.45 ± 0.21	1.52 ± 0.34 **	2.64 ± 0.30 *
Glucose 16.7 mM; Ca^{2+} 40 μM	3.45 ± 0.21	1.74 ± 0.32 **	2.01 ± 0.32 *
Glucose 16.7 mM; Ca^{2+} 40 μM Mannoheptulose, 20 mM	3.45 ± 0.21	2.48 ± 0.31 *	2.41 ± 0.34 *
Total cell			
Glucose 16.7 mM; Ca^{2+} 2.5 mM	4.02 ± 0.24	3.65 ± 0.31	5.51 ± 0.20 **
Glucose 16.7 mM; Ca^{2+} 40 μM	4.02 ± 0.24	3.84 ± 0.41	4.02 ± 0.18
Glucose 16.7 mM; Ca^{2+} 2.5 mM Mannoheptulose, 20 mM	4.02 ± 0.24	3.70 ± 0.41	4.01 ± 0.21
Multilamellar membrane system			
Membrane associated			
Glucose 16.7 mM; Ca^{2+} 2.5 mM	1.23 ± 0.23	1.25 ± 0.19	1.52 ± 0.17
Glucose 16.7 mM; Ca^{2+} 40 μM	1.23 ± 0.23	1.26 ± 0.19	1.64 ± 0.21
Glucose 16.7 mM; Ca^{2+} 2.5 mM Mannoheptulose, 20 mM	1.23 ± 0.23	1.25 ± 0.21	1.60 ± 0.21
Total cell			
Glucose 16.7 mM; Ca^{2+} 2.5 mM	1.65 ± 0.18	1.64 ± 0.18	2.70 ± 0.26 *
Glucose 16.7 mM; Ca^{2+} 40 μM	1.65 ± 0.18	1.68 ± 0.21	1.89 ± 0.29
Glucose 16.7 mM; Ca^{2+} 2.5 mM Mannoheptulose, 20 mM	1.65 ± 0.18	1.69 ± 0.22	1.74 ± 0.30

Ca^{2+} transport across the model membrane, it was shown that there was no change in this capacity of membrane lipids following high glucose exposure (Fig. 2, panel B). The extract of the whole islet showed a significant increase 10 and 15 min after exposure to high glucose concentration. This was prevented if the incubation of the islets was performed in the absence of added extracellular Ca^{2+} and by the presence of mannoheptulose.

Discussion

In this study the time-course of alteration in plasma membrane and whole cell lipid associated Ca^{2+} binding and/or ionophoretic species has been measured following exposure to high glucose concentration. Concurrent measurements of membrane and whole cell phospholipid changes following exposure to high glucose concentrations have been performed. A major finding was that exposure of neonatal rat islets to a stimulatory concentration of glucose leads to a rapid fall in the content of plasma membrane associated lipid species with the ability to bind Ca^{2+}, as measured by the degree of facilitation of Ca^{2+} entry into the organic phase of a bulk phase system. The distribution of Ca^{2+} in this system presumably reflects the sum total of ionic and electrostatic forces and ion chelation. Absence of added Ca^{2+} from the extracellular medium did not prevent this fall, suggesting that it is not a secondary consequence of Ca^{2+} influx from the extracellular medium. Measurement of concurrent changes in membrane phospholipids revealed a striking rapid and protracted fall in membrane polyphosphoinositides, as has been shown previously for intact adult rat islets, [14,15] and in a previous study by ourseves in neonatal rat islets [10]. There were no phospholipid changes seen following L-glucose, a hexose which does not stimulate insulin release from the islet [16]. Polyphosphoinositides added to the bulk phase system bound Ca^{2+} (Fig. 1), so the rapid fall in content of polyphosphoinositides could have contributed to the fall in Ca^{2+} binding activity of lipid species in the membrane fraction. However, it cannot be concluded that the temporal association seen between the membrane polyphosphoinositides and membrane Ca^{2+} binding activity implies that the altered polyphosphoinositides are solely responsible for the observed changes in lipid-associated Ca^{2+} binding activity. For example, phospholipid methylation, another membrane related event, has been shown to accompany glucose-stimulated insulin release [17]. In the bulk phase model system, Ca^{2+} binding differs between phosphatidylethanolamine, its N-methylated derivative phosphatidyl-*N*,*N*-dimethylethanolamine (PDE) and phosphatidylcholine, indicating that formation of PC from PE may result in a loss of Ca^{2+} binding within the islet. A decrease in PE, perhaps indicative of methylation, was seen in the plasma membrane fraction in the present study.

The second system employed for studying effects on Ca^{2+} binding or ionophoretic properties of endogenous lipids in the islet cells or islet membranes employed a multilamellar phospholipid membrane. The ability of added phospholipids or islet or islet membrane extracts to facilitate Ca^{2+} transport across the membrane separating two aqueous phases was taken to reflect their Ca^{2+} ionophoretic activity. Electrostatic binding with a lipophilic conformation and possibly membrane fusion and phase separation may contribute to ion transfer in this model system [2,18]. No change in the content of plasma membrane associated lipids with the property of facilitating Ca^{2+} transport across the membrane was seen following exposure to stimulatory glucose concentration. However, a delayed rise in the content of lipids with this property was observed in the whole islet cell, presumably reflecting an increase in intracellular lipids with ionophoretic properties. This increase was not observed when the islets were exposed to glucose in the absence of added extracellular Ca^{2+}, suggesting that it may have been dependent on the influx of extracellular Ca^{2+}. The rise in total islet content of Ca^{2+} binding activity in the bulk phase system paralleled the increase in Ca^{2+} ionophoretic activity in the membrane system, and may well reflect alteration in the same lipid species.

A number of phospholipid metabolites may be involved in the finding of an increase in intracellular Ca^{2+} ionophoretic activity. These include phosphatidic acid, formation of which is seen in the adult [19] and neonatal [12] islet following exposure to stimulatory glucose concentration and lysophosphatidic acid seen in the present study.

The time-course of the increase in the intracellular content of phosphatidic acid and lysophatidic acid was consistent with the rise in intracellular ionophoretic activity, and it has been demonstrated that both phospholipids have the ability to act as true ionophores in the membrane system [20]. Whether lysophosphatidic acid is derived from PA or arises de novo from glucose-derived triose metabolites cannot be determined in this study. The formation of PA and lysoPA is dependent on glucose metabolism as indicated by the attenuated response in the presence of mannoheptulose. In addition ionophoretic properties have been described for thromboxane A_2 [6] and leukotriene B_4 [20], products of the enzymic oxygenation of arachidonic acid by the cyclo-oxygenase system and lipoxygenase, respectively. These products are found in the glucose stimulated islet [21,22]. It is of particular relevance that Ca^{2+}-dependent phospholipase A_2 activation is considered the rate-limiting factor in the formation of arachidonic acid products and lysophosphatidic acid. We have shown that the formation of arachidonic acid products is a late event following glucose stimulation in the neonatal islet [12] and their formation is dependent on extracellular Ca^{2+} [23]. Reduced formation may contribute to the reduction in Ca^{2+} ionophoretic activity seen in the absence of Ca^{2+} ion in the present study.

Thus, two lipid related mechanisms may contribute to redistribution of calcium within the plasma membrane microenvironment. Phospholipid metabolism may result in the formation of species with altered affinity for Ca^{2+} or concomitantly phospholipid turnover may produce further lipid species which mobilize Ca^{2+} from intracellular soruces due to their ionophoretic nature. Both these mechanisms appear to obtain following glucose stimulation in the neonatal islet and may provide a significant source of cytoplasmic Ca^{2+}.

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