Endogenous ionophoretic activity in the neonatal rat pancreatic islet

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1. INTRODUCTION

Recent studies have focused attention on the ability of the pancreatic β cell to regulate the transposition of Ca^{2+} during stimulus recognition and the coupling of this to insulin release [1,2]. Some exogenous agents with an insulinotrophic effect may alter the ionophoresis of Ca^{2+} through processes involving native, endogenous ionophores [3], possibly derived from membrane phospholipids [4–7].

The pancreatic islets of the neonatal rat show a limited insulin release in response to glucose which is augmented in the first week following birth [8]. The aim of this study was to investigate a possible role for deficient lipid-associated calcium ionophoretic activity in the decreased glucose responsiveness of neonatal islets. Particular attention was paid to the possibility that islet phosphatidic acid might be important in determining this ionophoretic activity. This lipid has Ca^{2+} ionophoretic activity demonstrable in artificial systems [9,10], and has been postulated to be an important physiologic ionophore in certain systems [5–7].

Ionophore-mediated Ca^{2+} translocation can be studied using a 2 phase model consisting of an aqueous buffer containing the ion and an organic immiscible phase containing the ionophore [11,12]. We have employed this technique as one method to study the neonatal islet. To study a situation more analogous to the transfer of calcium from one aqueous phase to another across a predominantly phospholipid membrane, a second model system was developed. This was based on a preparation of phospholipid multilayered membranes whose characteristics resemble those of planar membranes and liposomes. The multilayered membranes formed in phospholipid-impregnated Millipore filters have been used extensively to investigate the structural, physio-chemical and electrical properties of phospholipid bilayers [13–16]. As ionophoresis could be expected through these membranes, the procedure was adapted to investigate the ability of pancreatic extracts to translocate Ca^{2+} across an intact lipid layer between 2 aqueous compartments.

2. MATERIALS AND METHODS

2.1. *Glucose-stimulated insulin release*

Islets from the pancreas of newborn (1-day-old) and 5-day-old infant Wistar rats were microdissected in bicarbonate buffer (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$ and 24 mM $NaHCO_3$ (pH 7.4) and 25 islets incubated in 1.0 ml of the same buffer containing bovine albumin (5 mg/ml) and glucose (1.6 or 16.7 mM) under O_2:CO_2 (95:5, v/v) at 37°C. Insulin released into the medium was determined by radioimmunoassay using rat insulin standards [17].

2.2. *Phosphatidic acid-stimulated insulin release*

Islets isolated from the pancreas of 1-day-old animals were preincubated in the presence of phosphatidic acid (1.0 μM) and glucose (1.6 mM) or glucose (1.6 mM) alone for 60 min at 37°C, under O_2:CO_2 (95:5, v/v). At the end of preincubation islets were washed once with bicarbonate buffer containing albumin and glucose (1.6 mM) and incubated with glucose (1.6 or 16.7 mM) for determination of insulin release.

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2.3 *DNA determination*

Islets were homogenized in bicarbonate buffer and the DNA content was determined fluorimetrically using 4′,6-diamidino-2-phenylindole [18].

2.4. *$^{45}Ca^{2+}$ ionophoretic activity*

2.4.1. Two phase system

$^{45}Ca^{2+}$ translocation from an aqueous phase consisting of 0.2 ml Hepes buffer (25 mM) containing NaCl (115 mM), KCl (5.0 mM) and $^{40}CaCl_2$ (2.5 mM) with trace amounts of $^{45}CaCl_2$ (5.0 μCi/ml) to a 0.2 ml toluene–butanol (7:3, v/v) organic phase containing a solubilized $CHCl_3$:CH_3OH (2:1, v/v) extract of microdissected islets was performed as in [12].

2.4.2. Multilamellar membrane system

Millipore filters (av. pore diam. 0.45 μM) were used as a support for a phospholipid multilayered membrane system prepared from a solution of phosphatidylserine (PS): phosphatidylcholine (PC) (9:1, 40 mg/ml) and either the lipid extracted from 25 microdissected islets using $CHCl_3$:CH_3OH (2:1, v/v) or phosphatidic acid (PA). All lipids were solubilized in benzene. Except for the inclusion of islet lipid, membranes were prepared using phosphatidylserine prepared from rat brain and phosphatidylcholine from fresh egg yolk as in [15]. The phospholipid adsorbed filter containing 1.94 ± 0.08 mg lipid/cm^2 was pre-soaked in buffer containing 2.5 μM $^{40}CaCl_2$ and clamped between 2 disposable glass vials, one containing Hepes buffer (25 mM) with $^{40}CaCl_2$ (2.5 mM) and $^{45}CaCl_2$ (5.0 μCi/ml), the other $^{40}CaCl_2$ (2.5 μM). In this manner a filter area of 225 mm^2 was exposed for calcium exchange. The system was mixed by rotation for 30 min at 24°C. Net transfer of $^{45}CaCl_2$ through the filter was taken to indicate ionophoretic activity.

2.5. *Phosphatidic acid content of glucose-stimulated islets*

Glucose-stimulated islets were frozen rapidly in glass tubes. Pooled islets were sonicated in chloroform:methanol (2:1, v/v) from the frozen state. Phosphatidic acid was separated by two-dimensional thin-layer chromatography and identified by comparison with purified 1-α-phosphatidic acid [19]. The phosphorus content of endogenous phosphatidic acid and co-chromatographed standard was estimated as in [20].

3. RESULTS

3.1. *Insulin release and islet phosphatidic acid content*

In islets stimulated with 16.7 mM glucose, insulin release rose significantly from 5.51 ± 0.07–12.4 ± 1.2 μU · 100 ng DNA^{-1} · 15 min^{-1} ($p < 0.001$, $n = 10$) between birth and 5 days of age (fig.1). Basal insulin release did not change over this period. Over the 5 day post-natal period islet phosphatidic acid content increased from 0.094 ± 0.047–0.266 ± 0.029 nmol/100 ng DNA ($p < 0.001$) in glucose-stimulated islets. A significant increase in glucose-stimulated insulin release was seen in islets from 1-day-old animals following preincubation with exogenous phosphatidic acid (fig.2.).

3.2. *Ionophoretic activity*

Using the first ionophoretic technique, calcium translocation into the organic phase of this 2 phase

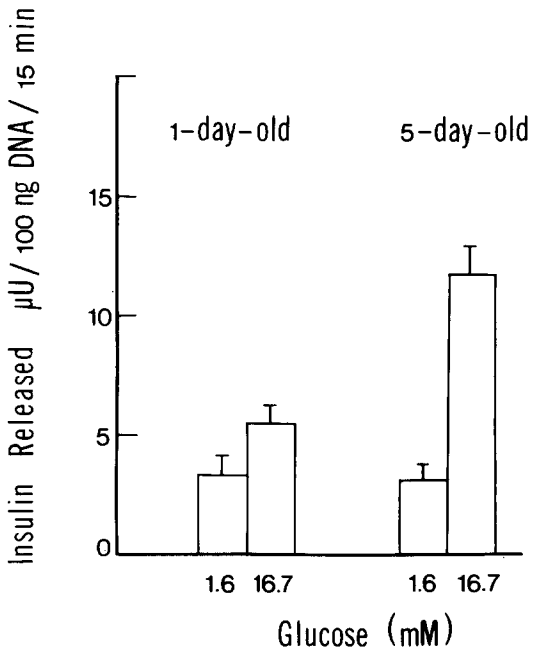

Fig.1. The effect of glucose stimulation on insulin release in islets from 1-day-old and 5-day-old rat pups. Values shown are mean ± SEM for 10 obs. at each experimental condition.

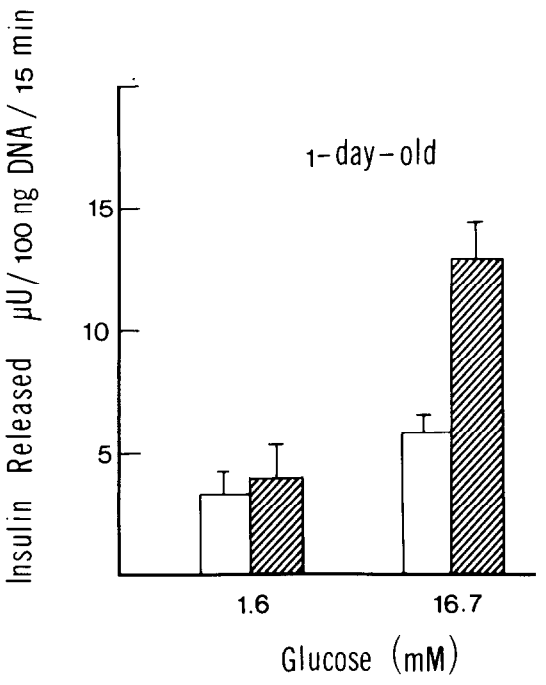

Fig.2. The effect of preincubation in the presence of phosphatidic acid (1.0 μM, ▨) on glucose-stimulated insulin release in islets from 1-day-old rat pups. Values shown are mean ± SEM for 4 obs. at each experimental condition.

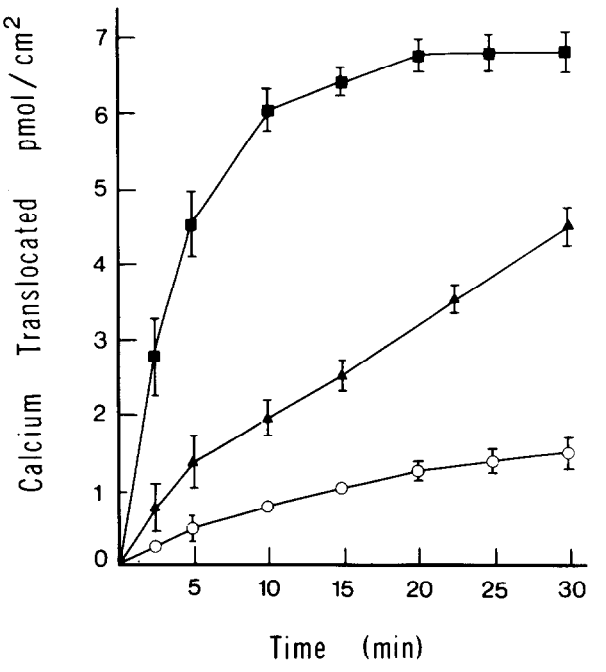

Fig.3. Effect of inclusion of islet lipid extract on calcium translocation through multilayered membranes. The time course for the translocation of $^{45}Ca^{2+}$ is shown for membranes consisting of: (■—■) PS/PC/CH_3Cl:CH_3OH extract of 5-day-old islets; (▲—▲) PS/PC/CH_3Cl:CH_3OH extract of 1-day-old islets, and (○—○) PS/PC prepared as in section 2. Values shown are mean ± SEM. The mean value is derived from 3 obs. on each of 4 individual islet extracts (n = 12).

Table 1

Effect of islet extract on the translocation of calcium from an aqueous phase

Extract added	n	Ca^{2+} Translocated (pmol)
1-day-old islets (25 islets, 635 ± 60 ng DNA)	10	104.0 ± 11.0
1-day-old islets following preincubation with phosphatidic acid (1 μM)	6	154.1 ± 12.7[a]
5-day-old islets (25 islets, 675 ± 67 ng DNA)	10	171.4 ± 15.6[a]
A23187 (1 μM)	10	40.0 ± 3.2
Phosphatidic acid (1 μM)	10	21.2 ± 2.8

model increased significantly ($p < 0.005$) over 5 days following birth (table 1). Increased translocation of Ca^{2+} by islet extracts of 1-day-old animals could be induced by preincubation of the islets with phosphatidic acid. Inclusion of the ionophore A23187 and phosphatidic acid, in the absence of

[a] $p < 0.005$ compared to 1-day-old islets (Student's distribution)

Values are corrected for the amount of calcium translocated in the absence of islet extract. This did not exceed 5% of total Ca^{2+} translocated. There was no significant difference between the DNA content of 1-day-old and 5-day-old islets. Values shown are mean ± SEM.

The values shown for A23187 and phosphatidic acid alone represent the effects of addition of those ionophores without islet extract to the aqueous and organic phases, respectively of the 2 phase system

cell extract, also caused translocation of aqueous Ca^{2+} in this 2 phase system.

Using the second model system, net calcium transport across the organic phase reconstituted from islet lipids was documented as an indication of ionophoretic potential (fig.3). Calcium was translocated through phospholipid-adsorbed filters in a time dependent manner. Under these experimental conditions pure phosphatidylserine:phosphatidylcholine membranes did not translocate Ca^{2+} effectively. Inclusion of the extract from islets of 5-day-old rats had a more marked effect in enhancing Ca^{2+} translocation than extracts from neonatal rats, supporting the results obtained from the simpler 2 phase system. The effect of inclusion of phosphatidic acid in the membrane system in the presence and absence of cell extract is shown in table 2. Phosphatidic acid increases the amount of Ca^{2+} translocated when compared to PS:PC containing membranes and enhances the Ca^{2+} translocation effected by islet extract. In addition preincubation of islets from 1-day-old animals with phosphatidic acid (1 μM) followed by washing in incubation buffer increased the ability of islet extracts to facilitate calcium exchange across the membrane (table 2, $p > 0.02$).

Table 2

Effect of phosphatidic acid or islet extract on the translocation of calcium through a model membrane

Membrane	Ca^{2+} Translocated (pmol . cm^{-2} . 15 min^{-1})
PS:PC	1.0 ± 0.3
PS:PC:PA (0.0025%)	5.2 ± 0.3
PS:PC:PA (0.005%)	8.4 ± 0.4
PS:PC:PA (0.025%)	12.1 ± 0.5
PS:PC:PA (0.0025%) 1-day-old islet extract	7.0 ± 0.7
PS:PC 1-day-old islet extract	2.4 ± 0.3
PS:PC 1-day-old islet extract from islets preincubated with PA (1 μM)	4.3 ± 0.7
PS:PC 5-day-old islet extract	6.3 ± 0.2

The quantity of PA is indicated as a % of membrane lipid (by wt) prepared as in section 2. Values are expressed as mean ± SEM (12 obs.)

4. DISCUSSION

Acquisition of increased responsiveness of neonatal rat islets to glucose in the 5 days following birth was associated with an increase in calcium ionophoretic activity in the islet extracts. Qualitatively similar results were obtained using the 2 phase system, and the multilamellar phosphatidylserine:phosphatidylcholine membranes supported on filters. In the latter system, the addition of islet extracts presumably contributes to a domain which facilitates Ca^{2+} translocation within the lipid adsorbed pore of the supporting filter.

Many candidates for endogenous ionophores have been suggested in different systems [9,10,21]. The demonstration that increased phosphatidic acid content of the islet coincided with an increase in endogenous ionophoretic activity, together with the effects of incubation of 1-day-old islets in medium containing phosphatidic acid on this activity suggested that the islet phosphatidic acid content may be important in determining endogenous calcium ionophoretic capacity. In turn, the concurrent stimulatory effect of this lipid on the insulin secretory response of the islet supports the postulate that the deficiency in endogenous ionophoretic activity in the neonatal islet may be important in determining the poor insulin response to glucose.

Findings from both ionophoretic techniques used here confirm that phosphatidic acid is an effective ionophore and suggest the presence in islet extracts of an ionophore with properties similar to phosphatidic acid. Using model lipid vesicles, divalent cations were shown to induce non-bilayer structures in phosphatidic acid containing membranes [22]. Calcium transport may be facilitated by the formation of such non-bilayer lipid structures [23]. Taken together with recent studies suggesting a biologic function for phosphatidic acid as a calcium ionophore [4–6] these findings support the concept that this phospholipid may be a critical component in determining calcium transport in the islet. However, other products of phospholipid metabolism may be present in the islet extract and their contri-

bution to calcium ionophoretic activity cannot be excluded.

Whatever the chemical nature of the endogenous lipid-associated ionophoretic activity, our data suggest that deficient Ca^{2+} availability due to a quantitative decrease in this activity may be responsible for the failure of neonatal islets to respond normally to glucose. Acquisition of glucose responsiveness between 1 and 5 days of post-natal life coincides with and may be dependent on an increase in this native ionophoretic activity.

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