Molecular and Cellular Endocrinology, 71 (1990) 49–54
Elsevier Scientific Publishers Ireland, Ltd.

MOLCEL 02291

Changes induced by glucose in the plasma membrane properties of pancreatic islets

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(Received 7 December 1989; accepted 12 March 1990)

Key words: Pancreatic islet; B cell membrane; Fatty acids lipid bilayer order; Fluorescence polarization

Summary

Partially purified membranes obtained from rat pancreatic isolated islets preincubated for 3 min with 3.3 and 16.6 mM glucose were labelled with 1,6-diphenyl-1,3,5-hexatriene to study fluorescence polarization. Other islets, incubated for 5 min with the same glucose concentration, were extracted and phospholipids separated by thin-layer chromatography. The composition of phospholipids of fatty acids was then studied by gas-liquid chromatography. Arrhenius plots of the microviscosity in membranes obtained from islets exhibited two components, a steeper slope below 18°C and a gentler slope above 18°C, indicating greater flow activation energy at temperatures below the transition point. Exposure of islets to 16.6 mM glucose significantly increased the flow activation energy (ΔE), below and above the transition point. Islets incubated for 5 min with 16.6 mM glucose showed an increase in the percentage composition of 12:0 and 18:2 together with a decrease in the 20:2 W6 and 22:3 W3 fatty acids esterified to phospholipids. Regardless of these changes, no significant alterations occurred in the proportion of saturated fatty acids or in the double bond index; these measurements therefore did not account for the effects of glucose concentration in flow activation energy.

The thermotropic changes reported here might be the consequence of some degree of disorder induced by glucose upon the membrane structure. This order alteration could either favor the membrane fusion which occurs during the emiocytosis or only reflects the consequence of such a process.

Introduction

Emiocytosis is the last step in the B cell secretory process triggered by glucose (Lacy, 1961). This step involves the fusion of the cell membrane with the secretion granule membrane (Orci, 1982). The regulatory mechanism of the fusion is unknown.

Experiments performed with liposomes have demonstrated that the fusion process is strongly influenced by changes in the membrane lipid composition (Roos and Chopin, 1985), spatial arrangement of phospholipids and cation composition of the environment (Duzgunes et al., 1981; Sundler et al., 1981). Electron microscopy studies of the

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pancreatic islets have shown that during exocytosis the zone of interaction between B secretory granule and plasma membrane was characterized by the absence of protein particles and increased cholesterol content (Orci, 1982). It had also been demonstrated that glucose induces significant changes in the islet membrane phospholipid turnover (Best and Malaisse, 1983; Gagliardino, 1983; Gagliardino et al., 1986) and fatty acid composition (Montague and Parkin, 1980; Cortizo et al., 1987). However, no conclusive data were reported regarding the effect of these composition and metabolic changes upon the physical properties of the islet membranes.

Several authors have demonstrated that microviscosity and other physical properties of liposomes and biological membranes can be estimated measuring the fluorescence polarization of a probe like 1,6-diphenyl-1,3,5-hexatriene (Shinitzky and Barenholz, 1978). This technique can be performed on small amounts of biological material. Using this technique we have studied the changes induced by glucose on the thermotropic transition temperature and energy flow activation of rat islet membranes. We also studied the effect of glucose on the fatty acid composition of islet phospholipids.

Material and methods

Chemicals

Collagenase was obtained from Serva Feinbiochemica, F.R.G.; aprotinin (Trasylol) (100,000 kIU) was kindly provided by Bayer Argentina; bovine serum albumin (BSA) fraction V from Sigma Chemical Co., U.S.A.; 1,6-diphenyl-1,3,5-hexatriene (DPH) from Aldrich, U.S.A. and tetrahydrofuran (THF) from Merck, F.R.G.

Standards of different lipids were obtained from P-L Biochemicals, U.S.A.

Isolation of islets and islet membrane preparation

Pancreases from normal fed male Wistar rats (150–200 g) were used to obtain isolated islets by collagenase digestion (Lacy and Kostianovsky, 1967). Groups of 600–700 islets were incubated in a microhomogenator (Tissue Grinder, Potter-Elvehjem, Teflon, size 18, rod OD 1/8″ and 0.5 ml capacity from Kontes Scientific Glassware/Instruments) for 3 min at 37 °C in 3 ml Krebs-Ringer bicarbonate (KRB) buffer, pH 7.4, containing 0.1% BSA and either 3.3 or 16.6 mM glucose concentrations. These glucose concentrations represent the basal and the stimulated conditions for islet function. The incubation was stopped by adding 9 ml of 250 μM EGTA-50 mM Tris-HCl buffer, at pH 7.24 and the suspension was centrifuged at $1700 \times g$ for 1 min. The supernatant was discarded and the islets washed twice with 0.25 M sucrose-50 mM Tris-HCl, pH 7.4 and homogenized in the same buffer by 200 passes of the plunger. This crude homogenate was used to obtain a partially purified plasma membrane preparation. The homogenate was centrifuged at $10{,}000 \times g$ for 1 min. The supernatant was removed and centrifuged at $150{,}000 \times g$ for 90 min. After removal of the supernatant, the pellet was rehomogenized in a small volume of the same buffer. Aliquots were used to measure protein according to the method of Lowry et al. (1951). As previously shown by our group (Gronda et al., 1987), this procedure results in a 3.5 times increase in the plasma membrane Ca^{2+}-ATPase activity as determined by the protein : activity ratio.

Fatty acid composition

Fatty acid composition was studied using groups of 150 islets incubated for 5 min at 37 °C in 1 ml of KRB buffer at pH 7.4 in the presence of 3.3 or 16.6 mM glucose. At the end of the incubation period the islets were sonicated at room temperature and the lipids extracted with chloroform/methanol (2 : 1, v/v) by the method of Folch et al. (1957). The washed lower phase was used for lipid analysis. Neutral lipids (NL) were separated from phospholipids (PL) by thin-layer chromatography on plates of silica gel G-60 using the solvent system chloroform/methanol/acetic acid/water (90 : 6 : 1 : 0.75, v/v/v/v) (Van Dorp et al., 1964). The spots were identified by iodine staining, scraped and eluted using Arvidson's method (1968) (chloroform/methanol/acetic acid/water (50 : 39 : 1 : 10, v/v/v/v)). The extracts were evaporated to dryness under N_2. Fatty acid methyl esters from the different lipid fractions were obtained by refluxing the extracts for 3 h at 65 °C with HCl-methanol according to the method of Stöffel et al. (1959) and separated into their multi-

ple components by gas-liquid chromatography. This technique was performed with a programmed temperature method (140–220 °C, 3 °C/min) in a GC-RIA Shimadzu, equipped with a column of Supelco 1-1851, 10% SP 2330 on 100/120 Chromosorb AW Zog and dual flame-ionization detectors. The peaks were identified running simultaneously appropriate standards.

Fluorescence polarization studies

The fluorescence of DPH was used to measure the islet membrane microviscosity (Shinitzky and Ingbar, 1976). For this purpose a solution of 2×10^{-3} M DPH in THF was diluted 1000-fold by injection into vigorously stirred 50 mM Tris-HCl buffer, pH 7.4. The DPH dispersion obtained was mixed 1 : 1 (v/v) with membrane suspensions (300 μg of protein/ml) and incubated for 3 h at 25 °C. A circulating water bath was used to regulate the membrane suspension temperature within ±0.1 °C from 10 to 30 °C. Steady-state fluorescence polarization measurements were performed in an SLM-AMINCO 4800 spectrofluorometer (Urbana, IL, U.S.A.). The concentration of DPH in the membrane suspension was at least two orders of magnitude below the levels where fluorescence depolarization due to energy transfer (Shinitzky and Ingbar, 1976) could occur. Anisotropy was measured at a sample concentration that shows a constant anisotropy value upon further dilution.

Sample excitation was performed with the smallest split compatible with a good signal-to-noise ratio. DPH was excited with plane-polarized light of 360 nm with a bandpass of 4 nm. A Corning filter 7-51 was employed in excitation to reduce the scattered second-order contribution from the excitation monochromator. Emitted light was detected using the classical T format (Weber, 1956). Shott KV 389 filters were used on the emission side of the instrument to isolate the emission band of DPH. In order to minimize photobleaching of DPH, sample illumination was avoided between determination.

$I_{\parallel}$ and $I_{\perp}$ are the fluorescence intensities of the emitted light, polarized parallel and perpendicular to the direction of the excitation beam respectively. The apparent microviscosity (η) of the lipid region of the membrane preparation at each temperature, was estimated using the modification described by Shinitzky and Barenholtz (1978) of the Perrin equation:

$$\bar{\eta} = C_{(r)} T \tau \left(\frac{r_0}{r} - 1 \right)^{-1}$$

where $C_{(r)}$ is function of the molecular shape and the location of the transition dipole for a given fluorescent probe, r_0 is the molecular anisotropy of the probe at infinite rigidity, r is the anisotropy of the probe measured at the experimental temperature, T is the absolute temperature, and τ is the excited state lifetime of the probe. Assuming $C_{(r)} \cdot T \cdot r$ to be constant at 2,4 poise and r to be 0.362 for DPH excited at 360 nm (Shinitzky and Ingbar, 1976), the following approximate expression for $\bar{\eta}$ (±15%) can be obtained:

$$\bar{\eta} = \frac{2.4\, r}{-0.362 - r} = \frac{2\,\mathrm{P}}{0.46 - \mathrm{P}} = \frac{I_{\parallel}/I_{\perp} - 1}{0.73 - 0.27 I_{\parallel}/I_{\perp}}$$

Phase transition in lipids is characterized by an abrupt change in fluidity with temperature. Using DPH, the temperature profile of the fluorescence polarization reveals phase transitions. A quantitative estimation of such a transition is obtained by a plot of ln η vs. $1/T$ (Shinitzky and Ingbar, 1976). The slope in each point of such a plot is proportional to the flow activation energy (ΔE). The ΔE derived from the change of η with temperature is the energy required to fuse the flowing lipid segments to furnish a 'hole' which is sufficient to maintain the flow process. This parameter can be considered as a criterion for the degree of order in the hydrocarbon core of lipid assemblies: an increase in order will decrease ΔE and vice versa (Shinitzky and Ingbar, 1976). A discontinuity in the plot provides the identification of temperature-dependent phase transition (T_t) within the membranes.

Lifetime studies

Lifetime determinations of DPH for the membrane preparations incubated in the presence of 3.3 or 16.6 mM glucose were performed directly with the cross-correlation phase fluorometer already mentioned in the previous section. Lifetimes were the average values calculated from phase (τ phase) and modulation (τ mod) with an excitation

wavelength of 360 nm modulated at 6 MHz. A KV 389 filter was used to isolate the DPH emission band. A suspension of cross-linked styrene divinyl benzene beads was employed as a zero lifetime reference.

The difference between τ phase and τ mod was approximately 3 ns indicating a large heterogeneity in the probe environment. However, the lifetime absolute values and their differences were the same for all the samples studied (τ phase = 4.9 and τ mod = 8.3 ns). Consequently, the same degree of heterogeneity was assumed for all of them and the anisotropy data was treated as an indication of membrane fluidity.

Statistical analysis

A computer program was developed to determine the best fits and the breakpoints of the Arrhenius plot obtained from the fluorescence polarization data. The criteria employed to select the best two line fit have been published in detail elsewhere (Livingstone and Schachter, 1980). Comparison of mean values between basal and stimulated conditions was performed using the unpaired Student's *t*-test for ΔE, T_t and percentage fatty acid composition.

Results

The lipid thermotropic transition of partially purified plasma membranes obtained from rat isolated islets and incubated in the presence of 3.3 or 16.6 mM glucose was studied in the range of 10–30 °C.

The values of the transition temperatures (T_t) and the flow activation energies (ΔE) are represented in Table 1. The data in each of six different membrane preparations as determined by the computer correlation program uniformly fit a two-slope plot. The plot of ln η vs. $1/T$ (Arrhenius plot) showed a breakpoint (T_t) representing the temperature at which a change in the membrane organization occurs (Fig. 1). The T_t was near 18 °C regardless of prior glucose exposure. However, as shown in Table 1, 16.6 mM glucose significantly increased the flow activation energy (ΔE) below and above the transition temperature.

Effects of glucose on phospholipid fatty acid composition of the whole islets are shown in Table 2. Preincubation of the islets during 5 min with

TABLE 1

ACTIVATION ENERGIES AND THERMOTROPIC TRANSITION TEMPERATURE FROM ARRHENIUS PLOTS

Values are expressed as means ± SEM of six independent experiments.

Glucose (mM)	ΔE_1 (kcal/mol)	ΔE_2 (kcal/mol)	T_t (°C)
3.3	3.91 ± 0.32	1.46 ± 0.34	19.0 ± 1.02
16.6	6.01 ± 0.59	3.55 ± 0.58	16.8 ± 1.28
3.3 vs. 16.6	$P < 0.02$	$P < 0.02$	N.S.

ΔE_1 = activation energy below transition temperature; ΔE_2 = activation energy above transition temperature; T_t = thermotropic transition temperature.

TABLE 2

PERCENTAGE FATTY ACIDS COMPOSITION OF ISLETS PHOSPHOLIPIDS FOLLOWING INCUBATION WITH 3.3 OR 16.6 mM GLUCOSE (MEAN ± SEM; $n = 8$)

Fatty acid	Glucose (mM)		3.3 vs. 16.6
	3.3	16.6	
12:0	0.48 ± 0.06	1.54 ± 0.34	$P < 0.02$
14:0	4.02 ± 0.59	4.46 ± 0.56	–
15:0	1.39 ± 0.16	1.25 ± 0.18	–
16:0	23.89 ± 2.10	26.30 ± 1.59	–
16:1	5.17 ± 0.59	5.16 ± 0.50	–
18:0	14.30 ± 0.93	14.98 ± 0.98	–
18:1	14.44 ± 0.99	15.38 ± 0.70	–
18:2	4.31 ± 0.02	6.94 ± 0.57	$P < 0.02$
18:3 w6	0.34 ± 0.28	–	–
18:3 w3	1.38 ± 0.26	0.76 ± 0.25	–
20:1 w9	1.24 ± 0.30	0.80 ± 0.14	–
20:2 w6	0.87 ± 0.18	0.35 ± 0.08	$P < 0.025$
20:3 w9	1.00 ± 0.33	0.53 ± 0.21	–
20:3 w6	1.13 ± 0.73	1.27 ± 0.70	–
20:4 w6	3.11 ± 0.88	2.71 ± 0.89	–
20:5 w3	3.75 ± 0.43	6.54 ± 1.69	–
22:2 w6	2.28 ± 1.09	0.80 ± 0.35	–
22:3 w3	10.35 ± 1.19	6.44 ± 0.70	$P < 0.05$
22:4 w3	3.01 ± 0.51	3.22 ± 0.50	–
22:5 w3	1.16 ± 0.04	1.39 ± 0.52	–
22:6 w3	8.00 ± 1.56	4.17 ± 0.64	–
Sat FA	44.07 ± 3.59	47.88 ± 3.27	–
DBI	154.89 ± 15.63	142.64 ± 17.34	–
R	3.90 ± 0.73	3.30 ± 0.60	–

Sat FA = proportion of saturated fatty acid; DBI = double bound index; R = ratio of DBI to sat FA.

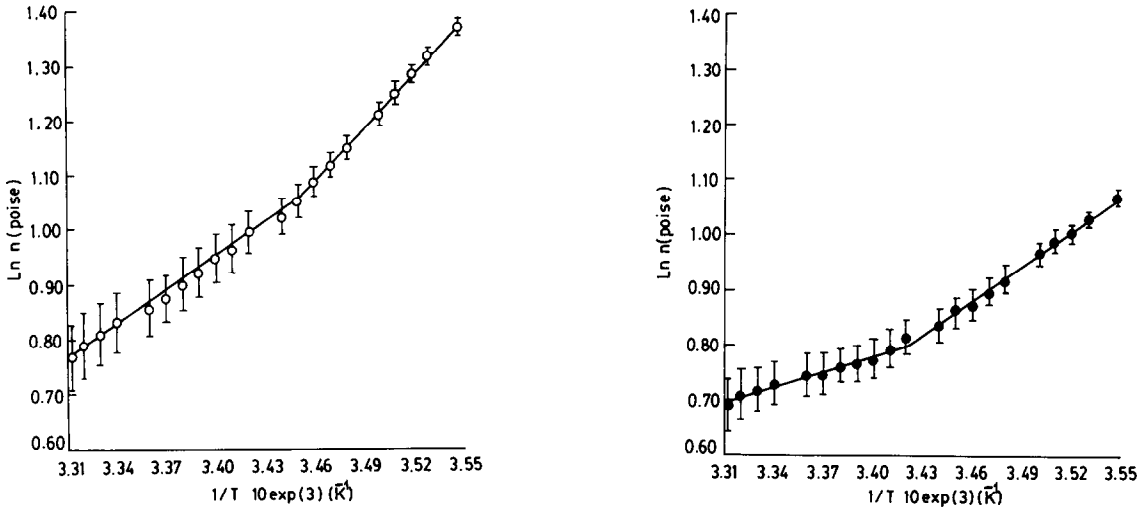

Fig. 1. Arrhenius plots were obtained as described in the text (see Statistical analysis) with data corresponding to membrane preparations obtained from islets preincubated with 3.3 mM (right panel, closed circles) or 16.6 mM glucose (left panel, open circles). Each value represents the mean ± SEM of six different experiments performed in triplicate. The η values corresponding to membranes obtained from islets preincubated with 16.6 mM glucose were slightly higher than those corresponding to islets preincubated with 3.3 mM glucose.

16.6 mM glucose produced a significant increase in the percentage composition of 12 : 0 and 18 : 2, together with a decrease in the 20 : 2 w6 and 22 : 3 w3 fractions. Despite these changes, no significant alterations occurred in the proportion of fatty acids which was saturated in the double bond index (DBI), or in the average 'membrane fluidity index' (R).

Discussion

Our results show that the membranes of isolated islets incubated briefly with high glucose concentrations undergo significant increases in flow activation energy, without significant changes in transition temperature or fatty acid composition of whole-cell phospholipids.

The thermotropic transition temperature values obtained for islet membranes are close to those previously reported for rat hepatocyte plasma membranes (Livingstone and Schachter, 1980). In those experiments, it has been demonstrated that the breakpoint temperature of whole membranes of hepatocytes or liposomes of membrane lipids were similar and represent a lipid transition. Hence, similar conclusions can be applied to the breakpoints found in our islet membrane preparation.

Studies of DPH in model bilayers suggest that changes in temperature mainly affect the hindrance of the rotation of the probe (Lakowicz et al., 1979a, b). Furthermore, hindrance of DPH rotations is particularly sensitive to lipid organization (Shinizky and Ingbar, 1976).

According to the definition of flow activation energy (see Material and Methods), this parameter serves as a criterion for the degree of order in the hydrocarbon core in such way that a decrease in its value indicates an increase in order (Shinitzky and Ingbar, 1976). Hence, the increases in flow activation energy of islets incubated with high glucose suggest the promotion of some degree of disorder in their membrane structure.

Previous studies have established that microviscosity of lipid regions depends on the degree of unsaturation of phospholipid acyl chains (Kimelberg, 1982), the sphingomyelin/phosphatidylcholine (PC) ratio (Shinitzky and Barenholz, 1974) and the cholesterol/phospholipid ratio (Shinitzky and Ingbar, 1976).

In this report, glucose did not produce significant changes in the degree of saturation of fatty

acids esterified to islet phospholipids. Montague and Parkin (1980) have reported that the cholesterol/phospholipid molar ratio remains unchanged in islets incubated with glucose in a concentration range from 0 to 20 mM. Since the percentage of islet phospholipid fractions is not affected by glucose (Cortizo et al., 1984) we could assume that the hexose would not modify the sphingomyelin/PC ratio. Thus, changes induced by glucose on the thermotropic properties of islet membranes could not be ascribed to gross alterations in lipid composition.

Using isolated pancreatic endocrine cells labelled with DPH, Deleers et al. (1981) reported a small decrease in fluorescence polarization when the cells were exposed for 1 min to an increasing concentration of 0–16.7 mM glucose suggesting a decrease in the islet membranes' microviscosity. Although of statistical significance, these results were handicapped, as stated by these authors, by the fact that (a) DPH could be internalized and consequently the polarization values obtained might be the average of changes occurring at the membrane level and in some intracellular organelles as well (Deleers et al., 1981; Grunberger et al., 1982), and (b) islet cells display a different secretory response than intact islets. Hence, the results obtained with such cells might not reflect exactly the events induced by glucose in the islets.

An early participation in the mechanism of insulin secretion has been ascribed to islet membranes, as a source of intracellular signals for hormone release. Our results might suggest that they also participate in its final step, the emiocytosis undergoing changes which favor its development or are just the consequence of such a process.

Acknowledgements

This work was partially supported by funds from CONICET, CICPBA and the Ministerio de Salud de la Pcia. de Buenos Aires.

We are also greatly indebted to Mrs. S. Lunati for secretarial support, Mr. G.G. Gagliardino and W. Platini for the computer analysis.

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