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GLUCOSE INDUCES MEMBRANE CHANGES DETECTED BY FLUORESCENCE POLARIZATION IN ENDOCRINE PANCREATIC CELLS

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SUMMARY : Pancreatic endocrine cells prepared from rat islets were labelled with 1,6-diphenyl-1,3,5-hexatriene and examined in a microviscosimeter. Glucose caused a dose-related decrease in fluorescence polarization. This decrease was observed within 1 min after increasing the concentration of glucose. These findings suggest that glucose affects membrane viscosity in pancreatic endocrine cells. At a glucose concentration of 16.7 mM, the estimated viscosity was 15 ± 3 per cent lower than basal value (2.01 ± 0.12 P).

Glucose induces a cascade of metabolic, ionic, motile and secretory events in endocrine pancreatic cells such as the insulin-producing B-cell (1). The present report reveals that glucose also induces membrane changes detected by fluorescence polarization in pancreatic endocrine cells.

MATERIALS AND METHODS

In each experiment, 500-700 islets were isolated by collagenase digestion (2) of pancreases removed from fed albino rats. The islets were disrupted into isolated cells by mechanical agitation for 10 min in a Ca^{2+}-free Hanks solution (3) containing EGTA (1.0 mM), bovine albumin (5 mg/ml) and collagenase (2 mg/ml). After filtration through a nylon screen (diameter approximately 60 μM) in order to remove cells aggregates and centrifugation for 10 min at 250 g, the cellular pellet was resuspended and preincubated for 240 min in 5 ml of medium 199 (medium n° 235, Gibco Biocult, Paisley, Scotland) containing glucose (8.3 mM), streptomycin (0.2 mg/ml), penicillin (500 U/ml) and heated calf serum (10 %, v/v). Aliquots (10 μl) of this medium were used for cell counting. After preincubation and a further centrifugation, the cells (4 to 6.10^5) were resuspended in 2.5 ml of a glucose-free Hanks solution containing Ca^{2+} (1.0 mM) and the lipophilic fluo-

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rescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH; Sigma, St. Louis, Missouri) at a 10^{-6} M concentration. After about 20-30 min, when the fluorescence intensity had reached a close-to-equilibrium value, the cell suspension was examined for fluorescence polarization in a Elscint MV_1a microviscosimeter (Elscint Ltd., Haifa, Israel). The concentration of glucose of the incubation medium (2.5 ml) was increased in a step-wise manner by addition of 85 µl of a concentrated solution of glucose (0.17 M). The apparent viscosity ($\bar{\eta}$) was calculated according to the equation

$$\bar{\eta} = 2p/(0.46 - p)$$

in which p represents the degree of fluorescence polarization (4).

RESULTS

When the temperature-dependency of the fluorescence polarization was monitored in the same batch of cells exposed to different concentrations of glucose, an obvious decrease in polarization was seen as the glucose concentration was increased from zero to 16.7 mM (Fig. 1, upper panel). In a series of 10 individual experiments, the estimated plasma membrane viscosity averaged 2.01 ± 0.12 P in the absence of glucose (basal value). By pairing analysis, a significant decrease in membrane viscosity averaging 67.1 ± 20.4 mP ($P < 0.01$) was observed in the presence of glucose 5.6 mM. Higher concentrations of glucose (11.1 and 16.7 mM) caused further increases in membrane fluidity (Table 1). At the highest concentration of glucose (16.7 mM), the effect of the sugar corresponded to a mean decrease in membrane viscosity of 15.3 ± 2.8 per cent below basal value. As judged from the mean absolute changes in viscosity, the effect of glucose was 3 to 4 times more marked in the range of concentrations between 5.6 and 16.7 mM than in a lower range of concentrations between zero and 5.6 mM. This pattern coincides with that of the curve relating insulin output to glucose concentration, in which case the steepest part of the dose-action relationship also corresponds to the 5.6 to 16.7 mM range of glucose concentrations (5).

The time-course of the glucose effect was examined in a separate series of experiments performed throughout at 37°C. The

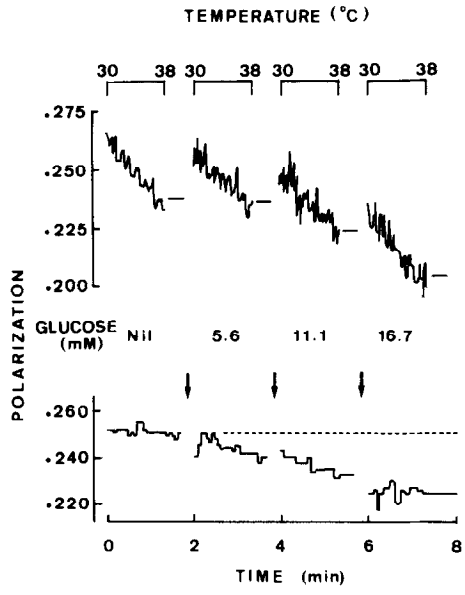

Fig. 1. Effect of glucose upon fluorescence polarization in isolated endocrine pancreatic cells. Upper panel : the same batch of cells was examined under steady-state conditions of exposure to four different glucose concentrations. At each concentration, the decrease in fluorescence polarization evoked by a progressive increase in the temperature of the incubation medium from 30 to 38°C (upper abscissa) was monitored over a period of approximately 10 min. The mean slope of each record was used to calculate the polarization value at a temperature of 37°C : such a value is indicated by the horizontal bar drawn at the right of each record. Lower panel : a batch of endocrine pancreatic cells was examined over 8 min (lower abscissa) at 37°C, the glucose concentration of the incubation medium being increased at the time shown by each vertical arrow. The horizontal dotted line refers to the mean basal value (no glucose) for fluorescence polarization. In this illustration, the original records, which refer to two distinct and representative experiments, were redrawn using a twofold (upper panel) and fourfold (lower panel) magnification of the ordinates/abscissae scale ratio.

glucose concentration was increased every 2 min in a stepwise manner (Fig. 1, lower panel). Within 1 min after increasing the glucose concentration, a seizable decrease in fluorescence polarization was noticed. The increment in insulin output evoked by a staircase increase in glucose concentration can also be detected within such a short period of stimulation (5). In control experiments including the addition of a glucose-free solution to the

Table 1. Mean values (± SEM) for the paired decrease in fluorescence polarization below basal value (no glucose) are expressed in absolute terms (milliunits). The basal polarization averaged 230.7 ± 7.0 milliunits. Membrane viscosities are derived from membrane fluorescence polarization values obtained at 37°C, under steady-state conditions of exposure to increasing concentrations of glucose. Mean values (± SEM) for the paired decrease in viscosity below basal value (no glucose) are expressed in both absolute (mP, millipoise) and relative (per cent) terms, and are shown together with their statistical significance (P) and the number of individual observations (n). The basal viscosity averaged 2,010 ± 120 mP.

Glucose (mM)	Paired decrease in fluorescence polarization (10^{-3})	in viscosity (mP)	per cent of basal viscosity	P	n
Nil	0	0	0		10
5.6	4.0 ± 1.3	67.1 ± 20.4	3.42 ± 1.12	< 0.02	10
11.1	10.6 ± 2.5	192.2 ± 46.8	8.76 ± 1.91	< 0.02	5
16.7	19.3 ± 3.7	300.9 ± 49.5	15.31 ± 2.74	< 0.001	10

incubation chamber, no decrease occurred in fluorescence polarization (data not shown).

DISCUSSION

The present findings indicate that glucose provokes a dose-related, rapid and sustained decrease in fluorescence polarization of DPH.

Some potential limitations of this study must be considered. First, our preparation represents a mixed population of endocrine cells (i.e. insulin-, glucagon-, somatostatin- and pancreatic polypeptide-producing cells). Nevertheless, since B-cells represent the large majority of endocrine pancreatic cells, our results are probably representative of the effect of glucose upon insulin-secreting cells. Second, the present study was conducted with isolated endocrine cells, which may display a secretory response

to glucose somewhat different from that of intact pancreatic islets (3,6). Third, since DPH may be internalized to a limited extent in cells (7), changes in the membrane fluidity of intracellular organelles could contribute to the observed response to glucose. However, we have purposedly restricted to 20-30 min the initial period of exposure to DPH in order to minimize the process of DPH internalization. Last, it should be kept in mind that the steady-state fluorescence polarization not solely depends on the viscosity, but may also be affected by the static orientational constraint of the label (DPH), as it is the case in certain cell types characterized by a high cholesterol/phospholipid molar ratio (8,9). In the pancreatic B-cell, however, the cholesterol/phospholipid molar ratio does not exceed 0.22/1.00, and is unaffected by glucose in the zero-20.0 mM range (10). Therefore, the changes in fluorescence polarization here observed are likely to correspond to a change in membrane fluidity.

The mechanism by which glucose increases membrane fluidity and the relevance of such a change to the process of stimulus-secretion coupling remain both to be elucidated. Because pancreatic endocrine cells were recently found to contain native ionophores (11) and because the membrane fluidity is a major regulatory factor of ionophore-mediated transport processes (12), it is conceivable that the glucose-induced change in membrane viscosity contributes to the remodelling of ionic fluxes evoked by the sugar in endocrine pancreatic cells.

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