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Effects of glucose and glucagon on the fructose 2,6-bisphosphate content of pancreatic islets and purified pancreatic B-cells

A comparison with isolated hepatocytes

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Glucose caused a sustained and dose-related increase in the fructose 2,6-bisphosphate content of isolated pancreatic islets, as well as of purified pancreatic B-cells. With isolated B-cells, the glucose saturation curve was sigmoidal and superimposable on that obtained with hepatocytes isolated from unfed rats. However, the response to glucose was notably faster in purified B-cells than in isolated hepatocytes. In contrast again with the situation prevailing in the liver, glucagon failed to decrease significantly the concentration of fructose 2,6-bisphosphate in either islets or purified B-cells. It is proposed that, in the process of glucose-stimulated insulin secretion, an early increase in fructose 2,6-bisphosphate formation may, by causing activation of 6-phosphofructo-1-kinase, allow glycolysis to keep pace with the rate of glucose phosphorylation.

Fructose 2,6-bisphosphate (Fru-2,6-P_2) is thought to play a critical role in the regulation of glycolysis and gluconeogenesis in hepatocytes (Hers & Van Schaftingen, 1982; Hers & Hue, 1983). Thus the Fru-2,6-P_2 content of hepatocytes increases in response to a rise in the extracellular concentration of glucose, leading to activation of 6-phosphofructo-1-kinase and inhibition of fructose-1,6-bisphosphatase. Even at high glucose concentrations, glucagon, by causing inactivation of 6-phosphofructo-2-kinase and activation of fructose-2,6-bisphosphatase, lowers the Fru-2,6-P_2 content of hepatocytes, and hence exerts effects opposite to those of glucose on the rates of glycolysis and gluconeogenesis. Fru-2,6-P_2 was also shown to be a potent stimulator of 6-phosphofructo-1-kinase in pancreatic-islet homogenates (Malaisse *et al.*, 1981*b*), and this preparation displays 6-phosphofructo-2-kinase activity (Malaisse *et al.*, 1981*c*). Unlike its liver counterpart, the latter enzyme is not inactivated by treatment of the islets with glucagon (Malaisse *et al.*, 1982*a*). Furthermore, and in agreement with the hypothesis that Fru-2,6-P_2 plays a role in the control of glycolysis in the B-cell, it was found that the concentration of this stimulator in the islets was greater in the presence of glucose than in its absence (Malaisse *et al.*, 1982*b*). These investigations were hampered, however, by the relative imprecision of the dosage as well as by the fact that B-cells represent only about 66–74% of the total number of endocrine islet cells (Baetens *et al.*, 1979). More recently two technological advances have opened new perspectives of research: firstly, the sensitivity of Fru-2,6-P_2 determination has been increased more than 100-fold (Van Schaftingen *et al.*, 1982; Van Schaftingen & Hers, 1983) allowing establishment of precise dose–response curves as well as time courses of the glucose effect; secondly, methods have been developed to purify pancreatic B- and non-B-cells selectively (Pipeleers & Pipeleers-Marichal, 1981; Van de Winkel *et al.*, 1982). An investigation that takes advantage of these new techniques is reported here.

Abbreviation used: Fru-2,6-P_2, fructose 2,6-bisphosphate.

Materials and methods

Pig glucagon was obtained from Novo (Bagsvaerd, Denmark).

Pancreatic islets were isolated by the collagenase technique from the entire pancreatic gland or, separately, from the ventral and dorsal regions of pancreases removed from fed albino rats (Orci *et al.*, 1976). In some experiments, the freshly isolated islets were immediately incubated in groups of 80–100 islets for 60min in 0.2ml of bicarbonate-buffered medium (Malaisse *et al.*, 1970) containing bovine albumin (5mg/ml) and, as required, glucose and glucagon. In other experiments, and for comparison, the islets were maintained for 20h in the same culture medium as that used for purified B-cells (see below), before being incubated for 60min at various glucose concentrations. After incubation and centrifugation (5000***g***, 30s), the medium was removed and the islets were homogenized in 50μl of 0.1M-NaOH.

Pancreatic-islet cells were purified by autofluorescence-activated cell sorting (Van de Winkel *et al.*, 1982) of rat islet-cell suspensions (Pipeleers & Pipeleers-Marichal, 1981). The purified B-cell fraction consisted of more than 95% single and viable B-cells, whereas the single non-B-cell fraction contained more than 80% A-cells, 15–20% D- and PP-cells and less than 2% B-cells (Van de Winkel *et al.*, 1982). The purified islet cells were cultured in bacteriological culture dishes for 20h in medium CMRL-1066 supplemented with 2mM-L-glutamine, 10% (v/v) heat-inactivated foetal-calf serum (Gibco, Paisley, Scotland, U.K.), penicillin and streptomycin (both 0.1mg/ml). After culture, the cells were collected by centrifugation, washed with Earle's Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] medium (Van de Winkel & Pipeleers, 1983) and distributed in polypropylene reaction tubes (Eppendorf 3810, capacity 1ml; 5×10^4 cells per tube); after a 3min centrifugation at 500***g***, the cells were resuspended in 1.0ml of Earle's Hepes medium (37°C) containing glucose (1.4–27.8mM). Incubations were performed at 37°C for 1–60min in a Forma CO_2 incubator, the gas phase being 7.5% (v/v) CO_2 in humidified air. After incubation, the tubes containing the islet cells were cooled on ice, and centrifuged (500***g***, 2min); the incubation medium was then removed and the purified islet cells were mixed with 50μl of 0.1M-NaOH.

Hepatocytes were isolated from 24h-starved rats by a modification (Bartrons *et al.*, 1983) of the method described by Seglen (1973); they were incubated at the indicated concentration of glucose in a Krebs–Henseleit bicarbonate buffer equilibrated with an O_2/CO_2 (19:1) gas phase, and further processed as described elsewhere (Bartrons *et al.*, 1983).

Fru-2,6-P_2 was assayed by its property to stimulate potato PP_i:fructose 6-phosphate phosphotransferase by a modification (Van Schaftingen & Hers, 1983) of the method described by Van Schaftingen *et al.* (1982). To check the specificity of this assay, several samples containing Fru-2,6-P_2 in either low or high concentrations were incubated for 30min at 20°C in the presence of 0.1M-HCl. This treatment resulted in the complete disappearance of the stimulator. All results are expressed as means (±S.E.M.) together with the numbers of individual observations (*n*). The statistical significance of differences between mean values was tested by use of Student's *t* test.

Results

Experiments with isolated islets

Over 60min incubation, glucose increased the Fru-2,6-P_2 content of either freshly isolated islets derived from the entire pancreatic gland or similar islets that had been cultured for 20h in the presence of 5.6mM-glucose (Table 1). In these two preparations, a glucose concentration as low as 1.4–1.7mM was sufficient to cause a sizeable increase in Fru-2,6-P_2 content above the basal value found in the absence of glucose (Fig. 1, Table 1), such an increase averaging 22% of that evoked by a much higher concentration of glucose (16.7–20.0mM). As shown in Fig. 1, the dose–response curve was close to a hyperbola, with a calculated half-maximal response at about 6.2mM-glucose. Exogenous glucagon (2.9μM) failed to affect significantly ($P > 0.1$) the Fru-2,6-P_2 content of freshly isolated islets derived from either the dorsal or ventral region of the pancreas and incubated at a high glucose concentration (Table 1). D-Glyceraldehyde and 4-methyl-2-oxopentanoate, which, at the concentrations used, are known to stimulate insulin secretion in isolated pancreatic islets, were without effect on the Fru-2,6-P_2 content of freshly prepared islets (Table 1).

Experiments with purified B-cells and comparison with isolated hepatocytes

As illustrated in Fig. 2(*a*), glucose increased the Fru-2,6-P_2 content of purified B-cells. At low glucose concentration (1.4–1.7mM), the Fru-2,6-P_2 content was relatively smaller than in isolated islets, allowing a 14-fold increase to be observed when the concentration of glucose was raised to 27.8mM. The saturation curve for glucose was clearly sigmoidal, with its greatest increment between 2.8mM- and 5.6mM-glucose, and an almost linear increase at higher glucose concentrations. These results were similar to those obtained with hepatocytes from unfed rats (Fig. 2*b*). Fig. 2 also shows that exogenous glucagon (14nM) failed

Table 1. *Effect of exogenous nutrients and glucagon on the Fru-2,6-P_2 content of freshly isolated or cultured islets*
The islets were derived from the entire pancreatic gland or separately from the dorsal and ventral regions of the pancreas, and incubated for 60min in the presence of the stated agent(s).

Type of islets (source)	Agent(s) (mM)	Fru-2,6-P_2 (fmol/islet)
Freshly isolated islets (entire pancreas)	Nil	3.9±0.3 (9)
	D-Glucose (16.7)	13.9±0.6 (9)
	D-Glyceraldehyde (10.0)	3.5±0.3 (9)
	4-Methyl-2-oxopentanoate (10.0)	3.1±0.4 (9)
Freshly isolated islets (dorsal pancreas)	D-Glucose (16.7)	14.1±1.2 (11)
	D-Glucose (16.7)+glucagon (0.003)	15.1±1.3 (12)
(ventral pancreas)	D-Glucose (16.7)	16.8±1.9 (6)
	D-Glucose (16.7)+glucagon (0.003)	12.9±1.2 (5)
Cultured islets (entire pancreas)	Nil	3.7±0.6 (3)
	D-Glucose (1.4)	9.7±0.9 (3)
	D-Glucose (20.0)	28.6±2.3 (3)

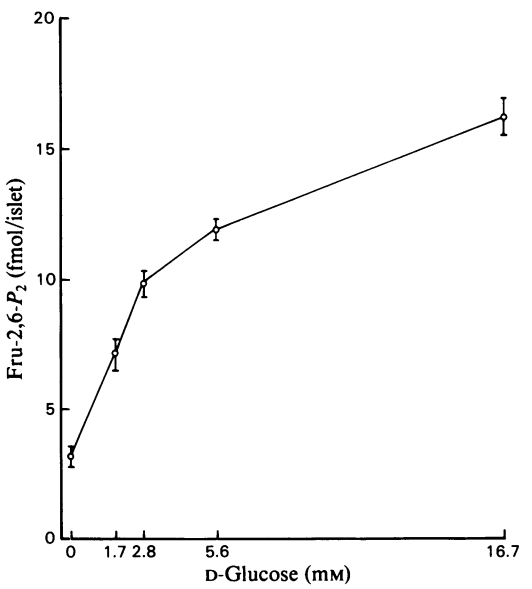

Fig. 1. *Effect of glucose on the Fru-2,6-P_2 content of freshly isolated islets incubated for 60min at the stated concentration of this hexose*
Mean values (±S.E.M.) refer to 5–16 individual determinations collected in five separate experiments, all results being normalized relative to the mean value found within the same experiment in the presence of 16.7mM-glucose. Such mean reference values averaged 16.2±2.9fmol/islet ($n = 5$).

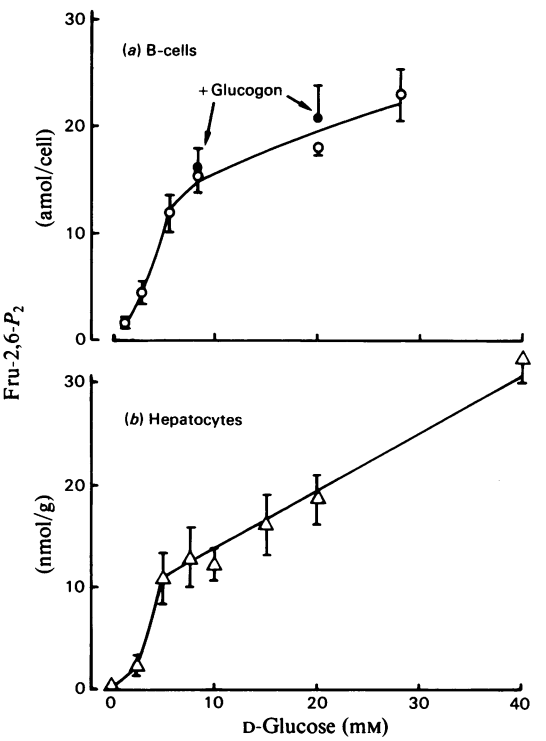

Fig. 2. *Effect of glucose concentration on the Fru-2,6-P_2 content of purified pancreatic B-cells (a) and hepatocytes (b) incubated for 40 (b) or 60 min (a)*
Glucagon (14nM) was also present in some experiments. Mean values (±S.E.M.) refer to three to eight individual determinations. The results in purified B-cells are derived from four separate experiments and were normalized relative to the mean value found within each experiment in the presence of 20.0mM-glucose. Such a reference value averaged 18.1±4.3amol/cell.

to decrease the concentration of Fru-2,6-P_2 in B-cells incubated in the presence of either 8.3mM- or 20.0mM-glucose. At the concentration used here, glucagon causes a half-maximal stimulation of

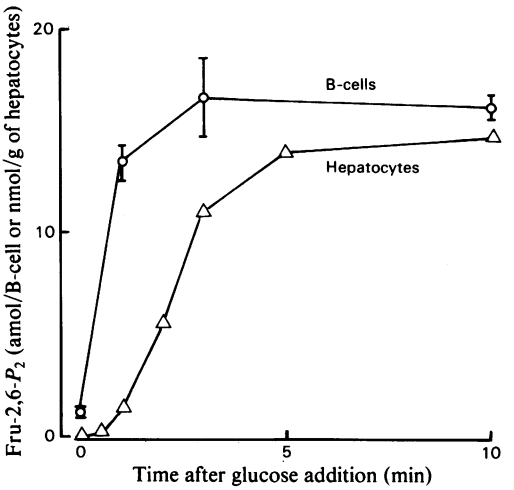

Fig. 3. *Time course of the effect of glucose to increase the Fru-2,6-P_2 content of purified B-cells and of isolated hepatocytes*

At zero time, the glucose concentration was raised from either 1.4mM (B-cells) or approx. zero (hepatocytes) to 20.0mM. Mean values (±S.E.M.) refer to three to six individual determinations (B-cells) or a single representative experiment (hepatocytes).

Table 2. *Effect of glucose on the Fru-2,6-P_2 content of purified islet non-B-cells*

Individual values ($n = 2$) or mean values (±S.E.M., if $n = 3$) are expressed as amol/cell.

D-Glucose (mM) Incubation time ...	Content 3 min	60 min
Nil	<0.1 (2)	<0.1 (2)
1.4	<0.1 (2)	<0.1 (2)
5.6	<0.1 (2)	0.2±0.1 (3)
20.0	0.2–0.3 (2)	0.8±0.1 (3)

cyclic AMP formation in our preparation of purified B-cells (Schuit & Pipeleers, 1983).

Fig. 3 shows that, in purified B-cells, the Fru-2,6-P_2 content reached nearly its maximal value within 1min after the glucose concentration was raised from 1.4 to 20.0mM. It reached 40.2±2.1% ($n = 3$) of such a maximal value within 3min after the glucose concentration was raised to only 5.6mM. This rapid response contrasts with the relatively slow effect of glucose on the concentration of Fru-2,6-P_2 in isolated hepatocytes (Fig. 3), as first reported by Van Schaftingen *et al.* (1980).

Experiments with islet non-B-cells

In a limited series of observations, the concentration of Fru-2,6-P_2 was measured in purified islet non-B-cells (5×10^4 cells/sample). The readings remained below the limit of sensitivity of our assay (<0.1 amol/cell), as long as the glucose concentration remained in the range 0–1.4mM. A time- and dose-related increase in Fru-2,6-P_2 content was observed, however, at higher glucose concentrations (Table 2). In considering these results, it should be kept in mind that the volume of islet non-B-cells (201±8fl/cell) is about one-quarter of that of B-cells (776±34fl/cell) as judged from the distribution space of [^{14}C]urea in excess of that of [6,6'(n)-^{3}H]sucrose (Gorus *et al.*, 1984).

Discussion

Effect of glucose on the concentration of Fru-2,6-P_2 in pancreatic islet cells

Because of the limited amount of endocrine pancreatic tissue readily available for experiments *in vitro*, the determination of its content of Fru-2,6-P_2 and the study of the effect of glucose thereupon requires an exquisitely sensitive analytical procedure, which was only recently developed (Van Schaftingen *et al.*, 1982; Van Schaftingen & Hers, 1983). The results of previous investigations, from which it was concluded that glucose either increases the Fru-2,6-P_2 content of isolated islets (Malaisse *et al.*, 1982*b*) or fails to do so (Matschinsky *et al.*, 1983), should therefore be considered with caution.

The present results unambiguously demonstrate that Fru-2,6-P_2 is formed in B-cells and that its concentration in these cells can be increased as much as 14-fold by glucose. The Fru-2,6-P_2 content was lower in islet non-B-cells than in B-cells, even if corrected for the difference in size of these two cell types. Nevertheless, glucose also caused a time- and dose-related increase in the Fru-2,6-P_2 content of islet non-B-cells. As expected, therefore, the effect of glucose on Fru-2,6-P_2 content was also evident in intact islets, in which the B-cells represent approx. 66–74% of the total number and about 82% of the total mass of endocrine cells (Baetens *et al.*, 1979).

The mean amount of Fru-2,6-P_2 found, at high glucose concentrations (16.7–20.0mM), in purified B-cells (18.1 amol/cell) and intact islets (16.2fmol/islet) is compatible with the knowledge that each islet contains approx. 1000 B-cells. Since the ratio of Fru-2,6-P_2 content at high to that at low glucose concentrations was higher in purified B-cells than in intact islets, we investigated whether the behaviour of purified non-B islet cells could account for such a difference. At the first glance, such was not the case. Indeed, in the islet non-B-cells, a low concentration of D-glucose (1.4mM) was not sufficient to cause any detectable increase in Fru-2,6-P_2 content. The latter finding could be explained by the fact that in islet non-B-cells, as

distinct from B-cells, the transport of glucose into the islet cells appears as a rate-limiting factor in the control of glucose metabolism (Gorus *et al.*, 1984). However, in comparing the results obtained in intact islets and purified B- and non-B-cells, it should be kept in mind that the behaviour of purified islet cells maintained in culture need not be identical with that of the homologous cells in intact islets. For instance, the existence of gap junctions between adjacent B- and non-B-cells (Orci *et al.*, 1975) could, by allowing the diffusion of glucose and other metabolites from one cell to another (Kohen *et al.*, 1979), modify the metabolic response of the non-B-cells to a change in extracellular glucose concentration.

In both purified B-cells and isolated hepatocytes, a sigmoidal curve related the Fru-2,6-P_2 content to the ambient glucose concentration in the low range of hexose concentrations, whereas a further and progressive increase in Fru-2,6-P_2 content was seen at higher glucose concentrations. The sigmoidal component of this relationship could be related, in part at least, to the moderately sigmoidal kinetics of glucokinase, an enzyme which is common to both types of cells (Meglasson *et al.*, 1983). Further work is obviously required to elucidate fully the determinants of the glucose-induced changes in Fru-2,6-P_2 content of these glucose-sensor cells.

The lack of glucagon effect

The capacity of glucagon to stimulate insulin release is synergistic with that of glucose (Malaisse *et al.*, 1967). It could be therefore expected that in B-cells glucagon would not cause the disappearance of Fru-2,6-P_2, as observed in hepatocytes (Van Schaftingen *et al.*, 1980). Accordingly, it was previously reported that exposure of the islets to either glucagon or theophylline, which are both known to cause cyclic AMP accumulation in the islets (Turtle & Kipnis, 1967; Montague & Cook, 1971), does not lead to inactivation of 6-phosphofructo-2-kinase (Malaisse *et al.*, 1982*a*). This is in good agreement with the present finding that glucagon fails to decrease the Fru-2,6-P_2 content of either isolated islets or purified B-cells. This lack of effect cannot be ascribed to an altered responsiveness of the islets or purified B-cells to glucagon, as could conceivably result from the collagenase treatment. Indeed, glucagon stimulates insulin release from both isolated islets and purified B-cells (Malaisse & Malaisse-Lagae, 1968; Pipeleers *et al.*, 1982). Moreover, at the concentration here used, glucagon was quite efficient in causing cyclic AMP accumulation in the purified B-cells (Schuit & Pipeleers, 1983). The results obtained with islets derived from the ventral region of the pancreas, which contains relatively few glucagon-producing cells (Orci *et al.*, 1976), and those obtained with purified B-cells further indicate that the failure of glucagon to affect the Fru-2,6-P_2 content of isolated islets cannot be ascribed to the release of endogenous glucagon during incubation of the islets.

Role of Fru-2,6-P_2 in the process of glucose-induced insulin release

Among the secretagogues tested in the present study, only D-glucose, but not D-glyceraldehyde or 4-methyl-2-oxopentanoate, provoked an increase in the islet content of Fru-2,6-P_2. This indicates that an increase in the concentration of the latter ester is not indispensable for insulin release to occur. However, a role for Fru-2,6-P_2 in the process of glucose-stimulated insulin secretion should not be overlooked. Indeed, in purified B-cells, the glucose-induced increase in Fru-2,6-P_2 content occurred rapidly enough to participate in the initial increase in glycolytic flux, which is known to precede the remodelling of cationic fluxes and subsequent stimulation of insulin release (Malaisse *et al.*, 1981*a*). In B-cells, the rapidity of Fru-2,6-P_2 accumulation in response to glucose was particularly striking when compared with its relative slowness in isolated hepatocytes. In the range of glucose concentration between 1.4 and 20.0mM, the dose–response relationship for the steady-state content of Fru-2,6-P_2 in purified B-cells was somewhat shifted to the left relative to the sigmoidal curve relating the rate of glycolysis in either isolated islets or purified B-cells to the ambient glucose concentration (Malaisse *et al.*, 1979; Gorus *et al.*, 1984). A similar situation was observed in isolated hepatocytes (Hue, 1982), but its interpretation is still obscure. It may be that, in these two types of cells, low concentrations of Fru-2,6-P_2 are poorly efficient in the control of glycolysis because the activator is protein-bound or because it needs to act synergistically with other metabolites, for instance fructose 6-phosphate.

Whatever the explanation, both the time course and dose–response relationship for the effect of glucose on the Fru-2,6-P_2 content of islet cells are compatible with the view that the concentration of this activator increases rapidly enough and to a sufficient extent to eliminate, in terms of either time or glucose concentration, any disparity between glucose phosphorylation and the production of fructose 1,6-bisphosphate from fructose 6-phosphate.

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