Abnormal glucose metabolism accompanies failure of glucose to stimulate insulin release from a rat pancreatic cell line (RINm5F)

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Glucose metabolism and insulin release were studied in isolated rat islets and in an insulin-producing rat cell-line (RINm5F). Intact islets displayed two components of glucose utilization, with glucose stimulation of insulin release being associated with the high-K_m component (reflecting glucokinase-like activity). Glucose failed to stimulate insulin release from RINm5F cells, which only displayed a single low-K_m component of glucose utilization. Only low-K_m (hexokinase-like) glucose-phosphorylating activity was found for disrupted RINm5F cells. These changes in glucose metabolism may contribute towards the failure of glucose to stimulate insulin release from RINm5F cells.

Although the mechanism by which glucose stimulates insulin release is not completely understood, most, but not all (Weaver *et al.*, 1978), evidence suggests that glucose metabolism is necessary for initiation of glucose-stimulated insulin release (Hedeskov, 1980). The findings that the trioses glyceraldehyde and dihydroxyacetone can stimulate insulin release (Hellman *et al.*, 1974) and that inhibition of glucose phosphorylation by mannoheptulose also inhibits insulin release (Halban *et al.*, 1980) lend support to such an involvement of glucose metabolism.

The insulin-producing rat cell line (RINm5F) (Bhathena *et al.*, 1982) has been found to respond to a variety of agents with appropriate changes in rates of insulin release (Praz *et al.*, 1983). It was, however, found that RINm5F cells showed only poor sensitivity to glucose despite pronounced sensitivity to glyceraldehyde (Praz *et al.*, 1983). These previous observations led us to suspect that RINm5F cells may display unusual properties of glucose metabolism compared with normal B-cells. The present study was thus aimed at a preliminary characterization of glucose utilization and phosphorylation by RINm5F cells.

Methods

Glucose utilization

Glucose utilization was measured by following the conversion of [³H]glucose into ^3H_2O (Ashcroft *et al.*, 1972) by using the methodology and controls described previously (Halban *et al.*, 1980).

(*a*) *RINm5F cells.* These were cultured in RPMI 1640 medium in multi-well test plates (type 3072;

Falcon, Oxnard, CA, U.S.A.; well diameter 6 mm) as described previously (Praz *et al.*, 1983). The cells (approx. 10^6 cells/well) were washed three times with a modified Krebs–Ringer bicarbonate buffer (Halban *et al.*, 1980) containing 10 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] and 2.5 mg of bovine serum albumin/ml (KRB/Hepes). To study glucose utilization, 0.2 ml of KRB/Hepes containing 7 μCi of [5-³H]glucose (New England Nuclear, Dreieich, West Germany)/ml, radioisotopically diluted to the desired total glucose concentration, was added to each well. After 1 h at 37°C, 0.1 ml of incubation buffer was taken to measure ^3H_2O production, and an additional 50 μl was taken for measurement of immunoreactive-insulin release after appropriate dilution. The cells were then washed three times in KRB/Hepes without bovine serum albumin, and finally 0.2 ml of 1 M-NaOH was added to each well to solubilize the cells for determination of cellular protein.

(*b*) *Isolated islets.* Islets were isolated from adult male Wistar rats and subsequently maintained in tissue culture for 3 days as described previously (Halban *et al.*, 1980), by using RPMI 1640 medium with the same supplements as for RINm5F cell culture (Praz *et al.*, 1983). After washing three times with KRB/Hepes, the cultured islets were transferred to multi-well test plates (ten islets/well) as for RINm5F cells. Then 0.1 ml of KRB/Hepes containing 43 μCi of [5-³H]glucose/ml radioisotopically diluted to the appropriate glucose concentration was added to each well. After 1 h at 37°C, 50 μl samples were taken for ^3H_2O equilibration and 25 μl samples for radioimmunoassay. Cultured islets from the same batch were transferred in parallel to 1 M-NaOH

(ten islets/0.1 ml) after washing in KRB/Hepes without bovine serum albumin for determination of total islet protein.

Glucose phosphorylation

The method of Trus et al. (1981) was used. RINm5F cells were suspended in buffer (Trus et al., 1981) at a density of 7.3×10^6 cells/ml and sonicated (Branson B-12 Sonifier, setting 3, 50 W, 3×10 s, on ice) and the sonicated cells were than kept on ice. Production of glucose 6-phosphate was assayed by following NADH production fluorimetrically by addition of glucose 6-phosphate dehydrogenase (from *Leuconostoc mesenteroides*; Sigma, St. Louis, MO, U.S.A.). For this assay, 2 ml of assay buffer (50 mM-Hepes, 100 mM-KCl, 7.2 mM-MgCl$_2$, 10 mM-NAD$^+$, 10 mM-β-mercaptoethanol, 0.5 mg of bovine serum albumin/ml, 5 mM-ATP and 0–100 mM-glucose, pH 7.7) was dispensed into glass cuvettes. The cuvettes were placed in a heated holder in a fluorimeter (Perkin–Elmer LS-3) and allowed to reach the reaction temperature (30–31 °C), and 40 μl of sonicated cells was then added. The coupled, secondary, reaction was started by the addition of 10 μl of glucose 6-phosphate dehydrogenase (final concn. in the cuvette 0.8 unit/ml), and the increase in fluorescence (340 nm excitation, 460 nm emission) was followed with time during the steady state (which was attained within 3 min and maintained for at least 30 min). The system was calibrated for quantitative determination of glucose phosphorylation by using NADH and glucose 6-phosphate standards (Trus et al., 1981). Control blanks (Trus et al., 1981) yielded apparent rates of NADH production that were less than 5% of the rate observed at the lowest glucose concentration studied in the presence of sonicated cells (0.01 mM), and rates of NADH reoxidation in the presence of sonicated cells were negligible.

Immunoreactive-insulin release and total cellular protein

Immunoreactive insulin was measured by a standard radioimmunoassay (Halban et al., 1980; Praz et al., 1983).

Total cellular protein of RINm5F cells and islets dissolved in NaOH was determined by the Coomassie Blue method with a commercially available kit (Bio-Rad Laboratories A.G., Glattbrugg, Switzerland), with human serum albumin as the standard.

Presentation of data

All data are presented as means ± S.E.M.

Results

Glucose utilization and immunoreactive-insulin release

Glucose utilization and insulin release were studied in parallel. For islets, glucose stimulated insulin release by up to 19-fold (Table 1), with half-maximal stimulation at 6–7 mM-glucose. Glucose utilization by islets (Fig. 1) showed a small increase in the range 0.1–1 mM-glucose, with a marked stimulation at higher glucose concentrations. A double-reciprocal plot ($1/v$ versus $1/s$) for these data could be resolved into two linear components: (1) ($r = 0.994$, $P < 0.001$ by linear regression analysis), apparent $K_m = 0.4 \pm 0.04$ mM (range 0.26–0.53), $V_{max.} = 0.03 \pm 0.004$ nmol of glucose/h per μg of protein (range 0.018–0.043); (2) ($r = 0.995$, $P < 0.001$), apparent $K_m = 9.3 \pm 2.3$ mM (range 2.3–17.5), $V_{max.} = 0.18 \pm 0.04$ nmol of glucose/h per μg of protein (range 0.07–0.34). These data are means ± S.E.M. for six independent experiments.

For RINm5F cells, glucose failed to stimulate immunoreactive-insulin release (Table 1), but the cells remained sensitive to glyceraldehyde, as shown in a separate series of experiments [immunoreactive-insulin release being 0.17 ± 0.01 and 0.38 ± 0.02 ng/h per μg of protein ($n = 10$) at 2.8 mM-glucose alone and at 2.8 mM-glucose plus 16.7 mM-DL-glyceraldehyde respectively].

RINm5F cells were, however, able to metabolize glucose actively, but with kinetics quite different from

Table 1. *Effects of glucose on release of immunoreactive insulin from isolated islets and RINm5F cells*
Isolated islets (after maintenance for 3 days in culture) or RINm5F cells were incubated for 1 h at 37 °C in KRB/Hepes at the given glucose concentration. The mean protein content for islets was 3.9 μg/ten islets per well and for RINm5F cells 13 μg/ten islets per well. The results are expressed as means ± S.E.M. for multiple observations (total numbers of observations in parentheses) taken from six and four independent experiments for islets and RINm5F cells respectively.

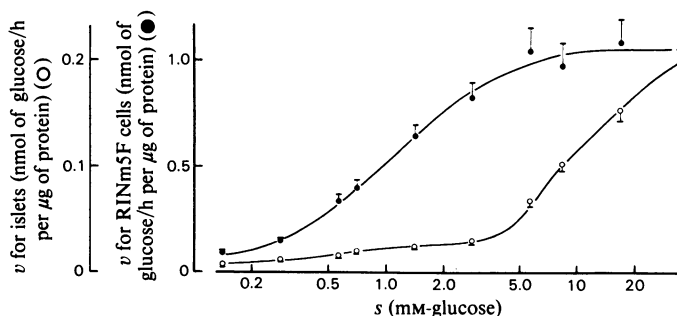| Glucose (mM)	Immunoreactive-insulin release (ng/h per μg of protein)	
	Isolated islets	RINm5F cells
0.14	0.42 ± 0.05 (20)	0.16 ± 0.02 (12)
0.28	0.43 ± 0.04 (24)	0.15 ± 0.02 (16)
0.56	0.38 ± 0.03 (23)	0.16 ± 0.01 (16)
0.70	0.54 ± 0.07 (23)	0.17 ± 0.02 (15)
1.4	0.42 ± 0.06 (20)	0.18 ± 0.02 (16)
2.8	0.48 ± 0.10 (23)	0.17 ± 0.01 (16)
5.6	1.12 ± 0.09 (24)	0.18 ± 0.02 (16)
8.3	5.53 ± 0.38 (24)	0.17 ± 0.02 (16)
16.7	8.03 ± 0.42 (24)	0.20 ± 0.02 (16)
33.4	7.38 ± 0.76 (8)	0.17 ± 0.01 (8)

Fig. 1. *Glucose utilization by isolated islets and RINm5F cells*

Glucose utilization was measured by following the conversion of $[5\text{-}^3H]$glucose into ^3H_2O. Isolated islets (after maintenance for 3 days in culture) or RINm5F cells were incubated, and the data are presented, as described in the legend to Table 1. The numbers of observations per data point were as indicated in Table 1. v is the rate of glucose utilization and s the glucose concentration present during the incubation. Note the different scales for the two preparations.
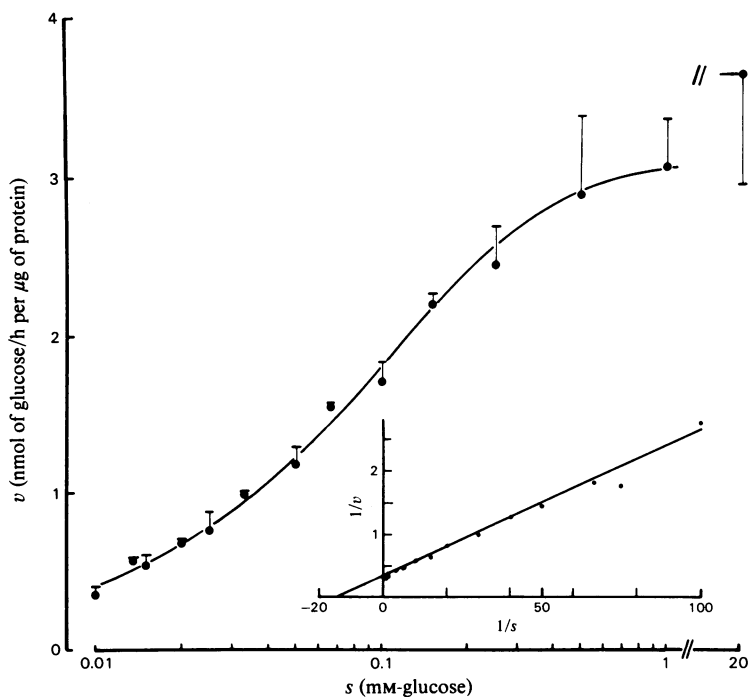

Fig. 2. *Glucose phosphorylation by sonicated RINm5F cells*

Glucose phosphorylation was measured by a fluorimetric method using glucose 6-phosphate dehydrogenase as a secondary exogenous enzyme for coupled production of NADH from glucose 6-phosphate generated by the sonicated cells during the incubation. The data are presented as means ± S.E.M. for four independent experiments. v is the rate of glucose phosphorylation at steady state, and s is the glucose concentration present during the incubation. The inset shows a double-reciprocal plot ($1/v$ versus $1/s$) of the data points.

those for islets (Fig. 1). The rate of glucose utilization by RINm5F cells was higher than that of islets at all glucose concentrations (note the different scales in Fig. 1), and a double-reciprocal plot ($1/v$ versus $1/s$) revealed only a single linear component ($r = 0.993$, $P < 0.001$) of glucose utilization, with an apparent K_m of 2.1 ± 0.4 mM-glucose (range 1.1–2.7) and a $V_{max.}$ of 1.5 ± 0.3 nmol/h per μg of protein (range 1.0–2.4) for four independent experiments.

Interpretation of the derived kinetic constants for glucose utilization by intact cells must, however, be subject to extreme caution, since glucose utilization reflects the activity of a number of interrelated metabolic pathways.

Glucose phosphorylation by sonicated RINm5F cells

RINm5F cells displayed a very active glucose-phosphorylation capacity (Fig. 2), which appeared highly sensitive to low concentrations of glucose. No statistically significant increase in the rate of phosphorylation was observed between 1 and 20 mM-glucose (Fig. 2), and in additional experiments no further increase was observed even when the glucose concentration was raised to 100 mM (results not shown). Re-expression of these data as a double-reciprocal plot ($1/v$ versus $1/s$; Fig. 2, insert) showed a straight line ($r = 0.991$, $P < 0.001$ by linear regression analysis), indicating an apparent K_m of 0.08 ± 0.01 mM-glucose (range 0.06–0.15) and a $V_{max.}$ of 3.2 ± 0.1 nmol/h per μg of protein (range 2.7–3.5) for four independent experiments.

Discussion

Glucose phosphorylation is thought to be the primary event in glucose recognition by pancreatic B-cells. Although islet cells have been shown to contain enzymes with characteristics typical of both hexokinase and glucokinase (Trus et al., 1981; Meglasson et al., 1983), glucokinase is taken to be the enzyme playing the key regulatory rôle in glucose-stimulated insulin release (Trus et al., 1981; Meglasson et al., 1983; Matschinsky & Ellerman, 1968). As a direct extension of these findings, it is logical to expect that, under conditions where B-cell glucokinase activity is attenuated (Trueheart-Burch et al., 1981) or even absent, the ability of glucose to stimulate insulin release may be equally affected.

In the present study, glucose utilization and phosphorylation by RINm5F cells have been investigated. This cell line was established in culture following heterotransplant in nude mice (Bhathena et al., 1982; Gazdar et al., 1980) of an X-ray-induced transplantable rat islet-cell tumour (Chick et al., 1977). We have previously observed a small stimulatory effect of glucose on immunoreactive-insulin release from RINm5F cells (Praz et al., 1983), an effect not reproduced in this present study. The complete absence of glucose-stimulated insulin release may reflect changes in the functional characteristics of RINm5F cells after prolonged periods in tissue culture.

Glucose utilization by intact RINm5F cells was defined by only a single component, with an apparent K_m of 2.1 mM-glucose. This relatively high value (compared with that observed for glucose phosphorylation by disrupted cells) could reflect rate-limiting steps in glucose metabolism distal to phosphorylation and/or a rate-limiting, and glucose-sensitive, hexose-transport system. Although glucose transport in islet cells has been shown to be rapid, with a high capacity (Hellman et al., 1971), it cannot be excluded that RINm5F cells differ from islet cells in this respect.

By using a similar protocol, others have shown that disrupted islets display two distinct glucose-phosphorylating systems (Trus et al., 1981; Meglasson et al., 1983). RINm5F cells, by contrast, show only one system, with low K_m, high affinity and high capacity. There was no evidence for a high-K_m, low-affinity, glucokinase-like system. Previous reports have indicated a $V_{max.}$ for glucose phosphorylation by the low-K_m (hexokinase-like) system in disrupted islet cells in the range of 12 mol of glucose/h per kg of DNA at 37°C (Trus et al., 1981), which, assuming a Q_{10} of 2 (Trus et al., 1981), approximates to 0.4 nmol of glucose/h per μg of protein at 31°C, with an apparent K_m of 0.03–0.05 mM-glucose. For RINm5F cells, the apparent K_m of 0.08 mM-glucose is thus comparable with that of the equivalent islet enzyme system. However, the $V_{max.}$ for RINm5F-cell hexokinase-like activity was 3.2 nmol/h per μg of protein (the calculations in the present paper being based on activity at 31°C), which is approx. 8 times that for islets. It seems reasonable to suggest that this difference is in major part responsible for the elevated rates of glucose utilization by intact RINm5F cells.

Although the finding of no further increase in glucose utilization by RINm5F cells over the range of glucose concentrations shown to stimulate islet insulin release, taken together with the failure of glucose to stimulate RINm5F-cell insulin release, may reflect glucokinase deficiency, it is premature to conclude that lack of this enzyme activity itself is the sole factor responsible. It could thus be suggested that elevated rates of glucose phosphorylation owing to the highly active RINm5F-cell hexokinase-like system may result in constitutively elevated amounts of key intermediates required for triggering insulin release, and, indeed, RINm5F cells at low glucose concentrations display characteristics typical of normal B-cells under stimulatory conditions (Praz et al., 1983). However, since these cells retain sensitivity towards glyceraldehyde, albeit somewhat attenuated compared with islets (Hedeskov, 1980; Hellman et al., 1974), it appears that changes in triose phosphate metabolism can still be evoked as in islets (Hedeskov, 1980; Ashcroft, 1980), despite the dramatically elevated rates of glucose phosphorylation. A comparison of the concentrations of glycolytic intermediates in RINm5F cells and normal B-cells may allow for identification of the factors normally responsible for glucose-stimulated insulin release.

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