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Characterization of glucose transport in an insulin-secreting cell line

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The rat insulinoma-derived RINm5F cell line retains many differentiated functions of islet β-cells. However, it fails to recognize glucose as an insulin secretagogue in the physiological concentration range. With this cell line, glucose-transport kinetics were investigated, by using a double-label technique with the non-metabolizable glucose analogue 3-O-methylglucose (OMG). RINm5F cells possess a passive glucose-transport system with high capacity and low affinity. Equilibration across the plasma membrane of extracellular OMG concentrations up to at least 20 mM is achieved within 2 min at 37 °C. The half-saturation of OMG uptake occurs at 32 mM. At lower temperatures OMG uptake is markedly retarded, with a temperature coefficient (Q_{10}) of 2.9. As indicated by efflux measurements, transport is symmetrical. Cytochalasin B at micromolar concentrations and phlorrhizin in millimolar concentrations are potent inhibitors of OMG uptake. Neutralization of the secreted insulin with antibodies does not alter OMG uptake kinetics. The glucose metabolism of RINm5F cells is much exaggerated compared with that of islet β-cells. Nonetheless, when measured in parallel to uptake, transport exceeds by far the rate of metabolism at glucose concentrations above 3 mM. Measurements of intracellular D-glucose reveal a lower intracellular glucose concentration relative to the extracellular in RINm5F cells. This seems to be due to abnormalities in the subsequent steps of glucose metabolism, rather than to abnormalities in hexose uptake. The loss of glucose-induced insulin release in RINm5F cells cannot be explained by alterations in hexose transport.

INTRODUCTION

Insulin-secreting cell lines are a convenient model for studies of hormone release mechanisms. One of these, the RINm5F cell line, is derived from a rat insulinoma (Gazdar *et al.*, 1980). Although RINm5F cells have only about 1% of the insulin content of native rat β-cells, they have retained over more than 5 years in culture many differentiated functions of pancreatic β-cells (Praz *et al.*, 1983; Wollheim & Pozzan, 1984; Dunne *et al.*, 1986). To validate the model system, it is necessary to characterize fully various biochemical functions, in particular to investigate possible differences from the normal β-cell. Whereas RINm5F cells have been demonstrated to respond to a variety of insulin secretagogues, the response to the main physiological stimulus, D-glucose, when detectable, is limited and variable (Praz *et al.*, 1983; Halban *et al.*, 1983; Gylfe *et al.*, 1983; Giroix *et al.*, 1985). These findings are associated with an increased glucose metabolism. In contrast with the sigmoidal dose relationship in islets, glucose utilization in RINm5F cells is hyperbolic and reaches maximal rates already at glucose concentrations of 3–5 mM (Halban *et al.*, 1983; Gylfe *et al.*, 1983). It is generally accepted that glucose has to be metabolized by the pancreatic β-cells to generate the signal for insulin release (Hedeskov, 1980; Sener & Malaisse, 1984; Meglasson & Matschinsky, 1986). Consequently, glucose uptake is a prerequisite for normal moment-to-moment detection of changes in extracellular glucose concentrations. This necessitates an efficient glucose-transport system to allow rapid adaptation of insulin-secretion rates for the fine tuning of blood glucose concentration.

In the early 1970s the existence of a high-capacity glucose-transport system was shown in mouse islets incubated at low temperatures (Hellman *et al.*, 1971). Intracellular and extracellular glucose concentrations equilibrate rapidly, since the rate of glucose transport exceeds, even at 8 °C, several-fold the rate of metabolism measured at 37 °C (Sehlin, 1981). Since the half-saturation of transport occurs clearly above physiological blood glucose concentrations, at about 50 mM-glucose in mouse islets (Hellman *et al.*, 1971), or at 75–100 mM in rat islets (McDaniel *et al.*, 1977), it was proposed that glucose transport has no regulatory function in islets.

Nevertheless, at physiological temperatures no full characterization of islet-cell glucose-transport kinetics has been performed, apart from studies giving just time courses of uptake in islets (Hellman *et al.*, 1974; Henquin & Lambert, 1976) and isolated islet cells (Lernmark *et al.*, 1975; Gorus *et al.*, 1984). This is mainly due to the difficulty of obtaining sufficient cell numbers from isolated islets. A second problem, the interference of glucose metabolism with transport measurements, can be circumvented by using non-metabolizable glucose analogues such as 3-O-methylglucose (OMG). This hexose is transported like glucose, but is not phosphorylated intracellularly or further metabolized (Hellman *et al.*, 1973; Sehlin, 1981; Gliemann & Rees, 1983).

The aim of the present study was to characterize glucose transport in RINm5F cells, especially at physiological temperatures. We wanted to investigate, in addition, whether an abnormal glucose uptake could be responsible for the loss of glucose-induced insulin secretion. To this end, the rates of glucose transport and metabolism were compared to exclude the possibility that glucose transport is rate-limiting for its metabolism.

Abbreviation used: OMG, 3-O-methylglucose.

METHODS

Cell culture

RINm5F cells were grown in plastic culture bottles in RPMI 1640 medium supplemented with 10% (v/v) fetal-calf serum, 100 i.u. of penicillin/ml and 0.1 mg of streptomycin/ml as described by Praz *et al.* (1983). On the morning of the day of the experiment, cells were detached with 0.025% trypsin, and isolated cells were allowed to recover in a 3 h spinner culture (Wollheim & Pozzan, 1984). Then cells were washed and resuspended in glucose-free modified Krebs–Ringer buffer containing 25 mM-Hepes, 5 mM-$NaHCO_3$ and 0.7 mg of bovine serum albumin/ml (basal medium; Wollheim & Pozzan, 1984). For the incubations, 10^5 cells/tube were employed.

Glucose uptake

Glucose uptake was measured by a method similar to that described for ^{45}Ca and inositol uptake (Wollheim *et al.*, 1977; Biden & Wollheim, 1986). In brief, 50 μl samples of the cell suspension were preincubated at 37 °C for 30 min in micro-centrifuge tubes on top of an oil layer [dibutyl phthalate/dinonyl phthalate (7:3, v/v) $\rho = 1.03$ g/ml] in glucose-free medium. The tips of the tubes contained 20 μl of 6 M-urea. Incubation was started by addition of 50 μl of prewarmed basal medium containing the non-labelled OMG, additions and 1.5 μCi of D-3-*O*-[^{3}H]methylglucose ([^{3}H]OMG) (or ^3H_2O) and 0.5 μCi of L-[1-^{14}C]glucose. Unless otherwise stated, all incubations were carried out at 37 °C. D-[U-^{14}C]Glucose was used for a control experiment at 7 °C with L-[^{3}H]glucose as extracellular marker. To terminate uptake, cells were separated from medium by rapid centrifugation through the oil layer in a micro-centrifuge (Greiner, Langenthal, Switzerland; 8000 g for 30 s). Radioactivity in the cut tips of the micro-centrifuge tubes was measured by liquid-scintillation counting, after addition of 5 ml of Hydroluma scintillant.

Uptake was calculated from the ^{3}H radioactivity by subtracting that originating from extracellular medium (estimated from the L-glucose space) after correction for channel crossover. The total intracellular volume was determined in each experiment by the water space in excess of the extracellular space.

The method used measured net uptake, consisting of uptake and an efflux component which was augmented with increasing intracellular OMG concentration, because the non-metabolizable OMG accumulates intracellularly. Since uptake was predominant and linear for the first 1 min, initial transport rates were estimated from 1 min incubations, which was a compromise between accuracy of the measurements and true unidirectional uptake. Therefore it should be taken into account that these measurements might underestimate the true rates.

Glucose efflux

After 30 min preincubation in glucose-free basal medium, about 50 × 10^6 cells were labelled for 30 min at 37 °C in 5 ml of medium containing 2 mM-OMG and 150 μCi of [^{3}H]OMG. L-[^{14}C]Glucose was used as extracellular marker. After rapid centrifugation (2 min at 150 g) of 1 ml samples containing about 10^7 cells, the pellet was resuspended in 3 ml of prewarmed basal medium without sugar and radioactivity. To terminate efflux, cells were separated from medium by centrifugation through oil as described for 'Glucose uptake'.

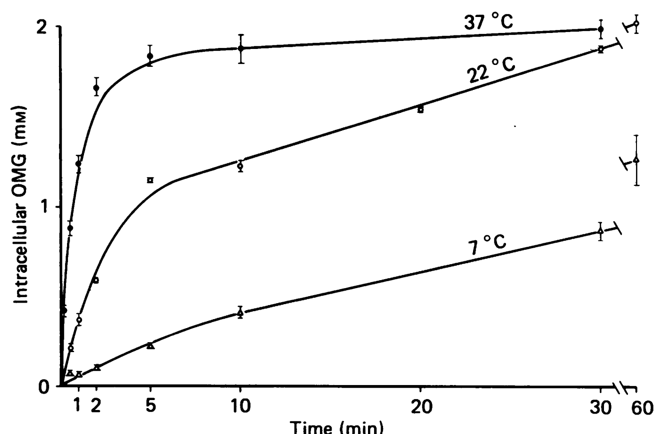

Fig. 1. Time courses of OMG uptake at different temperatures

RINm5F cells were incubated in the presence of 2 mM-OMG, 1.5 μCi of D-[^{3}H]OMG and 0.5 μCi of L-[1-^{14}C]glucose at 37, 22 and 7 °C. Uptake was terminated by centrifugation of the cells through an oil layer to separate cells from medium. Each point represents the mean ± S.E.M. for 16 observations at 37 °C and for eight observations for the lower temperatures.

Equilibrium exchange

To investigate glucose transport at zero net flux conditions, cells were preincubated in the presence of the same concentration of unlabelled hexose as that used for the incubation in uptake measurements. Efflux was measured against a medium containing the same concentration of unlabelled hexose as used for labelling the cells. Otherwise experiments were performed as described for 'Glucose uptake' and 'Glucose efflux'.

Glucose metabolism

Glucose utilization was measured as the conversion of D-[5-^{3}H]glucose into ^3H_2O (Ashcroft *et al.*, 1972), with the modifications described by Halban *et al.* (1980). Samples of 10^5 cells/vial were incubated in inner wells suspended in glass scintillation vials for 1 h in 100 μl of the usual Krebs–Ringer buffer containing 2 μCi of D-[5-^{3}H]glucose, radioisotopically diluted to yield appropriate final glucose concentrations. Metabolism was arrested by injection of 50 μl of 0.3 M-HCl. After overnight equilibration of the formed ^3H_2O with the 0.5 ml of water in the counting vials, the inner wells were removed and radioactivity was measured by liquid-scintillation counting with 10 ml of Hydroluma scintillant. Appropriate standards were used to prove that 90–95% of the theoretically expected radioactivity was recovered in the water. All incubations were carried out in parallel with OMG uptake experiments from the same cell preparation.

Intracellular D-glucose concentration

Approx. 10^7 cells/ml were incubated in medium containing 25 mM-D-glucose for 30 min at 37 °C under continuous stirring. At the end of the incubation, cells (100 μl samples) were separated from the medium by centrifugation through an oil layer as described under 'Glucose uptake', except that the micro-centrifuge tubes contained 40 μl of 0.5 M-$HClO_4$ in the tip instead of 20 μl of urea. The tips were cut below the oil layer, to avoid interference of oil with the fluorimetric analysis, and

20 μl of the tip solution was diluted into 2.2 ml of water. Simultaneously, the glucose content of 1 μl of supernatant from the incubation medium of the corresponding tube was determined by the same method.

Then 0.9 ml of each sample was added to assay buffer, containing 100 mM-Tris, 2 mM-$MgCl_2$, 1 mM-dithiothreitol, 0.6 mM-Na_2ATP, 0.2 mM-$NADP^+$, 0.04 unit of yeast glucose-6-phosphate dehydrogenase/ml and 0.28 unit of yeast hexokinase/ml, pH adjusted to 8.2 with HCl (Lowry & Passonneau, 1972). A second sample was always measured with assay buffer containing no hexokinase to correct for endogenous fluorescence. After 20 min at room temperature, fluorescence was determined in a Perkin–Elmer LS 3 spectrofluorimeter at an excitation wavelength of 340 nm and an emission wavelength of 460 nm. Standards (1 μl) were prepared from media containing 0, 5, 10, 15, 20 or 25 mM-D-glucose.

All results were corrected for extracellular contamination as judged from the L-[^{14}C]glucose distribution space and related to the ^3H_2O space determined in parallel in each cell preparation.

Statistics

All results are expressed as means ± S.E.M. Statistical analysis was performed by Student's t test for unpaired data.

Materials

Culture medium RPMI 1640 was purchased from Amimed (Basel, Switzerland) and fetal-calf serum from Gibco (Grand Island, NY, U.S.A.). Radiochemicals were obtained from New England Nuclear (Dreieich, Germany). OMG, cytochalasin B and yeast hexokinase were bought from Sigma (St. Louis, MO, U.S.A.), and phlorrhizin was from Fluka (Buchs, Switzerland). Dinonyl phthalate and dibutyl phthalate were from Merck (Darmstadt, Germany). Glucose-6-phosphate dehydrogenase, Na_2ATP, $NADP^+$ and dithiothreitol were obtained from Boehringer Mannheim (Mannheim, Germany). Guinea-pig anti-insulin serum 860-P was a gift from Dr. R. Gingerich (Linco Research, St. Louis, MO, U.S.A.). Hydroluma scintillant was bought from Fakola (Basel, Switzerland). All other chemicals used were of analytical grade.

RESULTS

The total intracellular water space of RINm5F cells was 90.2 ± 2.6 nl/10^5 cells (n = 56). The extracellular fluid trapped in the cell pellet after centrifugation was determined by using the L-glucose distribution space, which was 19.3 ± 1.8 nl (n = 19) after 10 s and 23.7 ± 2.1 nl (n = 17) after 30 min of incubation. This confirms the restriction of L-glucose to the extracellular space.

At 37 °C OMG was rapidly taken up by RINm5F cells. At concentrations of 2 mM-OMG in the medium, the sugar equilibrated across the plasma membrane within 2 min (Fig. 1). Identical results were obtained with 20 mM-OMG (results not shown). Even after 30 min incubation, the intracellular OMG concentration was never significantly higher than the extracellular concentration.

At lower temperatures, transport rates were markedly decreased. At 22 °C the intracellular OMG concentration equalled that in the medium only after about 30 min (Fig. 1). Finally, at 7 °C the intracellular hexose concentration was still increasing even after 1 h of incubation, and had reached only about 60% of the extracellular concentration (Fig. 1). The control experiment measuring the uptake of D-glucose was also performed at 7 °C to exclude an important influence of metabolism. The time course obtained for D-glucose uptake in RINm5F cells was identical with that for the OMG uptake at the same temperature (results not shown). Calculation of the initial transport rates from the 1 min value gave 1.1 ± 0.02 nmol/min per 10^6 cells at 37 °C (n = 16), 0.29 ± 0.04 nmol/min per 10^6 cells at 22 °C (n = 8) and 0.045 ± 0.01 nmol/min per 10^6 cells at 7 °C (n = 8). These data yielded a temperature coefficient (Q_{10}) of 2.9.

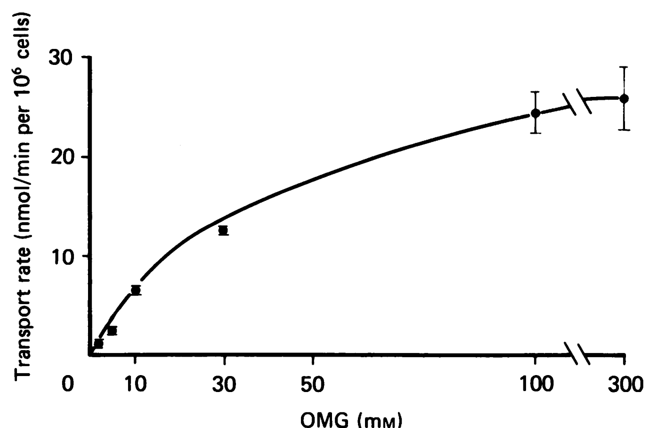

Fig. 2. Concentration-dependency of OMG uptake

RINm5F cells were incubated at 37 °C for 1 min with the respective OMG concentration as described for Fig. 1. The maximal transport rate was about 26 nmol/min per 10^6 cells, and the half-saturation of transport occurred at 32 mM-OMG. Results from experiments with OMG concentrations of 0.01, 0.03, 0.1 and 0.3 mM are not shown. Each point represents the mean ± S.E.M. for 12–16 observations.

The concentration-dependency of OMG uptake is shown in Fig. 2. Eadie–Hofstee analysis of the concentration-dependency revealed a maximal transport rate of about 26 nmol/min per 10^6 cells and a half-saturation of OMG transport at around 32 mM.

Inhibitors of hexose uptake were tested during 1 min incubations in the presence of 2 mM-OMG (Table 1). The mould metabolite cytochalasin B inhibited OMG uptake in a dose-dependent manner at concentrations of 1–30 μM, by 40–80% respectively. The onset of cytochalasin B inhibition was immediate, since 30 μM of the drug present already during the preincubation did not lead to a more pronounced inhibition. Phlorrhizin decreased the transport rate by 50% at 1 mM, and abolished uptake nearly completely at 10 mM. The solvent used, dimethyl sulphoxide (less than 3%), had no influence on uptake.

Next we investigated whether the hexose carrier of RINm5F cells belongs to the insulin-sensitive type of carriers present in adipocytes and myocytes or whether it is more similar to the liver type, which is insulin-independent (Carruthers, 1984). Therefore uptake was studied in the presence of anti-insulin antibodies to neutralize the secreted insulin during the experiment; 10 μl of anti-insulin serum was added to the cell suspension at the beginning of the preincubation. Its concentration was chosen so that 20 ng of insulin was

Table 1. Effect of cytochalasin B and phlorrhizin on OMG uptake

RINm5F cells were incubated for 1 min at 37 °C in the presence of 2 mM-OMG and of inhibitors as described in the Methods section for uptake. In one experiment cytochalasin B was also present during the preincubation period (30 min). The solvent used, dimethyl sulphoxide, did not influence uptake. Results are given as means ± S.E.M. for *n* experiments; n.s., not significant.

Addition	Transport rate (nmol/min per 10^6 cells)	*n*	Inhibition (%)	Significance versus control
Control	0.73 ± 0.06	8		
1 μM-cytochalasin B	0.49 ± 0.05	8	33	$P < 0.02$
10 μM-cytochalasin B	0.21 ± 0.03	8	71	$P < 0.001$
30 μM-cytochalasin B	0.11 ± 0.02	8	84	$P < 0.001$
30 μM-cytochalasin B present during preincubation	0.06 ± 0.02	4	91	$P < 0.001$ (n.s. versus 30 μM)
1 mM-phlorrhizin	0.33 ± 0.02	8	55	$P < 0.001$
10 mM-phlorrhizin	0.03 ± 0.01	8	96	$P < 0.001$
Control + solvent	0.68 ± 0.04	4		n.s.

Table 2. Effect of insulin antibodies on OMG uptake

RINm5F cells were incubated for 1 min at 37 °C in the presence of indicated concentrations of OMG as described in the Methods section for uptake. Anti-insulin serum was added after washing the cells at the beginning of the preincubation to neutralize secreted insulin. Each result represents the mean ± S.E.M. for eight observations: n.s., not significant.

OMG concn. (mM)	Transport rate (nmol/min per 10^6 cells)		Significance
	Anti-insulin serum present	Without antiserum	
10	5.9 ± 0.75	6.6 ± 1.3	n.s.
30	8.9 ± 1.6	10.3 ± 1.3	n.s.
100	24.0 ± 6.3	20.8 ± 3.8	n.s.

neutralized; this is more than 8 times the amount of insulin secreted during the experiment. At the tested OMG concentrations of 10, 30 and 100 mM, the transport rates were not different from those determined in parallel without anti-insulin antibodies present (Table 2).

When efflux of OMG from preloaded cells into sugar-free medium was measured, the intracellular OMG concentration was already 20% of the initial concentration after 1 min (Fig. 3). After 2 min virtually no intracellular OMG was detectable.

To investigate whether the loaded and the unloaded transporter have a different mobility through the plasma membrane, equilibrium exchange experiments were performed. For both uptake and efflux conditions, no difference could be seen in the time course at zero net flux with 2 mM-OMG, compared with the unidirectional flux conditions illustrated in Figs. 1 and 3 respectively (results not shown).

In some experiments in which the concentration-dependency of glucose uptake was measured, the glucose utilization was determined in parallel, as shown in Fig. 4. The maximal rate of glucose utilization was about 1.1 nmol/min per 10^6 cells. Already at 5 mM-glucose the cells displayed maximal metabolism. The half-maximal utilization occurred at around 1.3 mM. Comparison of the rates of OMG uptake (Fig. 2) with those of glucose utilization (Fig. 4) demonstrates that glucose uptake exceeds the rate of utilization at concentrations above 3 mM. At higher glucose concentration, that of intracellular D-glucose would therefore be expected to approach the extracellular concentration. To examine this, we measured intracellular D-glucose after 30 min of incubation at 37 °C in medium containing 25 mM-D-glucose. The intracellular glucose concentration was 11.1 ± 1.7 mM ($n = 12$), compared with that in the medium, 23.3 ± 0.3 mM ($n = 11$) at the end of the incubation.

DISCUSSION

The insulin-producing RINm5F cells possess an efficient hexose-transport system, which at 37 °C allows equilibration of OMG at concentrations of 2–20 mM

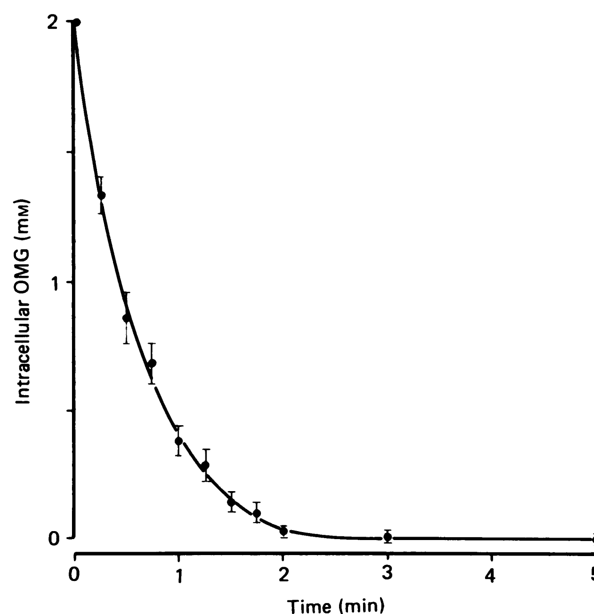

Fig. 3. Time course of OMG efflux at 37 °C

Efflux of OMG was measured at 37 °C from cells loaded with 2 mM-OMG as described in the Methods section. Incubations were terminated by separating the cells from the medium by centrifugation through an oil layer. Each point represents the mean ± S.E.M. for six observations.

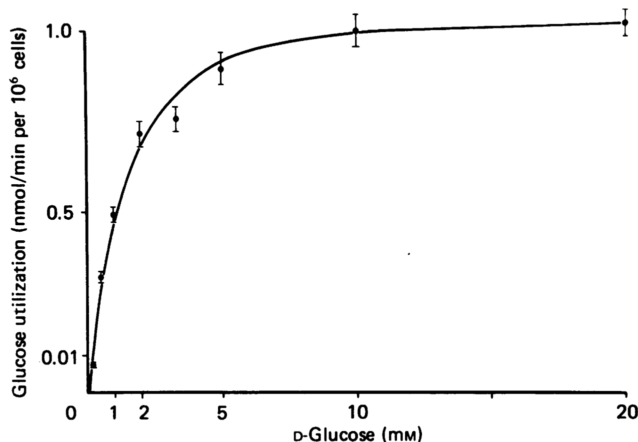

Fig. 4. Concentration-dependency of D-glucose metabolism

Glucose utilization was measured by monitoring the conversion of D-[5-^{3}H]glucose into ^3H_2O. RINm5F cells were incubated for 1 h at 37 °C in the presence of 2 μCi of D-[5-^{3}H]glucose radioisotopically diluted to the desired glucose concentration. The maximal rate of metabolism was 1.1 nmol/min per 10^6 cells, and the half-maximal rate occurred at 1.3 mM-glucose. Each point represents the mean ± S.E.M. for 12 observations.

across the plasma membrane within 2 min. OMG is transported by facilitated diffusion, since the characteristics for active transport, the transport against a concentration gradient, could not be seen in insulin-producing cells. Active hexose transport is only found in the intestinal brush border and in the kidney, where glucose is also co-transported with Na^+ (Stevens *et al.*, 1984; Silverman, 1976). On the other hand, in all tissues studied D-glucose uptake proceeds much faster than would be expected for simple diffusion (Carruthers, 1984). Moreover, uptake is stereospecific for D-glucose (Hellman *et al.*, 1971; Sehlin, 1981; Carruthers, 1984). Therefore L-glucose could be used as a marker of the extracellular space. The time course of OMG uptake in RINm5F cells at 37 °C is virtually identical with results from islets (Hellman *et al.*, 1974; Henquin & Lambert, 1976), dispersed islet cells (Lernmark *et al.*, 1975) and dispersed purified β-cells (Gorus *et al.*, 1984).

The half-saturation of OMG transport in RINm5F cells is above 30 mM. This observation compares well with previous reports describing K_m values of 50 mM- and 75–100 mM-glucose for mouse and rat islets respectively (Hellman *et al.*, 1971; McDaniel *et al.*, 1977). Our findings disagree with the statement of Malaisse *et al.* (1986) suggesting a K_m of 2 mM for glucose uptake in RINm5F cells at 7 °C.

No temperature coefficient for glucose transport in insulin-secreting cells has been reported so far; our results indicate a Q_{10} of 2.9. This observation coincides with the value estimated for erythrocytes (Sen & Widdas, 1962). Previous investigations in islets have only been performed at low temperatures. A $V_{max.}$ for glucose transport of 400 mmol/h per kg dry wt. of islets was observed at 8 °C in *ob/ob*-mouse islets (Hellman *et al.*, 1971). By using the Q_{10} of 2.9, this value can be approximated to a $V_{max.}$ of 54 nmol/min per 10^6 cells (it was estimated that one rat islet = 1 μg dry wt., one *ob/ob*-mouse islet = 5 μg, and one rat islet = 3000 cells). In rat islets incubated at 20 °C a $V_{max.}$ of 200 pmol/45 s per islet can be estimated from a Lineweaver–Burk plot (see Fig. 3 in McDaniel *et al.* 1977). The latter corresponds to 60 nmol/min per 10^6 cells at 37 °C. By using a Q_{10} of 2, these values become 18 and 26 nmol/min per 10^6 cells respectively. Thus these values compare favourably with the maximal transport rate of 26 nmol/min per 10^6 cells reported here for RINm5F cells. These resemblances to glucose uptake in islets at 37 °C contrast with a clearly higher temperature-dependency of RINm5F cells than of islets. The slow uptake of OMG at 22 °C was also observed by Meglasson *et al.* (1986).

Comparison of uptake and efflux shows no evidence for an asymmetry of glucose transport as is seen in erythrocytes (Widdas, 1980). The slightly faster efflux is explained by the fact that, because of infinite dilution in the extracellular medium, true unidirectional flux is measured under these conditions. In contrast, in uptake experiments net uptake is determined, which consists of uptake and an efflux component increasing with time. It was reported that OMG can be phosphorylated by mammalian hexokinase to a minor extent (Malaisse-Lagae *et al.*, 1986). In our experiments there is no evidence for a significant OMG metabolism. Firstly, even after 30 min of incubation in the presence of 2 mM- or 20 mM-OMG, the intracellular OMG concentration never exceeds the extracellular concentration. Secondly, the efflux experiments show a complete disappearance of trapped radioactivity within 2 min. Thus the accumulation of OMG 6-phosphate can be excluded.

Cytochalasin B and phlorrhizin are known inhibitors of hexose transport in many cell types, including islets (Hellman *et al.*, 1971, 1972, 1973; McDaniel *et al.*, 1974; Carruthers, 1984). Both substances exert a dose-dependent inhibition. In contrast with previous observations in islets (McDaniel *et al.*, 1974), the onset of the inhibitory effect of cytochalasin B was immediate in RINm5F cells and the transport inhibition was more pronounced. On the other hand, the phlorrhizin inhibition compares well with observations in islets (Hellman *et al.*, 1971, 1972, 1973).

We have shown that neutralizing the secreted insulin does not influence transport rates or affinity to glucose. This finding was not unexpected, since alterations of insulin secretion in islets never resulted in changes in glucose uptake (Hellman *et al.*, 1974). Therefore we conclude that the glucose transporter of insulin-secreting cells belongs to the same non-insulin-dependent subtype as the transporter in liver (Carruthers, 1984). The time courses of uptake and efflux in the equilibrium exchange experiments are not diffferent from unidirectional flux measurements. This indicates that the loaded transporter moves with about the same velocity as the unloaded one. This finding agrees with observations in *ob/ob*-mouse islets (Hellman *et al.*, 1971), adipocytes (Gliemann & Rees, 1983) and hepatocytes (Craik & Elliott, 1979). On the other hand, it has been reported for several cell types, including Novikoff hepatoma cells, that the loaded carrier moves faster than the unloaded (Plagemann *et al.*, 1981; Wheeler & Hinkle, 1985).

Glucose metabolism in the tumour-derived RINm5F cells is much exaggerated compared with islet β-cells and exhibits a hyperbolic rather than a sigmoidal dose–response relationship (Halban *et al.*, 1983; Gylfe *et al.*, 1983; Giroix *et al.*, 1985). When measured in parallel with glucose uptake at concentrations above 3 mM-glucose, the rate of metabolism is markedly lower than the transport rates. Therefore it is unlikely that glucose transport

has a regulatory role in the subsequent overall glucose metabolism. Consequently, at glucose concentrations above 3 mM, free glucose should equilibrate across the plasma membrane. When we measured intracellular free glucose under steady-state conditions the concentration was only about 48% of that in the medium. This confirms a report (Sener *et al.*, 1986), but contrasts with the situation in islets, where intracellular and extracellular glucose concentrations are nearly equal (Matschinsky & Ellerman, 1968; Sener *et al.*, 1986).

The failure of RINm5F cells fully to equilibrate D-glucose across the plasma membrane cannot easily be explained by abnormal glucose transport, because the time-dependent OMG uptake at 37 °C was similar to that reported for islet cells (Hellman *et al.*, 1974; Lernmark *et al.*, 1975; Henquin & Lambert, 1976; Gorus *et al.*, 1984). Another more likely explanation is the abnormal glucose-phosphorylating activity in RINm5F cells (Halban *et al.*, 1983; Giroix *et al.*, 1985; Vischer *et al.*, 1987). The observed glucose-phosphorylation rates in RINm5F cell homogenate of 3.2 nmol/h per μg of protein (Halban *et al.*, 1983) and of 49.7 munits/mg of protein (Vischer *et al.*, 1987) at 100 mM-glucose correspond to 11.8 and 11 nmol/min per 10^6 cells at 37 °C (by using a Q_{10} of 2 and 130 μg of protein/10^6 RINm5F cells) respectively. Thus the glucose-phosphorylating activity is close to the transport rate of about 22 nmol/min per 10^6 cells (Fig. 2), counteracting the full equilibration of D-glucose.

However, the decrease in intracellular glucose concentrations relative to extracellular cannot be marshalled as an explanation for the defective glucose-induced insulin release (Praz *et al.*, 1983; Halban *et al.*, 1983; Giroix *et al.*, 1985). For instance, at 23.3 mM-glucose in the medium, the observed intracellular glucose concentration of 11.1 mM is still clearly above the threshold concentration for insulin secretion in islets, of approx. 5 mM (Hedeskov, 1980; Meglasson & Matschinsky, 1986). It should be noted that even extracellular glucose concentrations up to 33.4 mM fail to elicit insulin release in these cells (Praz *et al.*, 1983; Halban *et al.*, 1983).

In conclusion, the failure of RINm5F cells to secrete insulin in response to increases in the glucose concentration in the physiological range cannot be explained by abnormalities in hexose transport, but rather by subsequent steps in glucose metabolism, such as the abnormal expression of glucose-phosphorylating enzymes (Vischer *et al.*, 1987).

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