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Pancreatic beta cell line MIN6 exhibits characteristics of glucose metabolism and glucose-stimulated insulin secretion similar to those of normal islets

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Summary. Glucose-stimulated insulin secretion, glucose transport, glucose phosphorylation and glucose utilization have been characterized in the insulinoma cell line MIN6, which is derived from a transgenic mouse expressing the large T-antigen of SV40 in pancreatic beta cells. Glucose-stimulated insulin secretion occurred progressively from 5 mmol/l glucose, reached the maximal level approximately seven-fold above the basal level at 25 mmol/l, and remained at this level up to 50 mmol/l. Glucose transport was very rapid with the half-maximal uptake of 3-O-methyl-D-glucose being reached within 15 s at 22 °C. Glucose phosphorylating activity in the cell homogenate was due mainly to glucokinase; the V_{max} value of glucokinase activity was estimated to be 255 ± 37 nmol · h^{-1} · mg protein^{-1}, constituting approximately 80% of total phosphorylating activity, whereas hexokinase activity constituted less than 20%. MIN6 cells exhibited mainly the high K_m component of glucose utilization with a V_{max} of 289 ± 18 nmol · h^{-1} · mg protein^{-1}. Thus, glucose utilization quantitatively and qualitatively reflected glucose phosphorylation in MIN6 cells. In contrast, MIN7 cells, which exhibited only a small increase in insulin secretion in response to glucose, had 4.7-fold greater hexokinase activity than MIN6 cells with a comparable activity of glucokinase. These characteristics in MIN6 cells are very similar to those of isolated islets, indicating that this cell line is an appropriate model for studying the mechanism of glucose-stimulated insulin secretion in pancreatic beta cells.

Key words: Clonal beta-cell line, insulin secretion, glucose transport, glucose phosphorylation, glucose utilization.

Impaired glucose-stimulated insulin secretion from pancreatic beta cells is thought to be one of the major causes of Type 2 (non-insulin-dependent) diabetes mellitus. Many investigations have therefore attempted to elucidate the mechanism of insulin secretion triggered by an elevated extracellular glucose concentration. However, the precise mechanism by which glucose stimulates insulin secretion from pancreatic beta cells has yet to be determined. Cellular heterogeneity and the limited availability of pancreatic islets have made progress in the field difficult.

Recent advances in gene manipulation technology provide tools for understanding cellular mechanisms. Clonal cell lines are preferable for these gene manipulations. Recently, we established an insulinoma cell line derived from a transgenic mouse, the MIN6 cell line. The amount of insulin secreted at 25 mmol/l glucose was six- to seven-fold higher than that obtained at 5 mmol/l glucose, suggesting that glucose-stimulated insulin secretion in MIN6 cells may retain a concentration dependence similar to that of normal islets [1]. In contrast, other reported insulin-producing cell lines such as RINm5F [2, 3], RINr [4], NIT-1 [5], βTC-1 [6, 7], βTC-3 [6, 8], IgSV195 [9], and HIT cells [10] do not respond to glucose, or respond at much lower glucose concentrations (1 to 5 mmol/l) than do normal islets. Although a recently developed cell line, INS-1, seemed to respond to the physiological range of glucose, only a 2.2-fold increase in insulin secretion was observed at a high glucose concentration [11]. Abnormal properties of glucose transport and/or phosphorylation have been reported for RINm5F [2, 3, 12–14, 15], RINr [12], βTC-1 [7], βTC-3 [8], IgSV195 [15], and HIT [15, 16] cells, leading to characteristics of glucose utilization which differ from those of normal pancreatic beta cells.

Considerable evidence has been collected that the glucose signal for insulin secretion is generated by glucose metabolism in beta cells [17, 18]. Recent studies have shown that glucose transport and phosphorylation steps are important for glucose-stimulated insulin secretion in pancreatic beta cells [18–20]. The present study was designed to characterize the properties of glucose transport, phosphorylation, utilization and glucose-stimulated insulin secretion in MIN6 cells and to determine whether MIN6 cells represent an appropriate model for investigat-

ing the mechanism of glucose-stimulated insulin secretion. The results indicate that glucose metabolism and the glucose concentration dependence of insulin secretion in MIN6 cells closely resemble those in normal beta cells, and that this cell line is thus a useful tool for studying glucose-stimulated insulin secretion in normal pancreatic beta cells.

Materials and methods

Cell culture: MIN6 cells were grown in Dulbecco's modified Eagle's medium (DMEM, 25 mmol/l glucose) equilibrated with 5% CO_2 and 95% air at 37 °C. The medium was supplemented with 15% fetal calf serum, 50 mg/l streptomycin and 75 mg/l penicillin sulphate. MIN6 cells used in the present study were harvested at passages 16–23.

Insulin secretion and insulin content assay. Insulin secretion was determined using a static incubation method in 5% CO_2 and 95% air at 37 °C. Two $\times 10^5$ cells were seeded in 1 ml DMEM (25 mmol/l glucose) in 24-well plates. After 48 h of culture, the medium was removed and cells were washed once with HEPES-balanced Krebs-Ringer bicarbonate buffer (119 mmol/l NaCl, 4.74 mmol/l KCl, 2.54 mmol/l $CaCl_2$, 1.19 mmol/l $MgCl_2$, 1.19 mmol/l KH_2PO_4, 25 mmol/l $NaHCO_3$, 10 mmol/l HEPES, pH 7.4) containing 0.5% bovine serum albumin (BSA) without glucose. Next, cells were preincubated for 0.5 h in HEPES-balanced Krebs-Ringer bicarbonate buffer with 0.5% BSA and 5 mmol/l glucose. After washing twice with HEPES-balanced Krebs-Ringer bicarbonate buffer, MIN6 cells were incubated for 2 h in HEPES-balanced Krebs-Ringer bicarbonate buffer supplemented with 0.5% BSA and varying concentrations of glucose. The media were then collected and assayed for immunoreactive insulin by RIA using rat insulin as a standard. To each well 200 µl of 1 mol/l NaOH was added to solubilize cells for determination of cellular protein content with a Bio-Rad protein assay kit. For measurement of cellular insulin content, 1 ml of acid ethanol was added to the wells which were then sealed with pressure-sensitive film. The extract was collected after 24-h incubation at 4 °C, diluted and assayed by RIA.

3-O-methyl-D-glucose transport: The method of Johnson et al. [21] was employed. MIN6 cells were detached by treatment with phosphate-buffered saline (PBS) containing 0.5 mmol/l EDTA for 5 min and incubated in PBS containing 2 mmol/l [^{14}C]urea (0.25 mCi/mmol) as an intracellular space marker at 37 °C for 20 min, followed by incubation at 22 °C for 10 min. Fifty microlitres of 1 mol/l glucose containing 10 mmol/l EDTA and 0.1% SDS (pH 8.0) was placed in the bottom of a 400 µl microfuge tube. This solution was overlaid with 150 µl of a dibutyl phthalate-dinonyl phthalate (4:1) mixture. Fifty microlitres of PBS containing 2 mmol/l [^{14}C]urea (0.25 mCi/mmol) and 20 mmol/l 3-O-[^{3}H]methyl-D-glucose (2.5 mCi/ mmol) was layered over the dibutyl phthalate-dinonyl phthalate phase. The tubes were then preincubated for 30 min at 22 °C. Uptake was initiated by addition of 50 µl of the cell suspension to the PBS layer containing labelled urea and 3-O-methyl-D-glucose. Uptake was terminated by centrifugation of the tubes for 15 s so that cells were sedimented to the 1 mol/l glucose-10 mmol/l EDTA-0.1% SDS solution. Then tubes were cut at the dibutyl phthalate-dinonyl phthalate layer and the part containing sedimented cells was used for determination of the uptake and the intracellular space. The uptake of L-[1-^{3}H]glucose was measured by the same procedure. Uptake was determined in quadruplicate at each concentration.

Glucose phosphorylation. Glucose phosphorylating activity was determined by the method of Trus et al. [18]. MIN6 cells were harvested with trypsin-EDTA, washed with PBS twice to remove glucose and homogenized with 7 strokes in a homogenizing buffer (20 mmol/l K_2HPO_4, 5 mmol/l dithiothreitol, 1 mmol/l EDTA, and 110 mmol/l KCl, pH 7.4) using a Potter-Elvehjem glass-Teflon homogenizer. A half aliquot of the homogenate was centrifuged at 12000 × *g* for 10 min at 4 °C and the supernatant was removed. Glucose phosphorylating activity and protein concentration were determined in both the homogenate and the supernatant. The supernatant and the total homogenate (containing 50–90 µg protein) were incubated for 90 min at 30 °C in the buffer containing 50 mmol/l HEPES, 100 mmol/l KCl, 7.4 mmol/l $MgCl_2$, 15 mmol/l β-mercaptoethanol, 0.5 mmol/l NAD^+, 0.05% BSA, 0.7 unit/ml glucose 6-phosphate dehydrogenase from Leuconostoc mesenteroides, 5 mmol/l ATP and varying concentrations of glucose (0.06, 0.15, 0.3, 0.6, 1, 6, 10, 15, 30, 60, and 100 mmol/l). The reaction was terminated by adding 1.6 ml of 500 mmol/l sodium bicarbonate buffer, pH 9.4, and NADH fluorescence was then measured. Phosphorylating activity was determined in triplicate at each concentration. In each assay, reagent blanks and tissue blanks were included. Reagent blanks consisted of assay reagents with either 0.6 or 60 mmol/l glucose in the absence of tissue. The experiments of tissue blanks were performed by incubation of tissue with either 0.6 or 60 mmol/l glucose in the absence of ATP. Fluorescence of both reagent blanks and tissue blanks was subtracted from the total fluorescence observed with a complete reagent with tissue. V_{max} and K_m for glucose were determined by analysis of the data with Eadie-Hofstee plots. On the Eadie-Hofstee plots, the least-squares method was used to draw a best-fitted line.

Glucose utilization. Glucose utilization was measured by following the conversion of [5-^{3}H]glucose into ^3H_2O [18]. MIN6 cells were seeded and preincubated using the same procedures as those used for the determination of insulin secretion. Cells were then incubated at 37 °C in 0.3 ml of HEPES-balanced Krebs-Ringer bicarbonate buffer with 0.5% BSA and varying concentrations of glucose (0.15, 0.3, 0.6, 1.0, 5.0, 10, 15, 25 and 50 mmol/l) containing 5 µCi/ml and 20 µCi/ml [5-^{3}H]glucose for lower ($\leq$ 1 mmol/l) and higher ($\geq$ 5 mmol/l) glucose concentrations, respectively. As blanks, 0.3 ml of media alone were incubated in parallel. After a 2-h incubation period, 0.1 ml of the incubation media was removed to a microtube and mixed with 20 µl of 1 mol/l HCl. The microtubes were then placed in 22-ml plastic scintillation vials containing 0.6 ml of distilled water. The vials were sealed and kept at 37 °C for 36 h to allow the ^3H_2O in the microtube to equilibrate with the water in the scintillation vial. Subsequently, the microtube was taken out and 10 ml of ACS II (Amersham Japan, Tokyo, Japan) was added to each vial. The rate of glucose utilization was calculated using the formula described by Ashcroft et al. [22]. Under these experimental conditions, the recovery from known amounts of ^3H_2O was 87 ± 4% (n = 3). Cellular protein content was determined after solubilizing cells with 200 µl of 1 mol/l NaOH.

Northern blot analysis. Total RNA was prepared using the guanidine isothiocyanate method. RNA was denatured with formaldehyde, separated by 1.0% agarose gel electrophoresis, and transferred onto Biodyne B nylon membranes (Pall BioSupport, Glen Cove, NY, USA). EcoT14I fragments of rat glucokinase cDNA (nucleotide 570–1616) were ^{32}P-labelled by random priming. Hybridization of the filters with the probe was carried out in 50% formamide, 5× SSC (1× SSC = 150 mmol/l NaCl, 15 mmol/l sodium citrate), 5× Denhardt's solution (1× Denhardt's solution = 0.02% polyvinyl pyrrolidone, 0.02% Ficoll, 0.02% BSA), 50 mmol/l sodium phosphate buffer, pH 7.0, 0.1% SDS and 100 µg/ml salmon sperm DNA at 42 °C. The filters were washed with 0.2% SDS, 0.5× SSC at 60 °C for 30 min and subjected to autoradiography.

Statistical analysis

Data are presented as mean ± SD, unless otherwise stated. Student's unpaired *t*-test was used to test for differences.

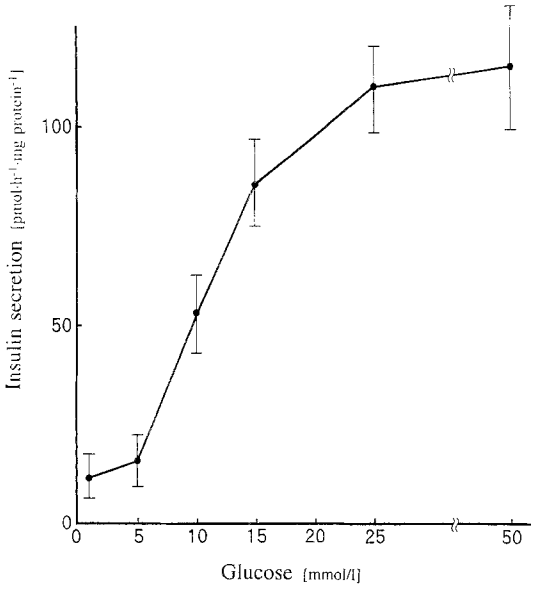

Fig. 1. Glucose-stimulated insulin secretion from MIN6 cells. Secreted insulin was measured in the medium after a 120-min incubation of MIN6 cells in the presence of the indicated concentrations of glucose. Data are expressed per mg cellular protein, extracted by solubilizing cells with 1 mol/l NaOH. Values are mean ± SD ($n = 4$)

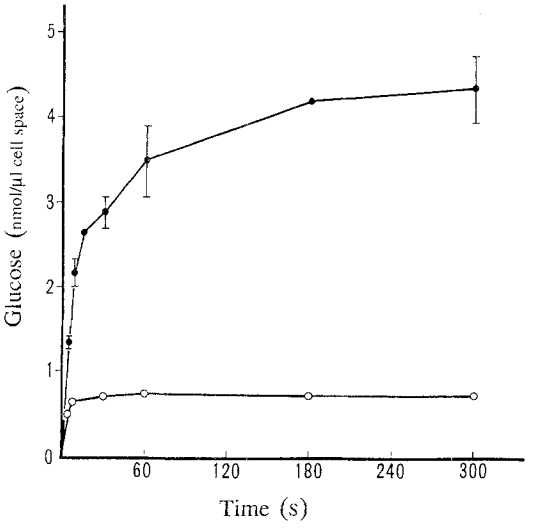

Fig. 2. Time course of the uptake of 3-O-methyl-glucose (●) and L-glucose (○) by MIN6 cells. Experiments were performed at a hexose concentration of 10 mmol/l at 22 °C. Each point with an error bar is mean ± SEM of three separate experiments and those without an error bar are the means of two separate experiments

Results

Glucose-dependence of insulin secretion

Insulin content in MIN6 cells was 3230 ± 690 pmol insulin per mg cellular protein ($n = 4$). This value was calculated to be 1010 pmol · 10^6 cells^{-1}, since the cellular protein content averaged 320 ± 42 µg · 10^6 cells^{-1} ($n = 4$). This amount is approximately 20% of the insulin content of mouse islet cells [23] and is comparable with that of recently established INS-1 cells [11]. Insulin secretion from MIN6 cells was increased when the extracellular glucose concentration exceeded 5 mmol/l and the half-maximum response was achieved at around 10 mmol/l glucose (Fig. 1). The amount of secreted insulin at 25 mmol/l glucose was approximately seven-fold greater than that at 5 mmol/l.

Glucose transport in MIN6 cells

The uptake of 3-O-methyl-D-glucose by MIN6 cells was rapid, as shown in Figure 2. The time required for the half-maximal uptake was less than 15 s and equilibration of the intracellular 3-O-methyl-D-glucose space was approximately 80% complete in 1 min at 22 °C. The distribution space of 3-O-[^{3}H]methyl-D-glucose over 5-min incubation was 0.70 ± 0.10 picolitre per cell (pl · cell^{-1}) ($n = 3$), whereas [^{14}C]urea distribution space over 30 min incubation was 1.78 ± 0.23 pl · cell^{-1} ($n = 3$). No significant increase in L-glucose uptake was observed during 5-min incubation, indicating that L-glucose in the cell pellets represented L-glucose trapped in the extracellular, rather than the intracellular, space. This L-glucose distribution space was 0.12 pl · cell^{-1} (mean of the results of two experiments). Intracellular 3-O-methyl-D-glucose space was estimated by subtracting L-glucose distribution space from 3-O-methyl-D-glucose distribution space. Although it is not clear why the distribution space of 3-O-[^{3}H]methyl-D-glucose was smaller than that of [^{14}C]urea in MIN6 cells, similar discrepancy was observed between the distribution space of glucose and that of urea in isolated islets [24]. It may be possible that the urea distribution space represents two or more intracellular compartments, one of which is intracellular 3-O-methyl-D-glucose space. These results on glucose uptake suggest that MIN6 cells contain a transport system with very high activity specific for D-glucose.

Expression of glucokinase in MIN6 cells

Total RNA from mouse brain, liver and MIN6 cells were probed with a rat glucokinase cDNA fragment (Fig. 3). An approximately 2.4 kilobase (kb) glucokinase mRNA was expressed in liver tissue, as reported previously [25], and an approximately 2.8 kb mRNA, slightly larger than the liver glucokinase mRNA, was detected in MIN6 cells. The size of this mRNA was identical to that reported previously for rat glucokinase mRNA in pancreatic islets [25]. Furthermore, amplification of glucokinase mRNA in MIN6 cells was performed by reverse transcription and

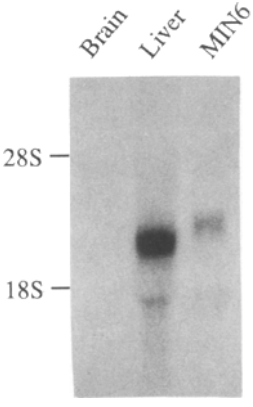

Fig. 3. Expression of glucokinase mRNA in MIN6 cells. Total RNA was extracted from C57 BL/6 mouse brain, liver, and MIN6 cells. 20 µg (brain, liver) or 5 µg (MIN6) of total RNA were electrophoresed in a 1.0% formaldehyde/agarose gel, transferred to a nylon membrane, and hybridized with rat glucokinase cDNA probe

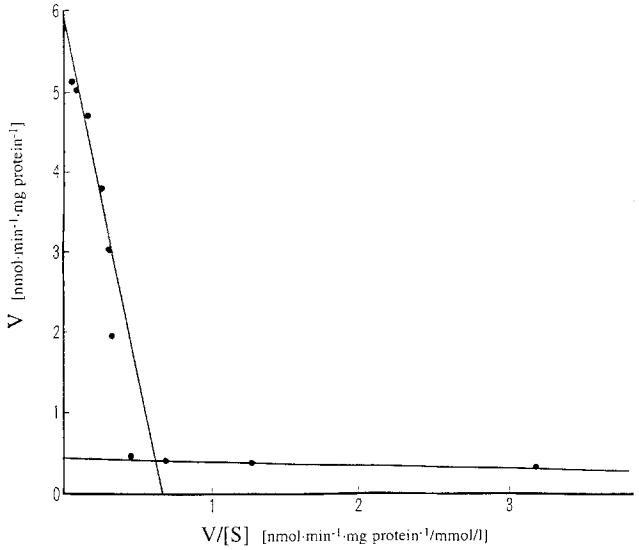

Fig. 4. Eadie-Hofstee plots for kinetic analysis of glucokinase and hexokinase activity in the supernatant of the MIN6 cell homogenate. Each point is the mean of triplicate measurements performed at 30 °C. A representative result from one of three independent experiments is shown. V, Reaction velocity; S, substrate concentration

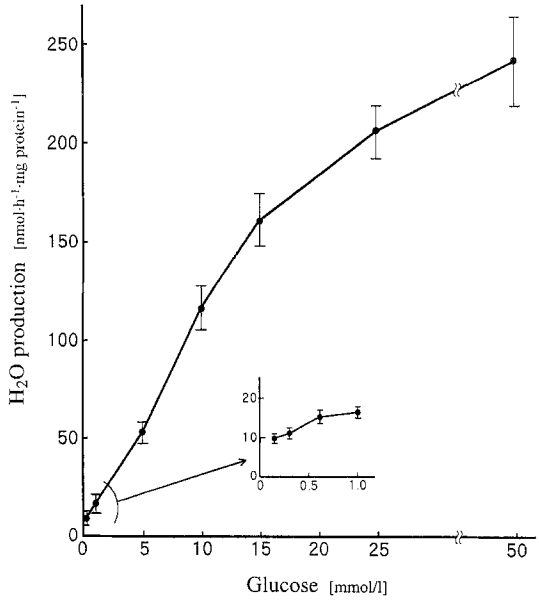

Fig. 5. Glucose utilization by MIN6 cells. Glucose utilization was measured by following the conversion of [5-^{3}H]glucose into ^3H_2O as described in Materials and methods. Data are expressed as the mean ± SD of three independent experiments, performed in triplicate for each concentration

polymerase chain reaction using a sense oligonucleotide corresponding to nucleotide 411 to 430 and an antisense oligonucleotide corresponding to nucleotide 659 to 678 of mouse glucokinase cDNA [26]. A 268 base-pair DNA fragment, of the expected size, was obtained and sequencing of the DNA fragment demonstrated that the deduced first 15 amino acid residues were identical to those of rat islet glucokinase (data not shown).

Glucose phosphorylating activity in the homogenate of MIN6 cells

Because a fraction of hexokinase may be bound to cytoplasmic organelles, glucose phosphorylating activity was measured both in the total cell homogenate and in the supernatant of the homogenate. The representative Eadie-Hofstee plots of glucose phosphorylating activity in the supernatant are shown in Figure 4. The Eadie-Hofstee plots revealed two components of glucose phosphorylating activity; glucokinase and hexokinase activity. The result at 6 mmol/l glucose deviated from the high K_m component, possibly reflecting a deviation in glucokinase kinetics from those of Michaelis-Menten model, i.e. glucokinase activity was lower than that expected from the Michaelis-Menten model at 6 mmol glucose. Kinetic parameters of glucose phosphorylating activity in MIN6 cells are summarized in Table 1. Glucokinase constituted approximately 80% and 90% of glucose phosphorylating activity in the total homogenate and in its supernatant of MIN6 cells, respectively. For a comparison, we measured glucose phosphorylating activity in the homogenate of MIN7 cells which exhibited only a small increase in insulin secretion in response to glucose despite the considerable levels of GLUT 2 expression [1]. MIN7 cells also had two components of glucose phosphorylating activity; glucokinase activity with V_{max} of 1.91 ± 0.29 nmol · min^{-1} · mg protein^{-1} and K_m of 12.4 ± 1.73 mmol/l and hexokinase activity with V_{max} of 2.31 ± 0.14 nmol · min^{-1} · mg protein^{-1} and K_m of 0.046 ± 0.012 mmol/l (n = 3). Thus, MIN7 cells have 4.7-fold higher hexokinase activity than MIN6 cells. MIN6 cells tend to have a higher glucokinase activity than MIN7 cells but the difference was not significant (p = 0.074).

Glucose utilization in MIN6 cells

Glucose utilization was studied at 0.15–50 mmol/l glucose concentrations. Glucose utilization by MIN6 cells exhibited a small increase in the range of 0.15–1 mmol/l glu-

Table 1. Kinetic characteristics of glucokinase and hexokinase in MIN6 cells

	Glucokinase		Hexokinase	
	V_{max} (nmol · min^{-1} · mg^{-1})	K_m (mmol/l)	V_{max} (nmol · min^{-1} · mg^{-1})	K_m (mmol/l)
Supernatant	5.58 ± 0.20	9.3 ± 0.9	0.52 ± 0.13	0.063 ± 0.009
Homogenate	2.58 ± 0.37	9.7 ± 1.7	0.49 ± 0.05	0.050 ± 0.014

Values are mean ± SD, n = 3

cose (9.78 ± 1.03 nmol · h^{-1} · mg protein^{-1} at 0.15 mmol/l glucose vs 16.5 ± 3.14 at 1.0 mmol/l glucose ($n = 3$), $p < 0.05$) with a marked stimulation at glucose concentrations greater than 5 mmol/l (Fig. 5). Eadie-Hofstee plots for these data could be resolved into two linear components; one with low K_m (apparent $K_m = 0.257 \pm 0.032$ mmol/l, $V_{max} = 20.8 \pm 4.2$ nmol · h^{-1} · mg protein^{-1}, $n = 3$) and the other with high K_m (apparent $K_m = 15.4 \pm 0.75$ mmol/l, $V_{max} = 289 \pm 18$ nmol · h^{-1} · mg protein^{-1}, $n = 3$).

Discussion

Glucose-sensing is a very important function of pancreatic beta cells and serves to maintain an appropriate blood glucose level. Impairment of glucose-sensing by beta cells results in an alteration in the glucose concentration dependence of insulin secretion in pancreatic beta cells, which may lead to Type 2 diabetes [27, 28]. Many lines of evidence have suggested that the glucose signal for insulin secretion is generated by the metabolism of transported glucose in beta cells. In this report, we have characterized the properties of transport, phosphorylation, and utilization of glucose in MIN6 cells and have demonstrated that these characteristics are very similar to those in normal pancreatic islets and beta cells.

The glucose concentration dependence of insulin secretion in MIN6 cells resembled that of isolated islets from rats [2, 29] and mice [30]. The fold-increase at a high glucose concentration is, however, lower in MIN6 cells (7.3-fold) compared with that in isolated mouse islets (~10–25-fold) [30, 31]. One of the reasons for the lower fold-increase in MIN6 cells may be a lack of suppression of insulin secretion by non-beta cells at a low glucose concentration. Reaggregated purified beta cells were reported to exhibit an enhanced insulin secretion compared with that of intact islets at a low glucose concentration, and thus the fold-increase in purified beta cells was smaller than that in islets and was comparable to that in MIN6 cells [32].

Glucose transport by MIN6 cells was very rapid, as in isolated islet cells of rats [21] and mice [33]. It has been reported that RINm5F [12–14], RINr [12] and HIT cells [12, 15], which did not respond to an elevated glucose concentration or responded only at much lower concentrations than the physiological level, exhibit a slow rate of glucose uptake. Interestingly, these clonal beta-cell lines have also been reported to express a considerable amount of GLUT 1 [26, 34, 35]. In contrast, MIN6 cells and normal beta cells express a high level of GLUT 2 and a barely detectable level of GLUT 1 [1, 36, 37]. Although it is not clear whether high levels of GLUT 2 expression without GLUT 1 are needed for normal glucose-sensing, these data on glucose uptake suggest the importance of rapid glucose transport for normal glucose-sensing in pancreatic beta cells. Since the V_{max} of GLUT 2 transport activity is much greater than that of GLUT 1 [38], the expression of GLUT 2 is advantageous for achieving a rapid glucose uptake. Glucose phosphorylating activity at 10 mmol/l glucose at 22 °C was calculated to be 0.082 nmol · 15 s^{-1} · 10^6 cell^{-1}, using the results obtained at 30 °C and a Q_{10} (correction factor) of 2 [39], whereas 3-O-methyl-D-glucose transport rate at 10 mmol/l glucose at 22 °C was calculated to be 3.15 nmol · 15 s^{-1} · 10^6 cell^{-1}. Thus, glucose transport rate was 38-fold greater than the rate of glucose phosphorylation in MIN6 cells.

Rapid glucose transport allows a rapid equilibration of intracellular and extracellular glucose concentrations. Thus, glucose phosphorylation progresses at the substrate concentration which is very similar to that of the extracellular space. In MIN6 cells, glucokinase is the major glucose phosphorylating enzyme, constituting about 80 % of total glucose phosphorylating activity. A high glucokinase activity in MIN6 cells may be the result of culture at a high glucose concentration (25 mmol/l), as glucokinase activity has been reported to be up-regulated by glucose [40]. It might be possible that an erroneously high glucokinase value could result if N-acetylglucosamine kinase was present in MIN6 cells. N-acetylglucosamine kinase was reported to exhibit glucose phosphorylating activity with a K_m for glucose of 370 mmol/l [41]. However, the Eadie-Hofstee plots for glucokinase were linear, suggesting that N-acetylglucosamine kinase does not contribute significantly to phosphorylating activity in MIN6 cells.

Approximately 50 % of the glucose phophorylating activity was attributable to hexokinase in $12000 \times g$ supernatant of homogenate of isolated islets from rats and mice (data not shown), which is in accord with previous reports [42]. In contrast, only 10 % of phosphorylating activity was attributed to hexokinase in MIN6 cells. Not only the relative value but also the absolute value of hexokinase activity was significantly lower in MIN6 cells (0.52 nmol · min^{-1} · mg protein^{-1}), as compared with the reported value in normal islets (3.0 nmol · min^{-1} · mg protein^{-1}) [42]. Despite a high level of hexokinase activity, the low K_m component of glucose utilization was a minor component in normal islets (15 % of total glucose usage) [18, 22]. One explanation for the apparent low activity of the low K_m component of glucose utilization is that hexokinase is largely inhibited by glucose 6-phosphate in islets. However, another possibility is that a considerable amount of hexokinase is present in non-beta cells, but not in beta cells, and that glucose phosphorylation is not a rate-limiting step for glucose utilization in non-beta cells. Indeed, there is good evidence that the pancreatic alpha cells are less accessible to glucose than the beta cells and that glucose transport, rather than the glucose phosphorylation step, might be rate-limiting for glucose utilization in alpha cells [43, 44]. If this is the case, the hexokinase content of pancreatic beta cells may be similar to that of MIN6 cells. Indeed, measurement of glucose phosphorylating activity in purified rat beta cells has suggested that hexokinase activity in beta cells was lower than the average value for cells of pancreatic islets [24]. It is interesting to note that in the liver, another tissue which expresses glucokinase, hexokinase activity is much greater in non-parenchymal cells than in hepatocytes [45].

Glucose utilization was much stimulated by glucose at concentrations greater than 5 mmol/l in MIN6 cells. The glucose concentration dependence of insulin secretion correlated closely with that of glucose utilization in MIN6

Table 2. Glucose-stimulated insulin secretion and glucose metabolism in various insulin-secreting cells

Cell line	Passage no.	Insulin content[a]		Insulin secretion				Glucose phosphorylation (%)[e]		Glucose utilization (%)[f]		
		per protein	per cell	Basal[b] (% content)	Fold-increase[c]	GLUT isoforms[d]						
						GLUT 1	GLUT 2	low-K_m	high-K_m	low-Km	high-Km	Reference
Islet	–		5100	~0.4	10–23	– ~ ±	+	45	55	15	85	23, 30, 31, 37
MIN6	16–23	3230	1010	0.5	7.3	±	+	16	84	7	93	This study
NIT-1	11		265	45	1.5	ND	ND	ND	ND	ND	ND	5
βTC-1	63, ?		54	?	2.5	+	–	ND	ND	58	42	6, 7
βTC-3	17–35	5330		0.5	1.9	+	–	ND	ND	ND	ND	8
IgSV195	?	?	?	?	1.0	ND	ND	79	21	75	25	9, 16
HIT-T15	55–75		172	0.35	8.3	± ~ +	± ~ +	25	75	80	20	10, 16, 35
RINm5F	?		2.5	22	1.2	+	–	~100	~0	~100	~0	2, 3, 16, 34
RINr	8–9		14.6	2.0	4.5	+	± ~ +	ND	ND	ND	ND	4, 26
INS-1	9–83		1550	5.0	2.2	ND	ND	ND	ND	ND	ND	11

[a] Insulin content was expressed as pmol per mg protein or pmol per 10^6 cells
[b] Insulin secretion per 1 h at a basal condition was expressed as % of cellular insulin content
[c] Insulin secretion at a high glucose concentration was expressed as fold-increase relative to that at a basal condition
[d] –, No expression; ±, low levels of expression; +, considerable levels of expression
[e] % contribution of low- and high- K_m system to total glucose phosphorylating activity in the supernatant of cell homogenates
[f] % contribution of low- and high- K_m system to glucose usage
ND, Not determined

cells. A similar correlation was also observed in normal islets. The V_{max} of glucokinase in the total homogenate of MIN6 cells at 37 °C was calculated to be 255 ± 37 nmol · h^{-1} · mg $protein^{-1}$ ($n = 3$), assuming that Q_{10} equals 2 [39]. This value is comparable to the V_{max} of the high K_m component of glucose utilization (289 ± 18 nmol · h^{-1} · mg $protein^{-1}$, $n = 3$), suggesting that glucokinase may have a role in determining the rate of glycolysis in MIN6 cells, as suggested by results obtained in normal islets [18].

Properties of glucose-stimulated insulin secretion and glucose metabolism in MIN6 cells and other insulin secreting cell lines are summarized and compared with those of isolated islets (Table 2). Except for the cellular insulin content and the glucokinase/hexokinase ratio in the supernatant of the cell homogenate, MIN6 cells and normal islets exhibit common features in the insulin secretion and glucose metabolism. Important characteristics distinguishing MIN6 cells from other cell lines are ample levels of GLUT 2 with very low levels of GLUT 1 and a high glucokinase activity with a low hexokinase activity in MIN6 cells. MIN7 cells have greater expression levels of GLUT 1 [1] and 4.7-fold greater hexokinase activity compared with MIN6 cells, while MIN6 cells and MIN7 cells have comparable levels of GLUT 2 and glucokinase activity. Thus, high levels of GLUT 1 expression or hexokinase, or both, may deprive MIN7 cells of normal responsiveness to glucose. These data further support the view that glucose transport and/or glucose phosphorylation play an important role for normal glucose-sensing. However, other abnormalities downstream from glucose-phosphorylating step are also possible in MIN7 cells.

Our data suggest that cellular components regulating the glucose concentration dependence of insulin secretion in MIN6 cells resemble those in normal islets both quantitatively and qualitatively. Thus, the MIN6 cell is a suitable model for studying the mechanism of glucose-stimulated insulin secretion. Further studies, in which these components are manipulated using molecular biological techniques, should help identify the mechanisms of glucose-stimulated insulin secretion in pancreatic beta cells.

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