Glucose Modulates Glucose Transporter Affinity, Glucokinase Activity, and Secretory Response in Rat Pancreatic β-Cells

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Pancreatic islets were cultured for 24 h in medium containing either low (1.4), normal (5.5), or high (16.7 mM) glucose, and then insulin secretion was measured at the end of 1 h incubation at 37°C. Insulin release in the absence of glucose was 64 ± 20, 152 ± 11, and 284 ± 30 pg · islet^{-1} · h^{-1} (mean ± SE, n = 6, G1.4 and G16.7 vs. G.5.5, $P < 0.05$) and the response to 22 mM glucose stimulation was 640 ± 136, 2460 ± 276, and 1890 ± 172 pg · islet^{-1} · h^{-1}, respectively (n = 6, G1.4 vs. G5.5, $P < 0.01$, G16.7 vs. G5.5, $P = 0.065$). The 50% maximal response of insulin secretion (increment over baseline) was reached at an average glucose concentration of 9.9 ± 0.7 mM in islets preexposed to G5.5, and at glucose 13.3 ± 0.9 and 4.8 ± 0.4 mM ($P < 0.05$ in respect to G5.5) in islets preexposed to G1.4 and G16.7, respectively. To investigate the molecular mechanism responsible for this altered glucose sensitivity, we measured, in parallel experiments, the kinetic characteristics of glucose transport, glucokinase, and glucose utilization. Glucose transport was measured by evaluating 3-*O*-methylglucose uptake. The apparent K_m of the low-affinity transporter (GLUT2) was 16.6 ± 2.4 mM in isolated pancreatic cells cultured at 5.5 mM glucose. In cells cultured at both low (1.4 mM) or high (16.7 mM) glucose concentrations, a significant change in the apparent K_m of this glucose transporter function was observed (24.4 ± 2.9 and 7.1 ± 0.6 mM, n = 5, $P < 0.05$ and <0.01, respectively), with no change in the V_{max} of the uptake. Under the same experimental conditions a concomitant change in glucokinase activity was observed: the enzyme V_{max} was 4.9 ± 0.32, 8.7 ± 0.79, and 15.8 ± 0.98 nmol · μg DNA^{-1} · h^{-1} in islets exposed to either 1.4, 5.5, or 16.7 mM glucose, respectively (mean ± SE, n = 5, G1.4 and G16.7 vs. G.5.5, $P < 0.05$), with no significant change in the enzyme K_m. In control islets glucose utilization, measured by ^3H_2O production from [5-^{3}H]glucose, had a K_m of 8.0 ± 1.7 mM and a V_{max} of 8.9 ± 0.41 nmol · μg DNA^{-1} · 2 h^{-1}. In islets exposed to either G1.4 or G16.7, the glucose utilization V_{max} was decreased and increased (5.3 ± 0.56 and 13.8 ± 0.98 nmol · μg DNA^{-1} · 2 h^{-1}, n = 5, $P < 0.05$), parallel with the changes observed in glucokinase activity. Moreover, the apparent glucose utilization K_m was increased in G1.4 preexposed islets (15.6 ± 1.3 mM) and decreased in G16.7 preexposed islets (3.7 ± 0.9 mM), parallel with the changes observed in the glucose transport K_m. These studies indicate that in vitro the sensitivity and responsiveness of pancreatic islets to glucose may be regulated by the ambient glucose concentrations. They support the concept that glucokinase plays a pivotal role in regulating glucose metabolism V_{max}, and, therefore, the β-cell responsiveness to glucose. In addition, they also indicate that changes in the affinity (K_m) of the glucose transporter are associated to changes in the K_m of glucose utilization, thus suggesting a possible role of glucose transport in determining the β-cell sensitivity to glucose. *Diabetes* 42:199–205, 1993

Glucose is the main regulator of pancreatic β-cell insulin release. For insulin secretion to occur, both glucose uptake and metabolism are required. In β-cells intracellular glucose rapidly equalizes with ambient glucose levels by a facilitated glucose transport system. The glucose transporter identified in pancreatic β-cells (GLUT2) has the unique property of a low affinity for glucose (1). The presence of a low-affinity (K_m ~17 mM), high-capacity glucose transporter allows a cellular glucose uptake proportional to a wide range of extracellular glucose concentrations (1–20 mM) and, therefore, determines the β-cell capacity to

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K_m, Michaelis constant; FCS, fetal calf serum; BSA, bovine serum albumin, Q_{10}, correction factor for increasing temperature.

appropriately respond to even extreme physiological glycemic changes (2). In β-cells glucose metabolism is regulated by glucokinase, a glucose-specific high-K_m enzyme that at glucose concentrations >5.5 mM, is the rate-limiting step for glucose metabolism and, therefore, for glucose-induced insulin release (3,4). Under these conditions, glucose transport, having a capacity that is largely in excess of glucose phosphorylation and metabolism, does not play a pivotal role in determining the secretory response. However, when the expression or function of the glucose transporter is significantly reduced, it may limit the access of glucose to glucokinase, thus preventing a normal glucose sensing and appropriate insulin secretion (2,5,6).

Both in vitro and in vivo studies indicate that chronic exposure to either low or high glucose concentrations may alter both the threshold of glucose-stimulated insulin release (glucose sensitivity) and the maximal responsiveness of pancreatic β-cells to glucose (7–11). Although changes in glucose responsiveness have been associated to changes in glucokinase activity (11), the mechanism underlying the difference in glucose sensitivity is unknown.

To better understand this problem, in this study we evaluated glucose transport, glucokinase activity, glucose utilization, and insulin secretion in rat pancreatic islets preexposed to different glucose concentrations for 24 h.

RESEARCH DESIGN AND METHODS

Male Wistar rats were purchased from Nossan (Milan, Italy). Culture medium CMRL-1066, heat-inactivated FCS, glutamine, and antibiotics were purchased from Gibco (Glasgow, UK). 3-*O*-[^{3}H]methyl-D-glucose, [^{3}H]-3OMG (specific activity 2.74 Ci/mmol), [U-^{14}C]sucrose (specific activity 673 mCi/mmol), and [5-^{3}H]glucose (specific activity 15.7 Ci/mmol) were purchased from Amersham (Amersham, UK).

Pancreatic islets were isolated from 200–250 g fed male Wistar rats and hand picked under stereomicroscope observation. Batches of 100 islets were maintained at 37°C in a 95% O_2/5% CO_2 atmosphere in a plastic Petri dish containing 3 ml of CMRL-1066, 10% heat inactivated FCS serum, and 2 mM glutamine and glucose at either 1.4, 5.5, or 16.7 mM (G1.4, G5.5, G16.7). After 24 h, groups of seven islets were transferred in Hank's balanced buffer (NaCl 136 mM, $CaCl_2$ 1.67 mM, $MgSO_4$ 0.8 mM, KCl 5.4 mM, Na_2HPO_4 0.35 mM, KH_2PO_4 0.45 mM, $NaHCO_3$ 4.2 mM) supplemented with HEPES HCl 10 mM, pH 7.35, at 37°C in the presence of various glucose concentrations, and after 1 h insulin was measured both in the medium and the acid-alcohol cell extracts.

Pancreatic cells were obtained from islets transferred to a Ca^{2+}-free Hank's balanced buffer at 30°C in the presence of trypsin (6.5 μg/ml) and DNAse (2 μg/ml) (9). The islet dissociation was stopped by adding Hank's balanced buffer containing 1.67 mM calcium and 1% newborn calf serum when most cells were in groups of three to seven. Cells from ~5000 islets were then cultured in CMRL-1066 medium in 48-multiwell dishes (120×10^3 cells per well). At 48 h, when cells were attached to the dish, the culture medium was gently replaced with fresh medium containing 1.4, 5.5, or 16.7 mM glucose, and cell culture continued for an additional 24 h. To measure [^{3}H]-3OMG transport, cells in monolayer were washed twice and incubated with Krebs-Ringer phosphate buffer (140 mM NaCl, 1.7 mM KCl 0.9 mM $CaCl_2$, 1.47 mM K_2HPO_4, 0.8 mM $MgSO_4$, and 0.5% BSA, pH 7.4) for 30 min at 15°C. The buffer was then replaced with 0.4 ml of fresh Krebs-Ringer phosphate containing increasing concentrations (0.1–60 mM) of 3OMG at a constant specific activity of 0.06 μCi/mmol. After 15 s the transport was stopped by washing the cells three times with ice-cold Krebs-Ringer phosphate con-

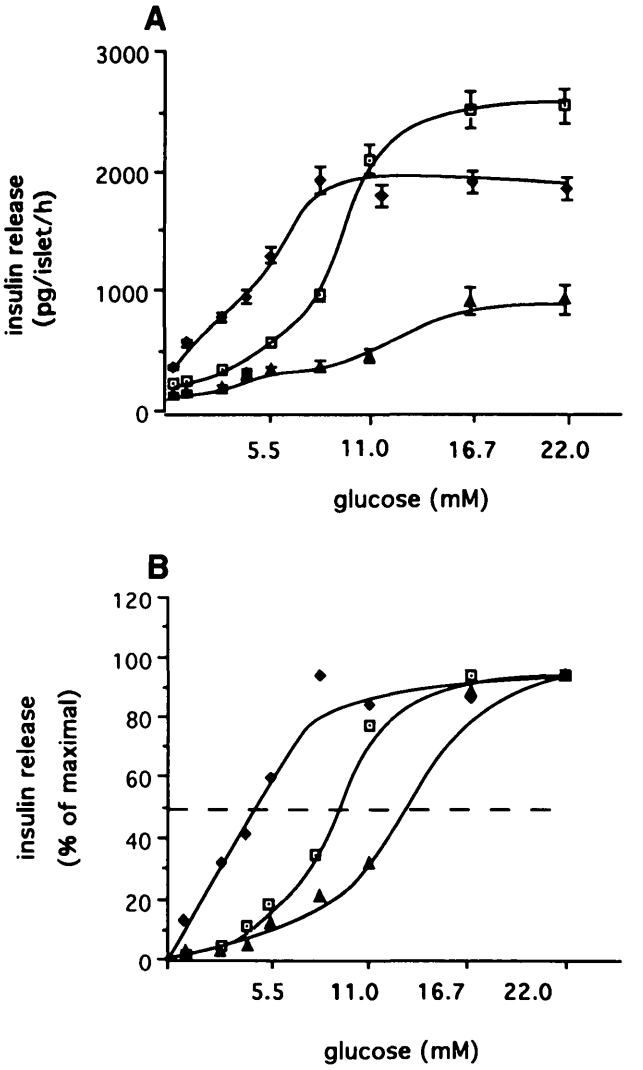

FIG. 1. Effect of preexposure to different glucose concentrations on insulin release from rat pancreatic islets. *A*: islets were cultured in CMRL-1066 containing glucose at either 1.4 (▲), 5.5 (□), or 16.7 (♦) mM. After 24 h, groups of 7 islets were transferred in Krebs-Ringer buffer (pH 7.35) at 37°C in the presence of various glucose concentrations and 1 h later insulin released in the medium was measured. Data are means ± SE of 6 separate experiments. *B*: data of insulin secretion from the top panel are presented as percentage of the maximal effect (increase over baseline) obtained in the 3 experimental groups.

TABLE 1
Effect of preexposure to different glucose concentrations on insulin secretion (pg · 10^{-3} cells · h^{-1}) from dissociated pancreatic cells

Glucose in buffer during acute stimulation (mM)	Chronic culture conditions (mM)		
	1.4	5.5	16.7
0	91 ± 11	97 ± 12	80 ± 11
2.8	86 ± 9	97 ± 14	138 ± 15
5.5	96 ± 12	135 ± 18	243 ± 19
11.0	124 ± 11	343 ± 21	323 ± 26
16.7	196 ± 15	480 ± 32	298 ± 21
22.0	192 ± 21	492 ± 34	319 ± 23

taining 0.5% BSA, 400 mM D-glucose, and 10 mM phlorizin, pH 7.4. Cells were then solubilized with 1 ml 0.1% sodium dodecyl sulfate (SDS), and the cell-incorporated radioactivity was counted and normalized to the DNA content (12). [U-^{14}C]sucrose uptake was performed to measure the extracellular space.

The glucose phosphorylation rate was determined by measuring the rate of glucose-6-phosphate formation in a fluorimetric assay (13). Pancreatic islets (~400) were homogenized in 300 μl of ice-cold buffer (pH 7.7) containing 20 mM K_2HPO_4, 1 mM EDTA, 110 mM KCl, and 5 mM dithiothreitol. The islet homogenate was then centrifuged for 10 min at 12,000 × *g*, and the glucose phosphorylation activity was measured in the supernatant.

The assay volume contained 4 μl of supernatant in 100 μl of HEPES HCl 50 mM (pH 7.7), 100 mM KCl, 7.4 mM $MgCl_2$, 15 mM β-mercaptoethanol, 0.5 mM NAD^+, 0.05% BSA, 2.5 μg/ml glucose-6-phosphate dehydrogenase, 5 mM ATP, and glucose concentrations ranging from 0.03 to 100 mM. In a typical experiment, glucose was added at 0.03, 0.06, 0.12, 0.25, and 0.5 mM to measure hexokinase activity and at 5, 7.5, 10, 15, 20, 25, 50, 65, 80, and 100 mM to measure glucokinase activity. The reaction was stopped after 1 h at 30°C by adding 1 ml of 500 mM sodium bicarbonate buffer, pH 9.4. Fluorescence was then measured at 460 nm (excitation at 340 nm). The V_{max} and K_m of hexokinase and glucokinase activities were calculated in each experiment by the Eadie-Hofstee plot. To calculate glucokinase activity, the V_{max} for hexokinase was subtracted from the activities measured at the concentrations >5 mM (13). A standard curve was obtained by incubating with the assay reagents 0.3–1.0 nmol glucose-6-phosphate and 1 nmol NADH (13). In each assay, tissue blanks were also performed by incubating either 0.5 or 100 mM glucose in the absence of ATP.

The utilization of glucose was determined by measuring the formation of ^3H_2O from (5-^{3}H)glucose (14). Groups of 10 islets were incubated in 40 μl of Krebs-Ringer bicarbonate buffer (118 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM MgSO4, 1.2 mM KH_2PO4, 25 mM $NaHCO_3$) supplemented with 10 mM HEPES (pH 7.4) containing 2 μCi D-(5-^{3}H)glucose, with glucose concentrations ranging from 5 to 20 mM. In a typical experiment, glucose was added at 5, 7.5, 10, 12.5, 15, and 20 mM. The incubation was performed in 1-ml glass vials inside an airtight-sealed 20-ml glass scintillation vial that contained 500 μl distilled water. After 2 h at 37°C, the reaction was stopped by adding 0.5 M HCl (100 μl injected through the rubber seal). The scintillation vials were then incubated overnight at 37°C, and the water radioactivity was measured. Blanks without islets were also conducted. Under these experimental conditions, the recovery from known amounts of ^3H_2O was fairly constant, ranging 50–60%.

Statistical analysis. Statistical significance was determined by the Student's *t* test for unpaired data.

RESULTS

Insulin secretion. In pancreatic islets exposed for 24 h to different glucose concentrations (either 1.4, 5.5, or 16.7 mM), insulin release in the absence of glucose was 64 ± 20, 152 ± 11, and 284 ± 30 pg · $islet^{-1}$ · h^{-1}, respectively (mean ± SE, $n = 6$, $P < 0.05$, G1.4 and G16.7 vs. G.5.5). The maximal response to glucose (22 mM) stimulation was 640 ± 136, 2460 ± 276, and 1890 ± 172 pg · $islet^{-1}$ · h^{-1}, respectively ($n = 6$, G1.4 vs. G5.5, $P < 0.01$, G16.7 vs. G5.5, $P = 0.065$) (Fig. 1*A*). The analysis of the insulin release dose-response curves (Fig. 1*B*) indicated that islet sensitivity to glucose was significantly affected by preexposure to different glucose concentrations. The 50% maximal response (increment over baseline) was reached at an average glucose concentration of 9.9 ± 0.7 mM in control islets (preexposed to G5.5), whereas in islets preexposed to G1.4 and G16.7 the 50% of maximal response was observed at a glucose concentration of 13.3 ± 0.9 and 4.8 ± 0.4 mM, respectively ($P < 0.05$ vs. G5.5).

Insulin secretion was measured also in monolayers of pancreatic cells exposed for 24 h at the three different glucose levels, and comparable results were obtained (Table 1). The 50% maximal response was reached at an average glucose concentration of 12.9 ± 0.5, 9.6 ± 0.6, and 4.7 ± 0.3 in islets preexposed to G1.4, G5.5, or G16.7, respectively ($n = 4$, G1.4 and G16.7, $P < 0.05$ vs. G5.5).

Glucose transport. Glucose transport was measured in pancreatic cells preexposed for 24 h at the same glucose concentrations used for insulin secretion studies. The kinetic characteristics of glucose uptake by pancreatic islet cells (measured at 15°C) were calculated according to the Eadie-Hofstee plot by adding increasing concen-

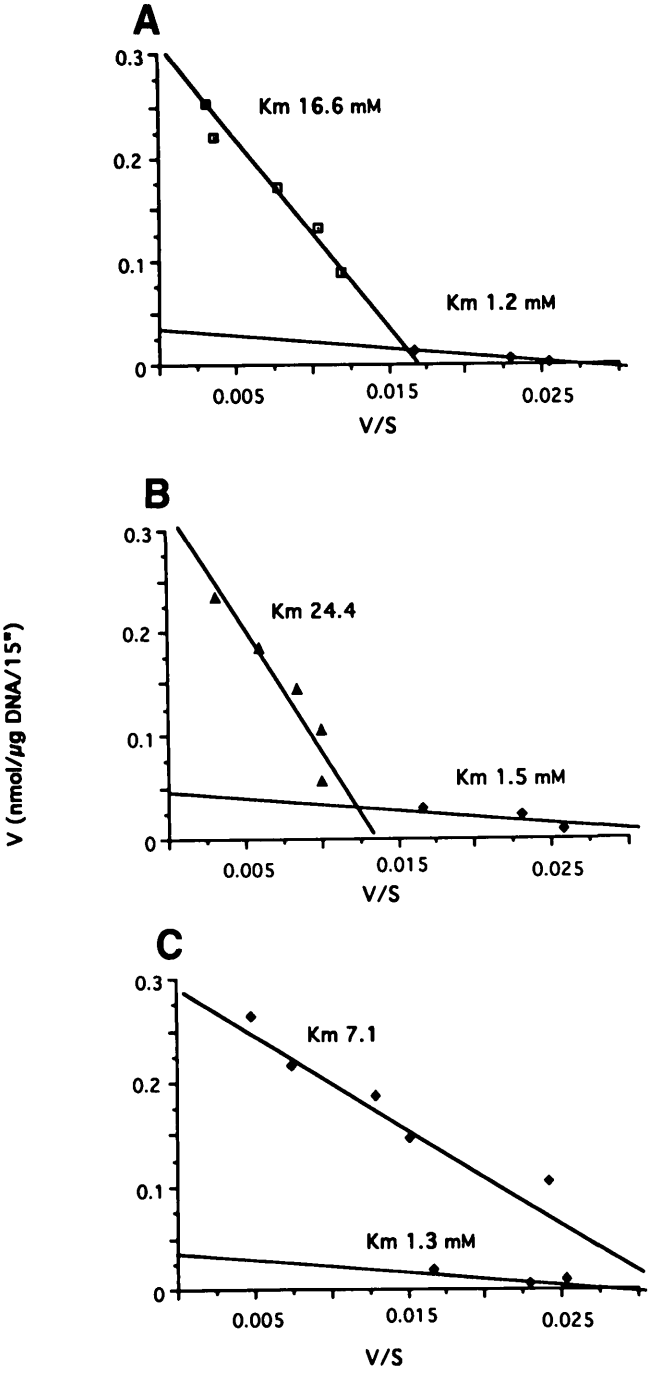

FIG. 2. Glucose transport kinetic in pancreatic islet cells. Cells were cultured in CMRL-1066 medium containing glucose at either 5.5 (*A*), 1.4 (*B*), or 16.7 mM (*C*). To measure [^{3}H]-3OMG transport, cells were washed twice and incubated in Krebs-Ringer phosphate buffer for 30 min at 15°C. The buffer was then replaced with 0.4 ml fresh Krebs-Ringer phosphate containing different concentrations of [^{3}H]-3OMG (range 0.1–60 mM). After 15 s, the transport was stopped by washing the cells with ice-cold Krebs-Ringer phosphate, containing 0.5% BSA, 400 mM D-glucose, and 10 mM phlorizin, pH 7.4. Counts were normalized to the DNA content. Results are presented according to the Eadie-Hofstee plot. A representative of 4 separate experiments is shown.

trations of 3OMG. In agreement with previous studies (1), in pancreatic cells cultured at 5.5 mM of glucose, two kinetically distinct components were identified (Fig. 2*A*): one with an apparent K_m of 16.6 ± 2.4 mM (n = 5) and another with a K_m of 1.2 ± 0.4 mM. The relative capacity of the low K_m component was 10–15%. The high K_m glucose transport function is believed to represent the GLUT2 transporter, a glucose carrier specific of β-cells among all pancreatic cells. Pancreatic cells cultured at both low (1.4 mM) or high (16.7 mM) glucose concentrations exhibited a significant change in the apparent K_m of this glucose transport function component in control islets cultured at 5.5 mM glucose. β-Cells preexposed to low glucose exhibited a lower affinity for glucose (K_m = 24.4 ± 2.9 mM, n = 5, $P < 0.05$ in control islets; Fig. 2*B*) and, in contrast, β-cells preexposed to high glucose exhibited a higher affinity (K_m = 7.1 ± 0.6 mM, n = 5, $P < 0.01$ in control islets; Fig. 2*C*). As a consequence of this higher affinity, glucose transport at 5 mM of glucose was doubled in islets exposed to high glucose in control islets (0.1 vs. 0.044 nmol · μg DNA^{-1} · 15 s^{-1}). No significant change in V_{max} was observed (Table 2). Furthermore, no change in the kinetic characteristics of the low K_m glucose transport function (believed to represent the glucose transporter of pancreatic cells other than β-cells) was observed in the three experimental groups (Fig. 2*C*).

Glucokinase activity. In control islets, cultured at 5.5 mM of glucose, glucokinase activity, measured at 30°C and at glucose concentrations ranging from 5 to 100 mM, showed a V_{max} of 8.7 ± 0.79 nmol · μg DNA^{-1} · h^{-1} and a K_m of 9.7 ± 1.0 mM (mean ± SE, n = 5; Fig. 3). Islets cultured at 1.4 mM glucose showed glucokinase activity with a V_{max} of 4.9 ± 0.32 nmol · μg DNA^{-1} · h^{-1} (n = 5, $P < 0.05$ in control islets) and a K_m of 10.2 ± 1.8 (Table 3). Islets cultured in the presence of 16.7 mM glucose showed a glucokinase activity with a V_{max} of 15.8 ± 0.98 nmol · μg DNA^{-1} · h^{-1} and a K_m that was not significantly different from control islets (Table 3). To calculate the ratio of glucose transport to glucokinase activity at the different glucose concentrations tested, we assumed a Q_{10} of two to adjust for the different temperature used for measuring glucose transport (15°C) and phosphorylation (30°C) (15). The calculated ratio varied at the various glucose concentrations tested, decreasing from 26 at 100 mM of glucose to 8 at 5 mM of glucose (Table 4).

Glucose utilization. In control islets cultured at 5.5 mM of glucose, glucose utilization, increased from 3.8 ± 0.12 at 5 mM of glucose to 7.4 ± 0.33 and 7.6 ± 0.28 nmol · μg DNA^{-1}2 h^{-1} at 15 and 20 mM of glucose, respectively (Fig. 4), with a calculated V_{max} of 8.9 ± 0.41 and a K_m of 8.0 ± 1.7 mM (mean ± SE, n = 7). In islets cultured at 1.4 mM of glucose, a decreased in glucose utilization was observed (Fig. 4), with a V_{max} of 5.3 ± 0.56 and a K_m of 15.6 ± 1.3 mM (n = 4, $P < 0.05$ in control islets). In islets cultured in the presence of 16.7 mM of glucose, an increase in glucose utilization was observed, with a V_{max} of 13.8 ± 0.98 nmol · μg DNA^{-1} · 2 h^{-1} and a K_m of 3.7 ± 0.9 mM (n = 4, $P < 0.05$ in control islets).

Reversibility of glucose-induced alterations. The changes observed in insulin secretion, glucose transport affinity, glucokinase activity, and glucose utilization in islets cultured at either 1.4 or 16.7 mM glucose were totally reversed by incubating these islets for additional

TABLE 2
Effect of preexposure to different glucose concentrations on glucose transport kinetic characteristics in pancreatic islet cells

Culture condition (mM)	Glucose transport V_{max} (nmol · μg $DNA^{-1} \cdot 15\ s^{-1}$)	K_m (mM)
1.4	0.29 ± 0.04	24.4 ± 2.9*
5.5	0.32 ± 0.04	16.6 ± 2.4
16.7	0.27 ± 0.05	7.1 ± 0.6†

*$P < 0.05$, †$P < 0.01$, in islets cells cultured at 5.5 mM of glucose.

24 h in CMRL-medium containing 5.5 mM of glucose (data not shown).

DISCUSSION

These data demonstrate that the ambient glucose levels may regulate both the threshold of glucose-induced insulin release (glucose sensitivity) and the maximal responsiveness of rat pancreatic β-cells to glucose. To investigate the molecular mechanism(s) responsible for these alterations, we studied, in parallel experiments, the kinetic characteristics of glucose transport, glucokinase, and glucose utilization.

Both high and low glucose concentrations in the culture medium affect glucose sensitivity. Islets cultured at high glucose, in addition to a shift to the left of the secretory dose-response curve to glucose, show a coordinate and reversible increase in glucose transporter affinity, glucokinase activity, and glucose utilization. Opposite changes are seen in islets cultured at low glucose. These data strongly suggest that these functions are

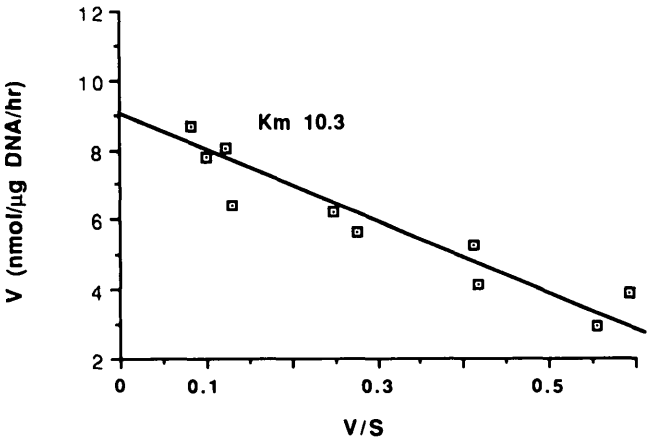

FIG. 3. Glucose phosphorylation kinetics in pancreatic islets. Islets (~400) cultured in medium containing 5.5 mM of glucose were homogenized, centrifuged, and glucose phosphorylation activity measured in the supernatant by evaluating the rate of glucose-6-phosphate formation in a fluorimetric assay. Data are presented according to the Eadie-Hofstee plot. A representative of 5 separate experiments is shown. In a typical experiment the following glucose concentrations were used: 0.03, 0.06, 0.12, 0.25, and 0.5 mM to measure hexokinase activity and 5, 7.5, 10, 15, 20, 25, 50, 65, 80, and 100 mM to measure glucokinase activity. In calculating this activity, the V_{max} for hexokinase was subtracted from the activity measured at glucose concentrations >5 mM.

TABLE 3
Effect of preexposure to different glucose concentrations on glucokinase activity of pancreatic islets

Culture condition (mM)	V_{max} (nmol · μg $DNA^{-1} \cdot h^{-1}$)	K_m (mM)
1.4	4.9 ± 0.32*	10.2 ± 1.8
5.5	8.7 ± 0.79	9.7 ± 1.0
16.7	15.8 ± 0.98*	9.3 ± 1.2

*$P < 0.05$ in islets cultured at 5.5 mM of glucose.

related in pancreatic islets, although the specific role of the glucose transport and the glucokinase activity in determining the difference in glucose sensitivity is still unclear. Because the rate of glucose metabolism is the major determinant of glucose-induced insulin release (3,4), and our data show that a change in the glucose transport K_m is associated with a change in the same direction of the glucose utilization K_m, it is possible that glucose transporter affinity may play a role in determining the observed shift in the dose-response curve of glucose-induced insulin release. For instance, in islets cultured at high glucose, an increased affinity (lower K_m) of the glucose transporter will increase β-cell glucose uptake at glucose concentrations ranging from 3 to 6 mM. The increased glucose availability may increase glucose phosphorylation and utilization and, in turn, stimulate insulin release at glucose levels that, under normal circumstances, are not stimulatory or less stimulatory. A lower threshold of insulin release in response to glucose will be the resulting effect. This mechanism may reflect what has been observed in experimental models like the mouse insulinoma βTC-3 and the hamster-transformed HIT β-cells. In both these cultured cell lines, the dose dependency for glucose-induced insulin release is shifted to the left in comparison to normal islets (16,17). Both these cell lines abnormally express the low K_m glucose transporter GLUT1, which allows glucose transport at much lower glucose concentrations, and may determine, by this mechanism, the increased sensitivity of glucose-induced insulin release. In addition, these cells also have a high glycolytic rate, which may contribute to the observed shift in the dose-response curve of glucose-induced insulin release (18). How an increase in

TABLE 4
Ratio of glucose transport to glucose phosphorylation at different glucose concentrations in pancreatic β-cells cultured at 5.5 mM of glucose

Glucose (mM)	Transport (nmol · μg $DNA^{-1} \cdot h^{-1}$)	Phosphorylation (nmol · μg $DNA^{-1} \cdot h^{-1}$)	Ratio
5	31.6	3.8	8
15	121.7	5.2	23
100	230.4	8.7	26

To calculate the ratio of glucose transport vs. glucokinase activity at the different glucose concentrations tested, we assumed a Q_{10} of 2 to adjust for the different temperature used for measuring glucose transport (15°C) and phosphorylation (30°C).

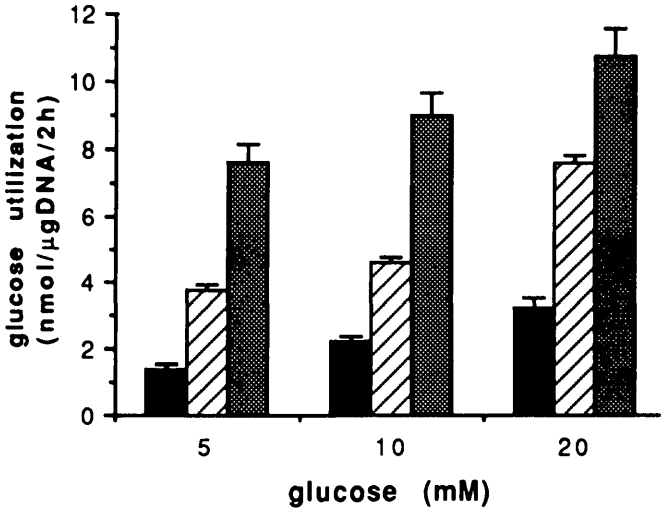

FIG. 4. Effect of preexposure to different glucose concentrations on glucose utilization in rat pancreatic islets. Islets were cultured in CMRL-1066 containing glucose at either 1.4 (*solid bars*), 5.5 (*hatched bars*), or 16.7 (*shaded bars*) mM. After 24 h, groups of 10 islets were incubated in 40 μl of Krebs-Ringer buffer containing 10 mM HEPES, 2 μCi D-[5-^{3}H]glucose, and at various glucose concentrations (5, 7.5, 10, 12.5, 15, and 20 mM). The utilization of glucose was determined by measuring the formation of ^3H_2O from [5-^{3}H]glucose. Glucose utilization at 5, 10, and 20 mM of glucose in the 3 experimental groups is shown (mean ± SE of 4 separate experiments).

glucose transport may possibly increase glucose phosphorylation and metabolism remains to be determined, because in β-cells glucose transport largely exceeds glucose phosphorylation. When we calculated the ratio of glucose transport to glucose phosphorylation, we found a change from 26 at the highest glucose concentration tested (100 mM) to 8 at the lowest concentration tested (5 mM). Similar values have been previously reported for the V_{max} of the two functions (1,15). Therefore, at a glucose concentration near to the threshold of glucose-induced insulin release (~5 mM) glucose transport still exceeds glucose phosphorylation. It is possible, however, that a certain transport/phosphorylation ratio is necessary to stimulate insulin release, and that the increased glucose transport efficiency at low glucose concentrations in islets preexposed to high glucose makes the glucose-sensing mechanism more effective. An additional explanation is that at low environmental glucose levels (1–4 mM), other enzymes (e.g., hexokinase and phosphofructokinase) may also play a role in regulating glucose metabolism and the consequent insulin release in β-cells (15); in islets preexposed to high glucose, the increased transport observed at low glucose concentrations may activate these enzymes. However, the fact that changes in glucose transport and glucose utilization K_m are associated do not necessarily establish a cause-effect relationship. Alternative hypotheses can be made: for instance the observed increase in glucokinase activity may be responsible for the shift in the insulin secretion dose-response curve. As a matter of fact, even small changes of glucose phosphorylation have been associated with a shift in the insulin secretion sensitivity to glucose stimulation (19).

A large body of evidence indicates that glucokinase activity and β-cell responsiveness to glucose are strictly related (3,4). Accordingly, in islets exposed to low glucose we found decreased glucokinase activity and decreased glucose utilization, which was parallel with the decreased islet responsiveness to glucose. In contrast, in islets cultured at high glucose, an increased glucokinase activity and glucose utilization did not correspond with an increase in insulin secretion. An alteration in the insulin secretion pattern after chronic exposure to high glucose has already been reported (8,9,20–22), and may be due to an impairment in one (or >1) distal event(s) of glucose-induced insulin release (23,24).

Altogether, these data raise the possibility that the two major components of the pancreatic β-cells glucose-sensing mechanism may play a complex and interrelated role in determining pancreatic β-cell response to glucose. The role of the two components may change under different conditions. Although the glucokinase activity (V_{max}) acts as the major determinant of β-cell responsiveness, the affinity (K_m) of the glucose transporter might influence β-cell sensitivity to glucose, at least under certain conditions.

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