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Rapid communications

Inhibition of the high-affinity glucose transporter GLUT 1 affects the sensitivity to glucose in a hamster-derived pancreatic beta cell line (HIT)

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Summary. HIT is a hamster-derived beta-cell line which in contrast to normal beta cells that only express the high K_m GLUT-2 glucose transporter, also expresses the low K_m glucose transporter GLUT 1. In HIT cells the abnormal glucose transport mechanism is associated with a marked shift to the left of the glucose-induced insulin release dose-response curve. We have used this cell model to investigate whether changes in glucose transport affect the glucose-induced insulin release. HIT cells were first incubated with a concentration of cytochalasin B (0.4 μmol/l) that selectively inhibits the GLUT-1 but not the GLUT-2 transporter. The consequences of blocking glucose phosphorylation and insulin release were studied. Exposure to 0.4 μmol/l cytochalasin B for 1 h caused a selective loss of the low K_m transport: the calculated V_{max} of GLUT 1 was reduced from 1726 ± 98 to 184 ± 14 pmol · mg protein^{-1} 5 min^{-1} (mean ± SEM, $n = 6$, $p < 0.005$), while no major difference in the high K_m (GLUT-2) transport was observed. In cytochalasin B exposed HIT cells the glucose phosphorylating activity (due to hexokinase and glucokinase) was unaffected. In these cells, however, the dose-response curve of glucose-induced insulin release was significantly shifted to the right: the 50% of maximal response (increment over baseline) was reached at an average glucose concentration of 2.9 ± 0.2 mmol/l (vs 0.6 ± 0.01 mmol/l in control HIT cells mean ± SE, $n = 5$, $p < 0.05$) and the maximal effect was reached at 11.0 mmol/l glucose (vs 2.8 mmol/l in control HIT cells $p < 0.005$). These results are consistent with the hypothesis that the affinity of the glucose transport system may contribute to determination of the glucose threshold concentration that triggers insulin secretion.

Key words: GLUT 1, GLUT 2, glucokinase, glucose sensitivity, insulin release, HIT cells.

The "glucose sensor" is a complex system that allows the pancreatic beta cell to adequately secrete insulin in response to changes in the environmental glucose. How the different components of this system determine the maximal responsivity to glucose (glucose responsiveness) and the threshold concentration of glucose that initiates insulin secretion (glucose sensitivity) is not completely understood. A number of experimental findings suggest that glucose phosphorylation (due to glucokinase) rather than glucose transport, is the major determinant of glucose responsiveness [1]. The mechanism(s) which regulate glucose sensitivity are less clear. We have recently observed that chronic exposure of rat beta cells to different glucose concentrations modulates GLUT-2 affinity, and that these changes are associated with a consensual shift of the dose-response curve of glucose-induced insulin release [2]. This observation is compatible with the hypothesis that the affinity of the beta cell glucose transport might play a role in determining the threshold of glucose-induced insulin release.

To further investigate this hypothesis, we used HIT pancreatic cells, a hamster-derived beta cell line which, in contrast to normal beta cells, abnormally expresses the high-affinity glucose transporter GLUT-1 in addition to GLUT-2 [3]. This cell line also has an altered glucose utilization pattern [4]. These HIT cell abnormalities are associated with a marked shift to the left of the dose-response curve of glucose-induced insulin release. In this cell model we investigated whether exposure to a concentration of cytochalasin B (0.4 μmol/l) which selectively inhibits the GLUT-1 but not the GLUT-2 transporter [5] would affect HIT cell sensitivity to glucose.

Materials and methods

HIT pancreatic cells in permanent culture were grown in Ham F-12 medium (Gibco, Glasgow, UK) containing 10% fetal calf serum and 11 mmol/l glucose. All experiments were performed within culture passages 74–80.

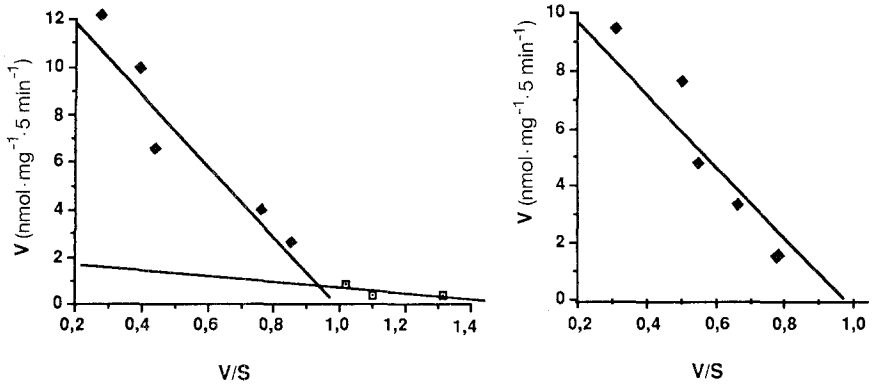

Fig. 1. Glucose transport kinetic in HIT cells. 3-O-[^{3}H]methyl-D-glucose transport was measured in HIT cells incubated in KRP buffer either in the absence (left panel) or in the presence (right panel) of 0.4 µmol/l cytochalasin B (see Materials and methods). The high affinity glucose transport (□) is abolished by cytochalasin B, while the low affinity transport (◆) is unaffected. Results are presented according to the Eadie-Hofstee plot. A representative plot of six separate experiments is shown V, Reaction velocity; S, Substrate concentration

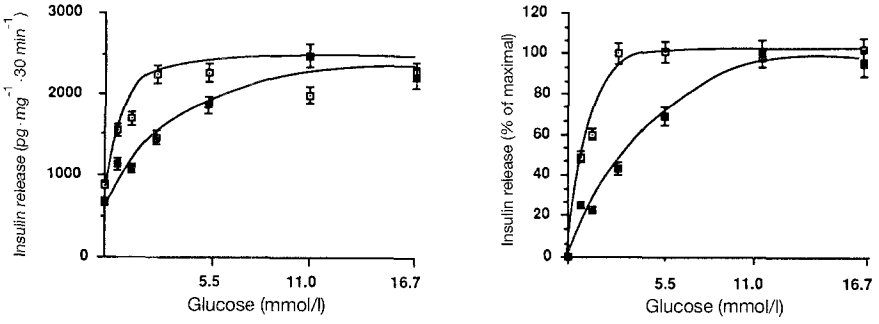

Fig. 2. Effect of pre-exposure to cytochalasin B on glucose-induced insulin release from HIT cells. Left panel: Cells incubated for 1 h either in the absence (□) or in the presence (■) of 0.4 µmol/l cytochalasin B were stimulated with increasing glucose concentrations for 30 min and then insulin released in the medium was measured. Data represent the mean ± SEM of five separate experiments. Right panel: data from the left panel are presented as percent of the maximal effect (increase over baseline)

Glucose transport

HIT cells were washed twice and incubated with Krebs-Ringer-Phosphate (KRP) buffer for 1 h at 37 °C, in the presence or absence of 0.4 µmol/l cytochalasin B. The buffer was then replaced with 0.4 ml of fresh KRP containing increasing concentrations (0.1–60 mmol/l) of 3-O-[^{3}H]methyl-D-glucose (Amersham, Amersham, Bucks., UK, specific activity 2.74 Ci/mmol) at a constant specific activity of 0.06 µCi/mmol. After 5 min at 24 °C the transport was stopped by washing the cells three times with ice-cold KRP containing 0.5% bovine serum albumin, 400 mmol/l D-glucose and 10 mmol/l phlorizin, pH 7.4 [2]. Cells were then solubilized with 1 ml 0.03% sodium dodecyl sulphate (SDS), the cell-incorporated radioactivity was counted and normalised to the protein content. [U-^{14}C]-sucrose uptake was measured to correct for radioactivity in the extracellular space.

Glucose phosphorylation

HIT cells were washed twice and incubated for 1 h at 37 °C in KRP either in the presence or the absence of 0.4 µmol/l cytochalasin B. The glucose phosphorylation rate was determined by measuring the rate of glucose 6-phosphate formation in a fluorimetric assay [2]. The reaction was stopped after 1 h at 30 °C by adding 1 ml of 500 mmol/l sodium bicarbonate buffer, pH 9.4. Fluorescence was then measured at 460 nm (excitation at 340 nm). To calculate the glucokinase activity, the V_{max} for hexokinase was subtracted from the activities measured at concentrations higher than 5.0 mmol/l. A correction factor, Q10 of 2 was used when calculating the ratio of glucose transport to phosphorylating activity in order to adjust for the use of different temperatures when measuring glucose transport (24 °C) and phosphorylation (30 °C) [1].

Insulin secretion

HIT cells were washed twice and incubated for 1 h at 37 °C either in the presence or the absence of 0.4 µmol/l cytochalasin B. Cells were then incubated for 30 min in KRP containing increasing glucose concentrations [3]. The insulin released in the buffer was measured by radioimmunoassay using rat insulin as a standard, and the data were expressed as pg of insulin released per 30 min per mg cell protein.

Statistical analysis

Statistical significance was assessed by Student's *t*-test for unpaired comparisons.

Results

3-O-[^{3}H]methyl-D-glucose uptake

When increasing concentrations of 3-O-methyl-D-glucose were added to HIT cells, the Eadie-Hofstee plot showed two kinetically distinct components (Fig. 1, left panel): one with an apparent Michaelis constant (K_m) of 1.1 ± 0.2 mmol/l (mean ± SEM, $n = 6$), and another with an apparent K_m of 14.1 ± 2.6 mmol/l. The relative capacity of the low K_m component was approximately 15–20%. HIT cells preincubated with 0.4 µmol/l cytochalasin B showed a selective loss of the low K_m transport, with no major change in the high K_m transport (Fig. 1, right panel). In six separate experiments the calculated V_{max} of GLUT-1 after exposure to cytochalasin B was reduced from 1.73 ± 0.09 to 0.18 ± 0.01 nmol · mg protein^{-1} · 5 min^{-1} (mean ± SEM, $p < 0.005$).

Glucose phosphorylation

Two kinetically distinct components of the glucose-phosphorylating activity were seen in HIT cells. One component had an apparent K_m of 0.16 ± 0.02 mmol/l and a V_{max} of 13.4 ± 2.1 nmol · mg · protein^{-1} · 90 min^{-1} (mean ± SEM, $n = 6$), the other one had an apparent K_m of 7.6 ± 0.6 mmol/l and a V_{max} of 54.0 ± 4.1 nmol · mg protein^{-1} · 90 min^{-1}. Pre-exposure to 0.4 μmol/l cytochalasin B did not affect either the low (K_m 7.9 mmol/l, V_{max} 51.5 ± 3.2 nmol · mg protein^{-1} · 90 min^{-1}, $n = 6$) or the high affinity ($K_m = 0.19 \pm 0.02$, V_{max} 12.6 ± 2.7, $n = 6$) glucose-phosphorylating activity.

When the ratio of glucose transport to phosphorylating activity was calculated at different glucose concentrations, different values were found. Glucose phosphorylation exceeded glucose transport by a factor of approximately 2 at glucose concentrations lower than 2.0 mmol/l. In contrast glucose transport was in excess by a factor of approximately 2 at 5 mmol/l and by a factor of approximately 5 at 10 mmol/l glucose.

Insulin secretion

Under basal conditions in HIT cells the insulin secretion pattern in response to glucose was shifted to the left compared to that observed in normal rat beta cells: half maximal stimulation (EC_{50}) was reached at a glucose concentration of 0.6 ± 0.01 (mean ± SEM, $n = 5$) and maximal effect was observed at 2.8 mmol/l glucose (Fig. 2). HIT cell exposure to 0.4 μmol/l cytochalasin B for 1 h, together with the selective loss of the low K_m glucose transport, caused a change of glucose sensitivity. The EC_{50} was reached at an average glucose concentration of 2.9 ± 0.2 mmol/l (vs 0.6 ± 0.01 mmol/l in control HIT cells mean ± SEM, $n = 5$, $p < 0.05$) and the maximal effect was observed at 11.0 mmol/l glucose (vs control HIT cells $p < 0.005$) (Fig. 2). In contrast to glucose sensitivity, the maximal responsiveness to glucose was unaffected by exposure to cytochalasin.

Discussion

The present data indicate that HIT cell exposure to cytochalasin B causes a selective loss of the high-affinity component of glucose transport, a finding that has also been observed in normal rat beta cells [5]. Under these conditions a reduction of glucose sensitivity is observed, as indicated by a shift to the right of the insulin release dose-response curve to glucose.

An increased sensitivity to low glucose concentrations is a characteristic feature of several cultured insulinoma cell lines [6], and human insulinomas [7]. The mechanism causing this altered glucose sensitivity is unknown, but an abnormality of the glucose sensor is the leading candidate. In insulinoma cells an alteration of both components of the glucose sensor has been described: an abnormal glucose transporter expression (high level of the low-K_m GLUT-1) [3, 8, 9], and an abnormal phosphorylation pattern (i.e. prevalence of the low-K_m component, due to an altered intracellular distribution of hexokinase or to a different isoenzyme) [4]. Our study demonstrates that in HIT cells, when the low-K_m component of glucose transport is inhibited and, therefore, only the high-K_m GLUT-2 is functioning, the glucose concentration that initiates insulin release is enhanced, and a shift to the right of the glucose dose-response curve is observed. Since HIT cell preincubation with cytochalasin B caused no change in their glucose phosphorylating activity, these observations suggest that changes in the glucose transport system affinity per se may affect the HIT cell sensitivity to glucose.

Firm evidence has been found that glucose phosphorylation plays a major role in determining insulin secretion pattern in response to glucose, and Epstein et al. [10] have recently demonstrated that changes in the hexokinase activity may also affect glucose sensitivity. It seems reasonable, however, that glucose availability for the phosphorylating enzyme is a prerequisite for either hexokinase or glucokinase function. In particular at low glucose concentrations which are close to the threshold level for stimulating insulin secretion, hexokinase significantly contributes to phosphorylation of glucose [1]. Under these conditions glucose transport is rate-limiting for glucose metabolism in HIT cells, whereas in normal rat beta cells it exceeds glucose phosphorylation, but only slightly in comparison to higher glucose levels [2]. In cultured rat beta cells we have recently demonstrated a co-ordinate effect of the medium glucose concentration on both the glucose transporter affinity and the beta-cell sensitivity to glucose [2], a finding that is consistent with a possible role of glucose transport in influencing the threshold concentration of glucose which triggers insulin secretion. The present data are consistent with this possibility. Altogether these data support the possibility that both component of the glucose sensing mechanism (i.e. transport and phosphorylation) may contribute to determination of the threshold concentration of glucose that stimulates insulin secretion.

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References

1. Matschinsky FM (1990) Glucokinase as glucose sensor and metabolic signal generator in pancreatic β-cells and hepatocytes. Diabetes 39: 647–652
2. Purrello F, Buscema M, Rabuazzo AM et al. (1993) Glucose modulates the glucose transporter affinity, the glucokinase activity, and the secretory response in rat pancreatic islets. Diabetes 42: 199–205
3. Purrello F, Buscema M, Vetri M et al. (1991) Glucose regulates both glucose transport and the glucose transporter gene expression in a hamster-derived pancreatic Beta-cell line (HIT). Diabetologia 34: 366–369
4. Shimizu T, Knowles BB, Matschinsky FM (1988) Control of glucose phosphorylation and glucose usage in clonal insulinoma cells. Diabetes 37: 563–568
5. Johnson JH, Newgard CB, Milburn LJ, Lodish HF, Thorens B (1990) The high K_m glucose transporter of islets of Langerhans is functionally similar to the low affinity transporter of liver and has identical primary sequence. J Biol Chem 265: 6548–6551

6. Clark SA, Burnham BL, Chick WL (1990) Modulation of glucose-induced insulin secretion from a rat clonal β-cell line. Endocrinology 127: 2779–2788
7. Yasunami Y, Funakoshi A, Ono J, Miyazaki K, Jimi A, Konomi K (1987) In vitro study of cultured human insulinoma cells: evidence of abnormal sensitivity to glucose. J Clin Endocrinol Metab 65: 110–115
8. Brant AM, McCoid S, Thomas HM et al. (1992) Analysis of the glucose transporter content of islet cell lines – implications for glucose-stimulated insulin release. Cell Signal 4: 641–650
9. Seino Y, Yamamoto T, Inoue K et al. (1993) Abnormal facilitative glucose transporter gene expression in human islet cell tumors. J Clin Endocrinol Metab 76: 75–78
10. Epstein PN, Boschero AC, Atwater I, Xingang C, Overbeek PA (1992) Expression of yeast hexokinase in pancreatic beta cells of transgenic mice reduces blood glucose, enhances insulin secretion, and decreases diabetes. Proc Natl Acad Sci USA 89: 12038–12042

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