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The glucose sensor in HIT cells is the glucose transporter

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The nature of the rate-limiting step for glucose utilization by the clonal insulin-producing cell line HIT-T15 has been investigated. In contrast to the situation in islets of Langerhans, we find that the HIT cell glucose metabolism is limited by the rate of entry of glucose into the cell. This is evidenced by the low rate of sugar transport and by the marked reduction in the rate of glucose utilization elicited by inhibitors of the glucose transporter. As judged by competition with glucose, the HIT cell glucose transporter also transports mannose, 2-deoxyglucose and 3-*O*-methylglucose but not L-glucose or *N*-acetylglucosamine. The K_m for glucose of the glucose transporter, measured as the concentration of glucose required for a half-maximal rate of glucose utilization, is 4.3 mM, similar to the concentration reported to give half-maximal insulin release. Glucose-stimulated insulin release from HIT cells is inhibited by phloretin or cytochalasin B but not by mannoheptulose. We conclude that the secretory responses of HIT cells are consistent with the substrate-site hypothesis, but that, in contrast to normal B-cells, the glucose sensor which confers concentration-dependence and specificity to sugar-stimulated insulin release, is the glucose transporter.

Insulin secretion; Glucose transport; B-cell line; (Pancreatic B-cell)

1. INTRODUCTION

In the B-cells of the islets of Langerhans glucose transport into the cell is non-rate-limiting [1]; the rate-limiting step for sugar metabolism is hexose phosphorylation [2]. The high K_m (8 mM) for glucose utilization by the B-cell [1,3] is a consequence of the presence of a high K_m glucokinase [1,4,5]. Since changes in glucose metabolism rates are causally related to insulin release [2], the glucokinase is regarded as the glucose sensor conferring concentration-dependence and specificity to the insulin secretory response to sugars [2,6]. The cloned B-cell line HIT-T15 is an important model for studying insulin secretory mechanisms because these cells, which can be grown in culture in essentially unlimited amounts, retain a secretory response to glucose [7-9]. We [9] and others [8] have noted, however, that the K_m for glucose of the glucose-stimulated insulin release in HIT cells is lower than in normal B-cells: this difference has been attributed to slow uptake of the sugar [10]. If glucose entry is indeed rate-limiting in HIT cells then it follows that modification of the rate of transport of the sugar should produce parallel effects on glucose utilization and hence insulin release. In other words the glucose-sensor in HIT cells would be the glucose transporter. The present study provides evidence for this concept.

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2. MATERIALS AND METHODS

HIT-T15 cells were cultured in RPMI 1640 containing 10% foetal calf serum and antibiotics as described [9]. For measurement of rates of glucose utilization and insulin release 5×10^5 cells were seeded in 1 ml medium in 24-well multiwells. After culture for 3-6 days, the culture medium was

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removed and replaced by a Hepes-buffered bicarbonate medium [11] supplemented with 5 mg/ml albumin. After 30–60 min the latter was aspirated and replaced with a similar bicarbonate medium containing test substances. When glucose utilization was to be measured as the formation of ^3H_2O from [5-^{3}H]glucose [3] the medium contained 1 μCi [5-^{3}H]glucose. After 45–90 min at 37°C in an atmosphere of humidified air, samples of the media from each well were taken and centrifuged at 4°C for 5 min at 500 × g to sediment any free floating cells. Aliquots of the supernatant were diluted with phosphate buffer containing 2 mg/ml albumin and merthiolate and stored frozen until assayed for insulin by a charcoal-based radioimmunoassay [12]. Further 100 μl aliquots of the supernatant were acidified by the addition of 20 μl N·HCl and placed in small tubes inside stoppered scintillation vials containing 0.5 ml H_2O. After equilibration overnight at 37°C, the ^3H_2O recovered in scintillation vials was measured by liquid scintillation spectrometry.

For measurement of sugar uptake, 10^5 cells were incubated in 100 μl Hepes-buffered bicarbonate medium containing albumin (5 mg/ml) and either ^{3}HOH (10 μCi/ml), 3-O-[U-^{14}C]methylglucose (0.5 mM; 2–10 μCi/ml), [U-^{14}C]sucrose (0.5 mM; 2–10 μCi/ml) or [2-^{3}H]glucose (0.5 mM; 2–10 μCi/ml). After incubation at 37°C, cells were spun through 100 μl of a mixture of dibutyl- and dinonylphthalate (3:1) into 20 μl of 6 M urea in a Beckman microfuge tube. The tubes were frozen in liquid N_2 and their tips cut off; tip contents were dissolved in 10 ml Opti-fluor (Packard Instruments) for measurement of radioactivity by liquid scintillation spectrometry using a Packard Tricarb with the external standard method for quench correction. The water and sugar spaces of the HIT cells were calculated after correction for the contamination of the cell pellet by the incubation medium, using [U-^{14}C]sucrose as extracellular space marker.

3. RESULTS

The intracellular water space of HIT cells was 0.81 ± 0.12 pl/cell (n = 6). The uptake of 3-O-methylglucose was slow: after 5 min incubation the 3-O-methylglucose space was only 33% of the intracellular H_2O space. After 20 min incubation 3-O-methylglucose had attained 76% equilibration. [2-^{3}H]Glucose uptake was also slow, averaging after 5 and 20 min respectively 26 and 54% of the intracellular H_2O space.

Rates of glucose utilization by HIT cells were linear for 2 h and were dependent on the extracellular glucose concentration over the range 0.5–20 mM. A double-reciprocal plot was linear and indicated a K_m for glucose of 4.3 mM (fig.1). Table 1 shows the effects of various agents on glucose utilization. Mannoheptulose (20 mM) did not significantly reduce the rate of glucose utilization at 5 mM glucose. However, glucose utilization was severely depressed by the inhibitors of glucose transport systems, phloretin (0.18 mM), phloridzin (5 mM) and cytochalasin B (50 μg/ml). Glucose utilization was also inhibited by caffeine (5 mM) or forskolin (10 μM). The effects of lower concentrations of phloretin and cytochalasin B are shown in table 2. As little as 0.1 μg/ml cytochalasin B produced significant inhibition of glucose utilization; phloretin was effective at 50 but not at 20 μM. The effects of other sugars on glucose utilization are given in table 3. Sugars were tested at a concentration of 20 mM in the presence of 1 mM glucose. No significant effect was observed with L-glucose or N-acetylglucosamine. A small inhibition occurred in the presence of galactose, fructose, goldthioglucose or 6-deoxygalactose. Glucose utilization was greatly depressed by the simultaneous presence of mannose, 3-O-methylglucose or 2-deoxyglucose.

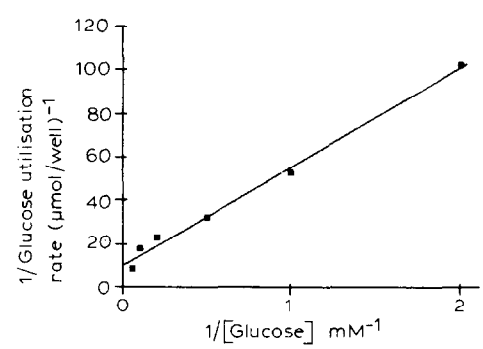

Fig.1. HIT cell glucose utilisation. Rates of glucose utilization were measured as the rate of formation of ^3H_2O from [5-^{3}H]glucose by HIT cells incubated for 2 h at 37°C. Rates were linear over this period. The data are plotted as a double-reciprocal plot of the mean values for 8 determinations at each glucose concentration.

Table 1

Effects of phloretin, phloridzin, cytochalasin B and caffeine on HIT cell glucose utilization

Agent	Concentration	Glucose utilization (nmol/well)
None	–	55.5 ± 3.4 (24)
Phloretin	0.18 mM	19.9 ± 1.6 (8)*
Phloridzin	5 mM	18.8 ± 0.6 (4)*
Cytochalasin B	50 μg/ml	2.3 ± 0.1 (4)*
Caffeine	5 mM	36.1 ± 1.4 (4)*
Forskolin	1 mM	16.6 ± 1.0 (4)*

* Significantly different from control $P<0.001$

HIT cells were incubated with [5-^{3}H]glucose (1 mM) and the additions shown for 1 h at 37°C. Glucose utilization was measured as the formation of ^3H_2O as described in the text. Data are given as mean ± SE for the number of observations shown

The effects of mannoheptulose, cytochalasin B and phloretin on insulin secretion are shown in table 4. In the presence of 5 mM glucose, insulin release was not inhibited by 20 mM mannohep-

Table 2

Effects of varying concentrations of phloretin and cytochalasin B on HIT cell glucose utilization

Agent	Concentration	Glucose utilization (nmol/well)	
None	–	52.2 ± 1.4 (6)	
Phloretin	10 μM	54.9 ± 3.1	
	20 μM	51.5 ± 3.1	
	50 μM	42.5 ± 0.9 (3)	*
	100 μM	30.6 ± 0.7	*
	200 μM	20.2 ± 0.7	*
Cytochalasin B	0.1 μg/ml	42.0 ± 6.3	
	0.2 μg/ml	39.7 ± 2.0	*
	0.5 μg/ml	23.3 ± 2.9	*
	1 μg/ml	16.1 ± 2.9	*
	5 μg/ml	6.0 ± 2.2	*
	10 μg/ml	6.3 ± 2.3	*

* Significantly less than control $P<0.001$

HIT cells were incubated with [5-^{3}H]glucose (1 mM) and the additions shown for 1 h at 37°C. Glucose utilization was measured as the formation of ^3H_2O as described in the text. Data are given as mean ± SE for 4 observations except where indicated otherwise

Table 3

Effects of sugars on HIT cell glucose utilization

Agent (20 mM)	Glucose utilization (% of control)
None	100 ± 6 (20)
Mannose	16 ± 7 (12)*
2-Deoxyglucose	13 ± 3 (8)*
3-*O*-Methylglucose	32 ± 5 (4)*
Galactose	78 ± 2 (8)*
6-Deoxygalactose	64 ± 2 (8)*
Fructose	85 ± 2 (8)*
Gold thioglucose	77 ± 8 (8)*
N-Acetylglucosamine	103 ± 4 (8)
L-Glucose	103 ± 4 (8)

* Significantly less than control ($P<0.001$)

All sugars are the D-stereoisomer except for L-glucose. HIT cells were incubated with [5-^{3}H]glucose (1 mM) and the sugars shown (20 mM) for 1 h at 37°C. Glucose utilization was measured as the formation of ^3H_2O. Data are expressed as % of control (no addition) rate in the same experiment and are given as mean ± SE for the number of observations shown. The mean absolute value of glucose utilization in these experiments was 27.7 nmol/well

Table 4

Effects of phloretin, cytochalasin B and monnoheptulose on glucose-stimulated insulin secretion by HIT cells

Agent	Concentration	Insulin release (% control)
None	–	100 ± 6 (20)
Phloretin	20 μM	92 ± 6 (8)
	50 μM	55 ± 2 (7)*
	100 μM	16 ± 1 (8)*
	200 μM	10 ± 1 (7)*
Cytochalason B	10 μg/ml	47 ± 8 (4)*
	100 μg/ml	44 ± 10 (4)*
Mannoheptulose	20 mM	93 ± 4 (7)

* Significantly less than control $P<0.001$

HIT cells were incubated in the presence of 5 mM glucose and the additions shown for 1 h at 37°C. Data are expressed relative to the mean control (no addition) rates of insulin release in the same experiment and are given as mean ± SE for the number of observations shown

tulose but was markedly decreased by 50–180 μM phloretin or 10–100 μg/ml cytochalasin B.

4. DISCUSSION

In confirmation of a previous study [10] we find that the uptake of sugars into HIT-T15 B-cells is slow, in marked contrast to normal rat or mouse islet cells [1,13,14]. Direct measurement of glucose in HIT cells also showed that the intracellular concentration remained lower than extracellular after 30 min incubation with 1 or 10 mM glucose [10]. Thus, the transport of sugars into the cell may represent the rate-limiting step for their utilization. If this is so, then agents inhibiting glucose transport should produce parallel inhibition of glucose utilization by HIT cells. This prediction has been verified in the present study. Both phloridzin and its aglycone phloretin inhibit mammalian glucose transporters in a variety of cells including islets of Langerhans [13,15,16]. Both agents markedly depressed HIT cell glucose utilization with phloretin being particularly potent. This situation contrasts with normal islets in which inhibition of glucose transport by phloretin is not accompanied by impaired glucose utilization because of the non-rate-limiting nature of sugar transport [2]. HIT cells glucose utilization was also inhibited by cytochalasin B which inhibits the glucose transporter in various tissues [17], including islets [18]. Caffeine also inhibited HIT cell glucose utilization as predicted from the inhibitory effect of the methylxanthine on sugar uptake in islets and other cells [14]. In contrast, mannoheptulose which inhibits glucose phosphorylation and glucose utilization in normal islets [2,3] did not inhibit glucose utilization in HIT cells. This finding is also consistent with the view that in HIT cells the rate-limiting step for glucose metabolism is transport rather than phosphorylation; it may also be, however, that mannoheptulose is only poorly taken up by HIT cells.

Some information on the characteristics of the HIT cell glucose transporter is afforded by the present study. Thus the K_m for glucose utilization of 4 mM reflects the affinity of the glucose transporter for the sugar. Moreover those sugars that reduce the rate of formation of ^3H_2O from [5-^{3}H]-glucose indicate those sugars which are recognised by the transport system. Neither L-glucose nor *N*-acetylglucosamine are substrates for the transporter. Galactose, 6-deoxygalactose, goldthioglucose and fructose compete only weakly with glucose for entry. However, mannose, 3-*O*-methylglucose and 2-deoxyglucose are well transported as evidenced by the marked inhibition of glucose utilization. This pattern of specificity is broadly similar to that in normal islets except that in the latter there is significant uptake of *N*-acetylglucosamine albeit at a much slower rate than glucose [2,19].

Since in the substrate-site hypothesis [2,20] there is close correspondence between rates of glucose utilization and rates of glucose-stimulated insulin release, the present study permitted the prediction that HIT cell insulin release should differ from that of normal islets in certain specific aspects. Firstly, the concentration dependence of glucose-stimulated insulin release should be lower than in normal islets, reflecting the affinity of the transport system rather than of glucokinase; this has already been noted [8,9]. Secondly, glucose-stimulated insulin release by HIT cells in contrast to that in normal islets should be inhibited by phloretin and cytochalasin B: this prediction was confirmed. Thirdly, and again confirmed here, mannoheptulose which markedly inhibits glucose-stimulated insulin release from normal islets should not inhibit release from HIT cells.

We conclude from these observations that the secretory behaviour of HIT cells is consistent with the substrate-site hypothesis but, in contrast with normal B-cells, the glucose sensor is the sugar transporter.

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