Biochimica et Biophysica Acta, 1063 (1991) 90–94

ADONIS 0005273691001386

BBAMEM 75172

Insulin regulates Na^+/glucose cotransporter activity in rat small intestine

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(Received 10 August 1990)

Key words: Insulin; Glucose transport; Sodium ion/glucose cotransporter; Streptozotocin-induced diabetes; Phlorizin binding; Brush-border membrane vesicle; (Rat small intestine)

In order to examine the involvement of insulin in the activity of Na^+/glucose cotransporter in rat small intestine, we compared Na^+-dependent uptake of D-glucose by brush-border membrane vesicles prepared from control, streptozotocin-induced diabetic, insulin-treated diabetic and starved diabetic rats. In four groups, the uptake of D-glucose showed a transient overshoot in the presence of Na^+ gradient between medium and vesicles (medium > vesicles). The overshoot magnitude was increased (1.8-fold of controls) in diabetic brush border membrane vesicles and recovered to the control level by the treatment of diabetic rats with insulin. In contrast, increased uptake of D-glucose in diabetic rats was not recovered by the starvation of diabetic rats although the blood glucose level was the same as that of controls. Furthermore, we attempted to examine phlorizin binding activities among four groups. Scatchard analysis indicated that phlorizin binding to diabetic brush border membrane vesicles was increased (1.6-fold of controls) without a change of the affinity for phlorizin as compared with controls. Increased binding of phlorizin to diabetic brush border membrane vesicles was also recovered to the control level by the treatment of diabetic rats with insulin, but not by starvation. These results suggested that the increased activity of Na^+/glucose cotransporter in diabetic rats was due to the increase of the number of cotransporter and that intestinal cotransporter was physiologically controlled by insulin, but not by blood glucose levels.

Introduction

Transport of D-glucose into mammalian cells is mediated by specific carriers [1,2] which have been classified into two types on the basis of the energy requirement; facilitated transporters and Na^+-dependent transporters. The rate of D-glucose uptake into adipocytes and skeletal muscle is increased by insulin [3–8]. The mechanism is in part explained by the translocation of intracellular glucose transporter to the plasma membrane [3]. Recently it has been known that there is a second activation following the translocation to the plasma membrane [9]. On the other hand, D-glucose transporter activity was decreased in hypoinsulinemic diabetic rat adipocytes [10] and the treatment of diabetic rats with insulin resulted in a hyperresponsive insulin-stimulated glucose transporter activity in the adipocytes [11]. Thus, the utilizing system of blood glucose by the adipocytes, which is regulated by insulin, is impaired in diabetes.

On the contrary, an energy-dependent uptake of D-glucose (Na^+/glucose cotransport) in small intestine of diabetic rats was greater than the corresponding uptake in controls [12–14]. At present, however, little is known about the mechanism leading to increase of the activity of the small intestinal Na^+/glucose cotransporter in diabetic rats. Furthermore, it is not clear whether the small intestinal Na^+/glucose cotransporter is regulated by insulin.

In this paper, we report that the increased activity of Na^+/glucose cotransporter in brush border membrane vesicles isolated from streptozotocin-induced diabetic rats was restored to that of controls by the treatment of diabetic rats with insulin, but not by starvation of the diabetic rats, although the blood glucose concentrations in diabetic rats were restored to those of controls under both conditions. These data suggest that insulin physio-

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logically controls Na^+/glucose cotransporter activity in small intestine.

Materials and Methods

Materials. D-[^{3}H]Glucose was obtained from American Radiolabeled Chemicals Inc. (St. Louis, MO). [^{3}H]Phlorizin and L-[^{3}H]glucose were from New England Nuclear (Boston, MA). Streptozotocin was kindly given from The Upjohn Company (Kalamazoo, MI). Insulin was obtained from Novo Industry (Denmark). Other chemicals were of highest purity available.

Animals and preparation of small intestinal brush-border membrane vesicles. Diabetes mellitus was induced in 250–300 g male Wistar rats with an i.v. injection of 65 mg/kg streptozotocin as previously described [15]. After the induction of diabetes mellitus, rats were fed ad libitum and rats were killed at 12–15 days after the injection. In indicated cases, some diabetic rats were subcutaneously injected with 5 IU of insulin every 12 h for 5 days and others were starved for 3 days. Concentrations of blood glucose in controls, streptozotocin-induced diabetic, insulin-treated diabetic and starved diabetic rats were 88.2 ± 3.1 mg/dl ($n = 39$), 375.6 ± 7.1 mg/dl ($n = 22$), 77.6 ± 7.0 mg/dl ($n = 21$) and 61.0 ± 5.8 mg/dl ($n = 15$), respectively. Body weight of controls, streptozotocin-induced diabetic, insulin-treated diabetic and starved diabetic rats were 337 ± 4 g ($n = 39$), 258 ± 5 g ($n = 22$), 317 ± 5 g ($n = 21$) and 232 ± 9 g ($n = 15$), respectively. Brush border membrane vesicles were isolated from the jejunum of above-mentioned groups by the method of calcium precipitation [16]. The membrane vesicles were suspended in 20 mM Tris-Hepes/100 mM mannitol buffer (pH 7.5).

Transport study. Transport of D-[^{3}H]glucose or L-[^{3}H]glucose into brush-border membrane vesicles was measured by a rapid filtration technique as previously described [15]. The reaction was initiated at room temperature by adding 50 μl of the above-mentioned buffer containing D-[^{3}H]glucose or L-[^{3}H]glucose and either 100 mM NaCl or KCl to 150 μl of the membrane vesicle suspension (5–10 mg/ml protein), which was preincubated for 10 min at 21°C. The reaction was terminated by filtering through cellulose nitrate filters (0.45 μm pore size) and rapidly washed with 20 ml of ice cold PBS. The radioactivity remaining on the filter was determined by liquid scintillation spectrometry.

Phlorizin binding study. Binding of [^{3}H]phlorizin to membrane vesicles was measured by a rapid filtration method. The reaction was initiated by adding 90 μl of 23 mM Tris-Hepes/115 mM mannitol buffer (pH 7.5) containing [^{3}H]phlorizin and either 100 mM NaCl or KCl to 10 μl of the membrane vesicle suspension (5–10 mg/ml protein). The reaction was performed at 21°C for 30 s and terminated by adding 3 ml of ice-cold PBS and filtering on cellulose nitrate filter (0.45 μm pore size) and rapidly washed with 20 ml of PBS. Na^+-dependent phlorizin binding activity was calculated by subtracting the activity in the presence of KCl from that in the presence of NaCl.

Enzyme assays and protein determinations. Sucrase activity was assayed by the method of Dahlqvist [17]. Alkaline phosphatase activity was assayed by the method of Fujita et al. [18]. Protein concentrations were determined by the method of Lowry et al. [19].

Results

Time-course of D-glucose uptake by small intestinal brush border membrane vesicles

The time course of D-glucose up take by intestinal membrane vesicles isolated from control rats was shown in Fig. 1. In the presence of an initial 100 mM NaCl gradient between medium and vesicles (medium > vesicles), the uptake of D-glucose showed a transient overshoot which reached a maximum in about 20 s. The transient overshoot of D-glucose uptake was not observed in the presence of 100 mM KCl. Furthermore, no overshoot in the uptake of L-glucose was observed in spite of the presence of an initial 100 mM NaCl gradient between medium and vesicles (medium > vesicles). These data indicated that the uptake of glucose occurred in a stereospecific manner. On the other hand, the overshoot magnitude of D-glucose uptake was increased 1.8-fold in the intestinal brush border membrane vesicles from streptozotocin-induced rats as com-

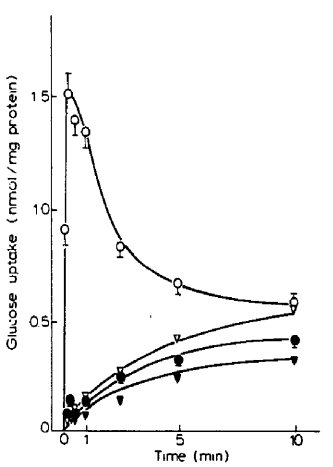

Fig. 1. Time-course of glucose uptake by brush-border membrane vesicles from control rat small intestine. Brush-border membrane vesicles were preincubated at 21°C in 20 mM Tris-Hepes/100 mM mannitol buffer (pH 7.5) for 10 min. Vesicles were subsequently incubated at room temperature with either 1 mM D-[^{3}H]glucose (○, ●) or 1 mM L-[^{3}H]glucose (▽, ▼) in the presence of either 100 mM NaCl (○, ▽) or 100 mM KCl (●, ▼). Each value is the mean ± S.E. of five experiments performed in duplicate determinations.

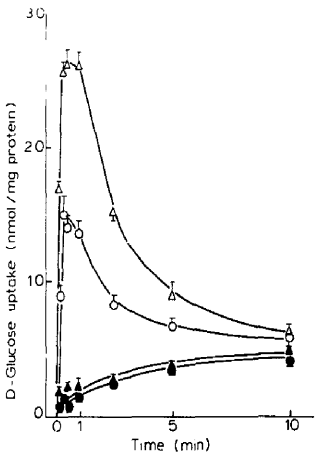

Fig. 2. Time course of D-glucose uptake by brush-border membrane vesicles from control and streptozotocin-induced diabetic rats. Brush-border membrane vesicles from small intestine of control (○, ●) and streptozotocin-induced diabetic rats (△, ▲) were preincubated at 21°C in 20 mM Tris-Hepes/100 mM mannitol buffer (pH 7.5) for 10 min. Vesicles were subsequently incubated at room temperature with 1 mM D-[^{3}H]glucose in the presence of either 100 mM NaCl (○, △) or 100 mM KCl (●, ▲). Each value is the mean ± S.E. of five experiments performed in duplicate determinations.

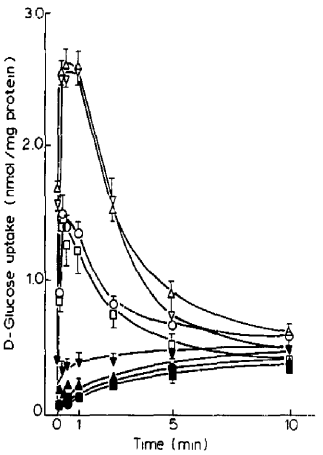

Fig. 3. Time-course of D-glucose uptake by brush border membrane vesicles from control, streptozotocin-induced diabetic, insulin-treated diabetic and starved diabetic rats. Brush-border membrane vesicles from control (○, ●), streptozotocin-induced diabetic (△, ▲), insulin-treated diabetic (□, ■) and starved diabetic rats (▽, ▼) were preincubated at 21°C in 20 mM Tris-Hepes/100 mM mannitol buffer (pH 7.5) for 10 min. Vesicles were subsequently incubated at room temperature with 1 mM D-[^{3}H]glucose in the presence of either 100 mM NaCl (○, △, □, ▽) or 100 mM KCl (●, ▲, ■, ▼). Each value is the mean ± S.E. of five (control, streptozotocin-induced diabetic rats and insulin-treated diabetic rats) and three (starved diabetic rats) experiments performed in duplicate determinations.

pared with controls (Fig. 2). The enrichment in the activities of sucrase and alkaline phosphatase, which are marker enzymes in the brush border membrane, in controls and diabetes was not significantly different from each other (Table I). These results excluded the possibility that a difference in the enrichment of brush border membrane vesicles was the cause for the increase in D-glucose uptake. Furthermore, Na^+-independent D-glucose up take into the membrane vesicles from diabetic rats was not different from that in controls, indicating that the intravesicular volume from both vesicles was almost similar.

TABLE I

Alkaline phosphatase and sucrase activities in homogenates, basolateral membranes (BLM) and brush border membrane vesicles (BBMV) from small intestine of control and streptozotocin-induced diabetic rats

Enzyme activities are expressed as the mean ± S.E. (μmol/mg protein per min). The numbers in paratheses indicate the numbers of experiments performed in duplicate determinations.

	Alkaline phosphatase		Sucrase	
	control (n = 13)	diabetes (n = 11)	control (n = 13)	diabetes (n = 11)
Homogenate (H)	1.17 ± 0.10	1.21 ± 0.10	0.06 ± 0.01	0.08 ± 0.01
BLM	1.33 ± 0.13	1.09 ± 0.08	0.06 ± 0.01	0.07 ± 0.01
BBMV	8.91 ± 0.57	8.61 ± 0.66	0.62 ± 0.06	0.79 ± 0.06
Enrichment (BBMV/H)	7.6	7.1	10.3	9.9

Recovery from the above-mentioned increase in the Na^+/glucose cotransporter activity in diabetic brush border membrane vesicles by insulin treatment

As shown in Fig. 3, the increased Na^+/glucose cotransporter activity in diabetic brush border membrane vesicles was restored to that of controls by the treatment of diabetic rats with insulin. In order to ascertain if the effect of insulin is mediated by the lowering of the blood glucose levels, we attempted to reduce the blood glucose level by starvation of diabetic rats for 3 days. Although the blood glucose level was completely lowered to the normal level by starvation, the uptake of D-glucose by brush-border membrane vesicles from starved diabetic rats was almost the same as that in the diabetic rats (Fig. 3). On the other hand, Na^+-independent uptake of D-glucose by the vesicles in these four groups was not different from each other. These results suggest that the effect of insulin on the decrease in Na^+/glucose cotransporter activity is not mediated by the hypoglycemic action to the epithelial cells in small intestine.

Comparison of Na^+-dependent [^{3}H]phlorizin binding among controls, diabetic, insulin-treated diabetic and starved diabetic rats

In order to further examine the effect of insulin on Na^+/glucose cotransporter in the small intestine, we

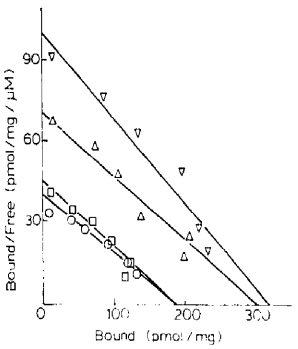

Fig. 4. Na^+-dependent phlorizin binding activity of brush border membrane vesicles form control, streptozotocin-induced diabetic, insulin-treated diabetic and starved diabetic rats. Brush-border membrane vesicles from control (○), streptozotocin-induced diabetic (△), insulin-treated diabetic (□) and starved diabetic rats (▽) were incubated at 21°C for 30 s in 23 mM Tris-Hepes/115 mM mannitol buffer (pH 7.5) containing [^{3}H]phlorizin, 5 mM $MgCl_2$ and either 100 mM NaCl or 100 mM KCl. Na^+-dependent phlorizin binding activity was calculated by subtracting the activity in the presence of KCl from that in the presence of NaCl. Data were expressed by Scatchard analysis of Na^+-dependent binding activity. Each value represents the mean of five (control), three (diabetes), four (insulin-treated diabetes) and two (starved diabetes) experiments performed in duplicate determinations.

attempted to compare with the binding activity of [^{3}H]phlorizin to membrane vesicles from four groups. Membrane vesicles were incubated for 30 s at 21°C in the medium varying phlorizin concentration from 1.0 μM to 12.0 μM. As shown in Fig. 4, Scatchard analysis of the binding data demonstrated that the K_d values for phlorizin were not significantly different between controls (4.7 μM), diabetes (4.3 μM), insulin-treated diabetes (4.2 μM) and starved diabetes (3.2 μM). On the other hand, the B_{max} value of diabetes group (300.0 pmol/mg protein) was also increased as compared with that of the control group (186.7 pmol/mg protein). Furthermore, the B_{max} value in the insulin-treated diabetes group (186.7 pmol/mg protein) was returned to the same as control value. In contrast, the B_{max} value in starved diabetes group (316.7 pmol/mg protein) was not different from that of diabetes. These results were coincident with the change in the Na^+/glucose cotransporter activity in the diabetes, insulin-treated diabetes and starved diabetes groups.

Discussion

In this communication we have indicated that the activity of small intestinal Na^+/glucose cotransporter from streptozotocin-induced diabetic rats is increased 1.8-fold as compared with controls (Fig. 2) and that the enhanced activity is due to the increase in the number of Na^+/glucose cotransporter protein (Fig. 4). Moreover, this increase was restored by the treatment of diabetic rats with insulin (Fig. 3). Previously we reported that the activity of renal Na^+/glucose cotransporter was decreased in streptozotocin-induced diabetic rats as compared with controls and that this decrease was restored to the control activity by the lowering of the blood glucose concentrations by both the treatment of diabetic rats with insulin and starvation, suggesting that renal Na^+/glucose cotransporter is regulated by the change in blood glucose concentrations [15]. On the contrary, the enhanced cotransporter activity in the small intestine from diabetic rats was not restored by the starvation. These results suggest that the change in the Na^+/glucose cotransporter in the small intestinal brush-border membranes by insulin is not mediated by the change in the blood glucose concentrations. At present, however, the reason why opposite changes are observed in the small intestine and the kidney is unclear.

The change in Na^+-dependent uptake of D-glucose in the brush border membrane is elicited not only by the activity of Na^+/glucose cotransporter but also by the electrochemical Na^+ gradient which is changeable in accordance with the passive influx of Na^+. On the basis of these considerations, Hopfer had concluded that the enhanced uptake of D-glucose by brush-border membrane vesicles from diabetic rats was due to a reduced passive Na^+ permeability [13]. If that is the case, the uptakes of all solutes which are dependent on the electrochemical Na^+ gradient must be increased as the result of diabetes. In previous studies, the increase in small intestinal Na^+-dependent uptakes of solutes including sugars [12–14] amino acids [20] and phosphate [21] had been shown in diabetes mellitus as compared with those in the controls. However, no increase in Na^+-dependent uptake of sulfate was observed in diabetic brush border membrane vesicles [14]. Thus, the increase in the Na^+-dependent uptake of D-glucose in diabetes seems to be not unequivocally explained only by a reduction in the passive Na^+ permeability.

Insulin stimulates glucose uptake into adipocytes, at least in part, via the translocation of the intracellular membranes to the plasma membranes [3,4,8]. To our knowledge, however, our present findings that the number of the small intestinal Na^+/glucose cotransporter molecule is changed in response to insulin are the first observation. At present, however, we have no explanation as to the mechanism of insulin action in the small intestine. Expression of Na^+/glucose cotransporter and/or translocation of this protein from intracellular pool to apical membranes may be concerned in the action.

It has been well known that the activity of insulin-responsive glucose transporter in rat adipocytes is decreased in diabetes mellitus [10]. This result is recently confirmed by the finding that the number of insulin-re-

sponsive glucose transporter protein is decreased as visualized by the immunoblotting with monoclonal antibody 1F8 [22]. Additionally, it is reported that diabetes mellitus markedly reduces insulin-responsive glucose transporter mRNA expression in rat adipocytes [23]. Since cDNA of the Na^+/glucose cotransporter from rabbit small intestine has been sequenced [24], it will be aimed to compare with mRNA expression of Na^+/glucose cotransporter in epithelial cells of the small intestine from control and diabetic rats.

Epithelial cells in the small intestine possess both apical and basolateral membranes, which are functionally and structurally distinct from each other. Since insulin receptor is reported to be located in intestinal basolateral membranes [25], it is very interesting that insulin bound to its receptor in basolateral membranes regulates Na^+/glucose cotransporter in apical membranes.

In conclusion, we report here that the enhanced activity of small intestinal Na^+/glucose cotransporter in diabetes mellitus is restored by the treatment of diabetic rats with insulin and that the effect of insulin is not mediated by its hypoglycemic action. An investigation on the restoration by insulin of the enhanced Na^+/glucose cotransporter activity in diabetes mellitus will provide new informations about insulin action in the small intestine.

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