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Decreased D-glucose transport across renal brush-border membrane vesicles from streptozotocin-induced diabetic rats

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The uptake of Na^+-dependent D-glucose by renal brush-border membrane vesicles (BBMV) isolated from streptozotocin-induced diabetic rats was decreased as compared with controls. Since a V_{max} of 4.8 nmol/mg protein per 30 s in diabetic BBMV was significantly decreased as compared with that of controls (V_{max} = 7.0 nmol/mg protein per 30 s) without changing an apparent affinity for D-glucose, the decrease in the Na^+-dependent D-glucose uptake in diabetic rats is likely to be due to the reduction in the number of the transporter. These results are also confirmed by the binding study of [^{3}H]phlorizin to diabetic BBMV. When the blood glucose level is lowered in diabetic rats by both the treatment with insulin and starvation, the decreased Na^+-dependent D-glucose uptake is returned to control level. These results suggest that Na^+-dependent D-glucose reabsorption through the apical membrane in proximal tubular kidney cells is dynamically regulated by the change in blood glucose level.

Introduction

Uptake of D-glucose by mammalian cells is mediated by specific carriers [1,2]. The existence of at least four different glucose transport systems has been known in terms of the uptake mechanism, organ specificity, inhibitor specificity, molecular weight and hormone sensitivity. In these transporter systems, the stimulatory action of insulin on glucose uptake is observed in adipose cells [3–5] and skeletal muscles [6]. Additionally, it was reported that D-glucose transport activity was decreased in hypoinsulinemic diabetic rat adipocytes [7] and that the treatment of streptozotocin-induced diabetic rats with insulin resulted in a hyperresponsive insulin-stimulated glucose transport activity in the adipose cells [8].

On the other hand, Na^+-dependent uptake of various nutrients such as sugars [9,10] and amino acids [11] in small intestinal BBMV isolated from streptozotocin-induced diabetic rats is greater than corresponding uptakes in controls. Little is known, however, about the change in Na^+-dependent transport activity of D-glucose in renal BBMV isolated from streptozotocin-induced diabetic rats and the regulatory mechanisms.

In this study, we report that Na^+-dependent D-glucose transport activity in decreased in renal BBMV isolated from streptozotocin-induced diabetic rats as compared with controls and that this decline is likely to be regulated by the change in blood glucose concentrations.

Materials and Methods

Materials. D-[^{3}H]Glucose and [^{14}C]methylaminoisobutyric acid were obtained from American Radiolabeled Chemicals Inc. (St. Louis, MO) and [^{3}H]-phlorizin was 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 membrane vesicle preparation. Diabetic rats were induced in 250–300 g male Wistar rats with an i.v. injection of 65 mg/kg streptozotocin dissolved in 0.1 M citrate buffer (pH 4.5). After the induction of diabetes mellitus, rats were fed ad libitum before killing. In indicated cases, some diabetic rats were starved for three days, and others were subcutaneously treated with 10 IU of insulin every 12 h for 5 days. The glucose

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levels in plasma of control, streptozotocin-diabetic, insulin-treated diabetic and starved diabetic rats are 79.5 ± 3.5 mg/dl ($n = 4$), 360.0 ± 15.6 mg/dl ($n = 11$), 64.0 ± 10.9 mg/dl ($n = 7$), and 57.0 ± 6.0 mg/dl ($n = 8$), respectively. Brush-border membrane vesicles were isolated from the renal cortex of above-mentioned groups by the method of calcium precipitation [12]. The membrane vesicles were suspended in 20 mM Tris-Hepes/100 mM mannitol buffer (pH 7.5).

Transport studies. Uptake of D-[^{3}H]glucose and [^{14}C]methylaminoisobutyric acid by the freshly prepared brush-border membrane vesicles was measured by a rapid filtration technique. The reaction was initiated by adding 80 μl of the above-mentioned buffer containing either D-[^{3}H]glucose or [^{14}C]methylaminoisobutyric acid and either 100 mM NaCl or 100 mM KCl to 120 μl of the membrane vesicle suspensions (6.5 mg of protein per ml), which were preincubated for 10 min at 21° C. After the incubations indicated periods at room temperature, samples were filtered through cellulose nitrate filters (0.45 μm pore size) and immediately washed with 20 ml of ice-cold phosphate-buffered saline (PBS).

Na^+-dependent phlorizin binding. Binding of [^{3}H]phlorizin to brush-border membrane vesicles was assayed by a rapid filtration technique. The reaction was initiated by adding 90 μl of 40 mM Tris-Hepes/200 mM mannitol buffer (pH 7.5) containing [^{3}H]phlorizin and either 100 mM NaCl or 100 mM KCl to 10 μl of membrane vesicle suspensions (6.5 mg of protein per ml). Samples were incubated for 15 min at 21° C and then filtrated through cellulose nitrate filters (0.45 μm pore size) and immediately washed with 25 ml of ice-cold PBS. Na^+-dependent phlorizin binding activity was calculated by subtracting the activity in the presence of KCl from the activity in the presence of NaCl.

Protein determination. Protein was determined by the method of Lowry et al. [13].

Results

Time course of D-glucose uptake by renal BBMV

The time course of D-glucose uptake by renal BBMV isolated from normal rats was shown in Fig. 1A. An initial 100 mM NaCl gradient between medium and vesicles (medium > vesicles) induced a rapid uptake of D-glucose, which reached a maximum in about 1 min. On the other hand, the overshoot magnitude of D-glucose uptake was significantly decreased in the renal BBMV from streptozotocin-induced diabetic rats as compared with controls (Fig. 1B). As shown in Table I the enrichment in the activities of alkaline phosphatase, which is a marker enzyme in BBMV, in renal BBMV from controls and diabetic rats were not significantly different from each other. These data excluded the possibility that a different enrichment of BBMV was the cause for the observed changes in D-glucose uptake. In contrast, Na^+-independent D-glucose uptake in the vesicles from diabetic rats was not different from that in normal rats, suggesting that the intravesicular volume isolated from normal and diabetic rats was almost similar. Furthermore, the addition of phlorizin completely inhibited the Na^+-dependent uptake of D-glucose in both vesicles.

Na^+-dependent D-glucose uptake as a function of D-glucose concentration

In order to examine the controlling mechanism for the decrease in Na^+-dependent D-glucose transporter activity, Na^+-dependent D-glucose uptake was examined as a function of concentrations of D-glucose in BBMV isolated from control and streptozotocin-induced diabetic rats. Vesicles were incubated for 30 s at room temperature in the medium varying D-glucose concentrations from 0.5 to 15 mM to determinate initial rates and kinetic constants since Na^+-dependent D-glucose uptake was almost linear up to at least 30 s. Fig. 2

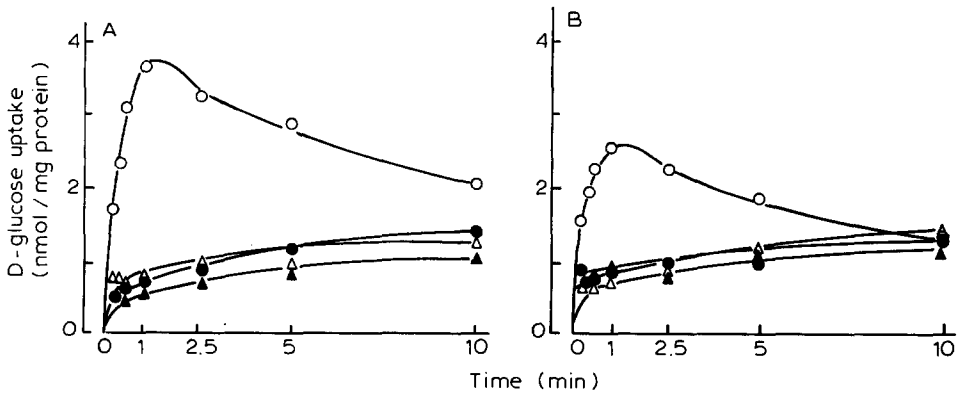

Fig. 1. Time course of D-glucose uptake by brush-border membrane vesicles. Membrane vesicles from control (A) and streptozotocin-induced diabetic rat kidney cortex (B) were preincubated at 21° C in 20 mM Tris-Hepes (pH 7.5) containing 100 mM mannitol for 10 min. Vesicles were subsequently incubated at room temperature with 1 mM D-[^{3}H]glucose in the presence of either 100 mM NaCl (○, △) or 100 mM KCl (●, ▲) with (△, ▲) or without (○, ●) 100 μM phlorizin.

TABLE I

Alkaline phosphatase activities in homogenates, basolateral membranes (BLM) and brush-border membrane vesicles (BBMV) from renal cortex of control and diabetic rats

Enzyme activities are expressed as the means ± S.E. (μmol/mg protein per 10 min). The numbers in paratheses indicate the numbers of experiments performed in duplicate determinations.

	Alkaline phosphatase activity	
	control ($n = 7$)	diabetes ($n = 8$)
Homogenate (H)	2.5 ± 0.2	2.0 ± 0.3
BLM	2.7 ± 0.7	2.6 ± 0.3
BBMV	15.4 ± 2.7	17.7 ± 3.0
Enrichment (BBMV/H)	6.2	8.9

shows that a V_{max} in vesicles from diabetic rats is 4.8 nmol/mg protein per 30 s from the figure, which is significantly decreased as compared with the control (V_{max} = 7.0 nmol/mg protein per 30 s) without changing an apparent K_m (about 0.9 mM) for D-glucose.

Recovery by insulin treatment and starvation of Na^+-dependent D-glucose uptake in diabetic BBMV

In order to clarify the mechanism by which Na^+-dependent D-glucose uptake was decreased in diabetic rats, we attempted to reduce the blood glucose level by two methods: (1) subcutaneous injection of insulin, (2) starvation for 3 days. Vesicles isolated from both insulin-treated and starved diabetic rats were incubated for 30 s at room temperature in the medium varying D-glucose concentrations from 0.5 to 15 mM to determine initial rates and kinetic constant. As shown in Fig. 3, both V_{max} values were returned to the control (Fig. 2) without a change in an affinity for D-glucose. These results suggest that Na^+-dependent D-glucose transporter in kidney epithelial cells is regulated by the change in the blood glucose.

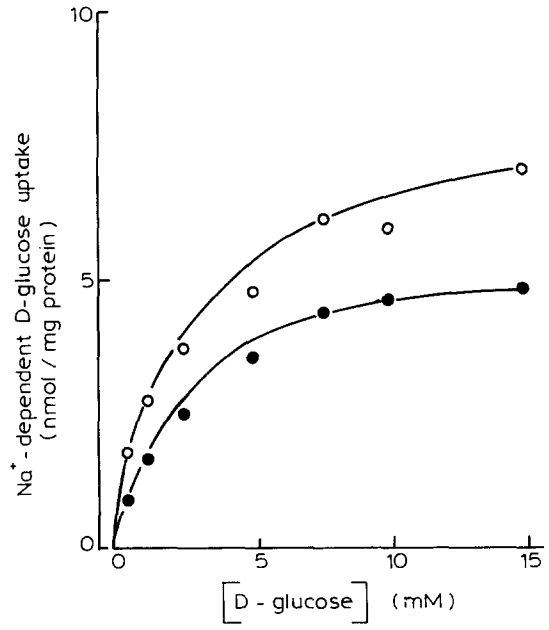

Fig. 2. Uptake of Na^+-dependent D-glucose by brush-border membrane vesicles from control and streptozotocin-induced diabetic rats as a function of concentrations of D-glucose. Membrane vesicles from control (○) and diabetes (●) were preincubated at 21°C in 20 mM Tris-Hepes (pH 7.5) containing 100 mM mannitol for 10 min. Vesicles were subsequently incubated at room temperature for 30 s with various concentrations of D-[^{3}H]glucose in the presence of either 100 mM NaCl or 100 mM KCl. Na^+-dependent D-glucose uptake was calculated by subtracting uptake in the presence of KCl from that in the presence of NaCl. Each point represents the mean of four experiments performed in duplicate determinations.

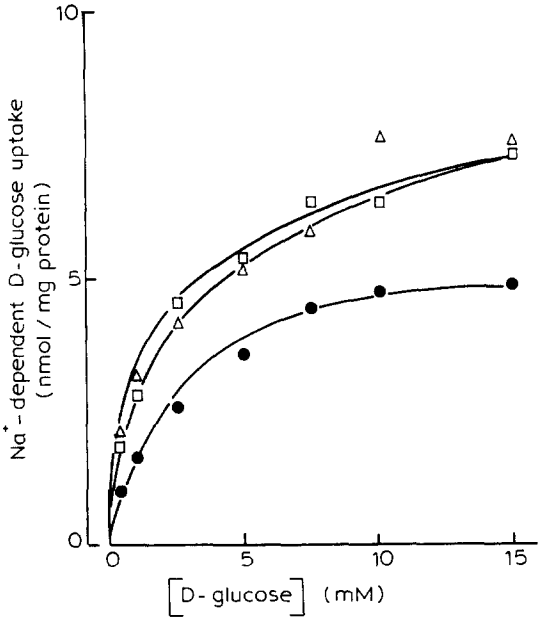

Fig. 3. Uptake of Na^+-dependent D-glucose by brush-border membrane vesicles from diabetes, insulin-treated and starved diabetic rats as a function of concentrations of D-glucose. Membrane vesicles from diabetes (●), insulin-treated (□) and starved diabetic rats (△) were preincubated at 21°C in 20 mM Tris-Hepes (pH 7.5) containing 100 mM mannitol for 10 min. Vesicles were subsequently incubated at room temperature for 30 s with various concentrations of D-[^{3}H]glucose in the presence of either 100 mM NaCl or 100 mM KCl. Na^+-dependent D-glucose uptake was calculated by subtracting uptake in the presence of KCl from that in the presence of NaCl. Each point represents the mean of four (diabetes and insulin-treated rats) or three (starved diabetic rats) experiments performed in duplicate determinations.

Na^+-dependent [^{3}H]phlorizin binding

In order to further confirm the decrease in the concentration of Na^+-dependent D-glucose transporter in renal BBMV from streptozotocin-induced diabetic rats, we studied on Na^+-dependent binding of [^{3}H]phlorizin to both preparations. As shown in Fig. 4A, Na^+-dependent [^{3}H]phlorizin binding in renal BBMV isolated from both normal and diabetic rats were saturable. A Scatchard analysis of the binding data (Fig. 4B) demonstrated that the K_d value for phlorizin was not significantly different in controls (1.3 μM) and diabetic BBMV

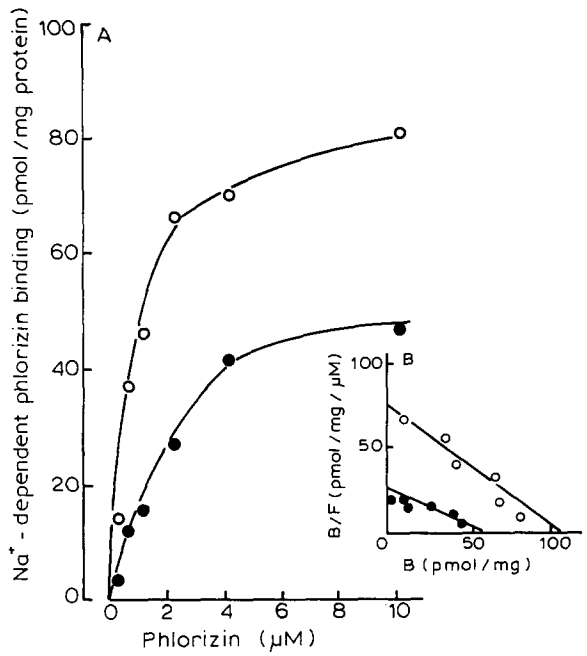

Fig. 4. Na^+-dependent phlorizin binding activity of membrane vesicles from control and streptozotocin-induced diabetic rats as a function of concentrations of phlorizin. (A) Membrane vesicles from control (○) and diabetes (●) were incubated at 21°C for 15 min in 36 mM Tris-Hepes (pH 7.5) containing 180 mM mannitol with various concentrations of [^{3}H]phlorizin in the presence of either 100 mM NaCl or 100 mM KCl. Na^+-dependent phlorizin binding activity was expressed by subtracting activity in the presence of KCl from that in the presence of NaCl. (B) Scatchard plots of Na^+-dependent phlorizin binding to membrane vesicles from control (○) and diabetes (●).

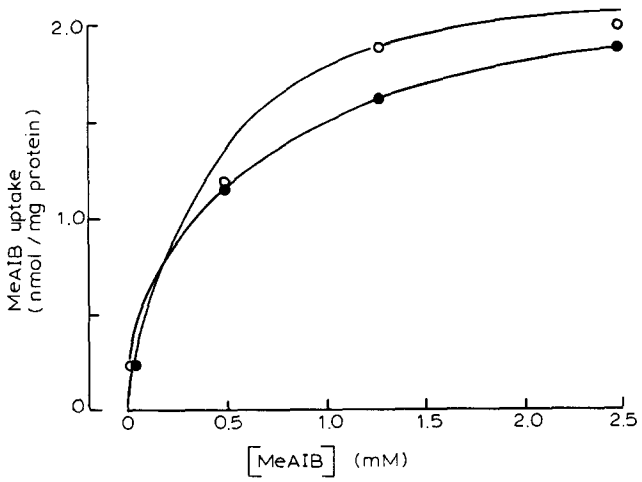

Fig. 5. Uptake of Na^+-dependent MeAIB by brush-border membrane vesicles from control and streptozotocin-induced diabetic rats as a function of concentrations of MeAIB. Membrane vesicles from control (○) and diabetes (●) were preincubated at 21°C in 20 mM Tris-Hepes (pH 7.5) containing 100 mM mannitol for 10 min. Vesicles were subsequently incubated at room temperature for 1.0 min with various concentrations of [^{14}C]MeAIB in the presence of either 100 mM NaCl or 100 mM KCl. Na^+-dependent MeAIB uptake was calculated by subtracting uptake in the presence of KCl from that in the presence of NaCl.

(2.0 μM). In contrast, the number of binding sites in diabetic BBMV (B_{max} = 56 pmol/mg protein) was reduced by 50% of controls (B_{max} = 104 pmol/mg protein).

Comparison of Na^+-dependent uptake of [^{14}C]methylaminoisobutyric acid (MeAIB) between control and diabetic rats

In order to investigate whether the uptake of D-glucose is specifically decreased in diabetes, we examined Na^+-dependent uptake of MeAIB, which is used as a substrate for system A, one of Na^+-dependent amino acid transport system in liver [14]. Na^+-dependent MeAIB uptake was examined as a function of concentrations of MeAIB in BBMV isolated from control and streptozotocin-induced diabetic rats. Vesicles were incubated for 1 min at room temperature in the medium varying MeAIB concentrations from 0.05 to 2.5 mM. No difference was observed in both apparent K_m and V_{max} between controls and diabetic rats (Fig. 5).

Discussion

It has been well studied that small intestinal Na^+-dependent uptakes of solutes including sugars [9,10], amino acids [11], phosphate [15] and bile salt [16] are increased in diabetic rats as compared with normal rats. On the other hand, the general properties of renal Na^+-dependent D-glucose transport system, including kinetics, requirement for cation and inhibition by phlorizin have been well investigated [17,18]. In addition, Hori et al. [19] indicated that Na^+-dependent D-glucose uptake by renal BBMV isolated from rats with uranyl nitrate-induced acute renal failure was decreased as compared with controls. However, there has been no indication that the active transport system of D-glucose in renal BBMV is changed in diabetic state. The present study indicates that Na^+-dependent D-glucose uptake in renal BBMV isolated from streptozotocin-induced diabetic rats is significantly decreased as compared with normal rats (Fig. 1A and B). This decrease is due to the reduction of the number of the Na^+-dependent D-glucose transporter, which is also confirmed by data of [^{3}H]phlorizin binding to BBMV isolated from streptozotocin-induced diabetic rats. On the other hand, there is no significant difference in amino acid uptake between renal BBMV isolated from control and that from diabetic rats, suggesting that D-glucose uptake is specifically decreased in diabetes, differing from the results for the small intestinal BBMV [11]. Recently the 75 kDa protein is solubilized from BBMV of pig kidney cortex and purified by using ion exchange HPLC and identified as a component of the Na^+/glucose cotransporter [20]. The purified cotransporter has a K_d value of 1.2 μM for phlorizin, which is close to the value in our present study (Fig. 4B).

In addition, this decrease in Na^+-dependent D-glucose uptake is likely to be regulated by the change in concentrations of D-glucose in the blood. In other words, this decrease in diabetic rats is returned to control by lowering a level of D-glucose in the blood in diabetic rats by either insulin treatment or starvation. Thus, it is very interesting that the expression, i.e. synthesis and/or degradation, of Na^+-dependent D-glucose transporter, involved in the reabsorption of D-glucose in proximal tubular kidney cells, is dynamically changed according to D-glucose concentrations in the blood. Although proximal tubular kidney cells possess both apical and basolateral membranes, which are functionally and structurally distinct from each other, it remains to be ascertained if the membranes actually interact with D-glucose i.e. the signal of glucose effects.

In cases of the facilitated hexose carrier, D-glucose uptake activity in response to the reduction of D-glucose concentrations in the medium is increased in a number of cultured cells [21–23]. Such an adaptation is controlled by two processes, both synthesis and degradation of the carrier protein. At present, however, we have little knowledge about the control mechanism for the change in Na^+-dependent D-glucose uptake in proximal tubular kidney cells in response to the level of the blood glucose. It is necessary to further explore the regulatory mechanism of glucose effects on an active transport of D-glucose in kidney epithelial cells, which in turn will provide new information about the renal reabsorption.

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