Diabetes Mellitus: Changes in the Transport Properties of Isolated Intestinal Microvillus Membranes

(membrane vesicles/glucose transport/sodium conductance/ionicophores)

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ABSTRACT Isolated, small intestinal microvillus membranes from normal and acutely diabetic rats were compared with respect to D-glucose transport. D-Glucose was accumulated to a greater extent by diabetic membrane vesicles when supplied with energy in the form of a NaCl or a NaSCN gradient across the vesicle membrane. The difference appeared to be caused by an inability of the diabetic membranes to maintain a higher driving force for active D-glucose transport and not by changes in the glucose "carrier." Increasing the glucose-independent Na+-conductance of the membrane with monactin or gramicidin D reduced the active accumulation of D-glucose by membrane preparations from both control and diabetic groups. Concentrations of monactin and gramicidin D in the incubation medium of membrane vesicles from diabetic animals could be adjusted so that their D-glucose transport became indistinguishable from that of membranes from normal animals not treated with ionophores.

These observations suggest the microvillus membranes as one site where changes occur in acute diabetes. In addition, the change in the transport properties of the isolated membranes offer a rational explanation for the simultaneous elevation of active intestinal sugar, amino acid, and bile salt transport observed for intact intestinal tissue.

Experimental diabetes was associated with an increased active transport of sugars, many amino acids, and bile salts in the gut (1–7). For example, insulin deficiency, induced by various means, approximately doubles the rate of D-glucose uptake from the small intestinal lumen and brings about an increased accumulation of nonmetabolizable analogues of D-glucose by the epithelial cells. These findings are contrary to those reported for tissues other than the small intestine, where a notable characteristic of experimental diabetes is the impairment of D-glucose transport (8).

Since the effects of insulin on adipose and muscle tissue have been linked to changes in the transport capacity of the plasma membrane for hexoses (9), it was decided to investigate the transport properties of the luminal plasma membrane of small intestinal epithelial cells from normal and diabetic animals. The luminal plasma membrane, i.e., the microvillus or brush border membrane, possesses a Na+-dependent and phlorizin-sensitive glucose transport system, and this has been well investigated for isolated and highly purified microvillus membranes from rat small intestine (10, 11). The membranes vesiculate and reseal after isolation, i.e., form an effective barrier between the suspension medium and an intravesicular space. The Na+-dependent glucose "carrier" mediates an uphill D-glucose transport into the intravesicular space of isolated membranes, provided osmotic energy in the form of an electrochemical gradient of Na+ is supplied such that the intravesicular Na+ activity is lower. This property is thought to constitute the biochemical counterpart to active accumulation and active transcellular sugar transport of the intact cell.

METHODS AND MATERIALS

Microvillus membranes were prepared from the pooled small intestinal mucosal scrapings of two to three rats as described (10). The methods to measure D-glucose uptake by the isolated membrane vesicles have been published (10, 11). The medium concentration of D-glucose was 1 mM in all experiments. Other experimental details are given in the legends. Diabetes mellitus was induced by injection of alloxan (50 mg/kg of body weight) into the tail vein of male albino rats (150–250 g of body weight). Control animals from the same batch received saline injections. Only animals with blood sugar levels of 300 mg/100 ml and above were considered diabetic. Diabetic or control animals were killed 4–8 days after injection, when transport changes are most pronounced (4).

In order to measure the cyclic AMP content of intestinal mucosa, the animals were killed by a blow on the head and subsequent cervical dislocation. After the abdominal wall had been opened, the intestine was chilled with ice-cold 0.9% saline. A jejunal (10 cm distal to the ligament of Treitz) and an ileal (10 cm proximal to the ileocecal valve) segment, each about 10 cm in length, were rinsed and mucosa was obtained with a special scraping device (12). The sample was placed immediately into 5% trichloroacetic acid, and the cyclic AMP content was determined by the method of Gilman (13). Protein determination was carried out on the material, precipitated by trichloroacetic acid, with the method of Lowry et al. (14). Initially, mucosal samples were removed at various times after death from adjacent segments of the same animal. Cyclic AMP levels were constant from 4 min (earliest time point) up to 8 min and were similar to values from ether-anesthetized animals right after onset of narcosis. Subsequently, all mucosal samples were obtained within the 8-min period after death. Since cyclic AMP levels per mg of protein were similar in jejunum and ileum, the data of both intestinal segments were pooled.

Statistical evaluations were carried out with the two-tailed t-test (15). Monactin was a gift of Ciba-Geigy, A.G., Basel, Switzerland. Gramicidin D was purchased from Sigma Chemical Co., St. Louis, Mo. Cyclic AMP-binding protein was obtained from Calbiochem A.G., Luzern, Switzerland. The sources for other materials have been given (10, 11).
RESULTS AND DISCUSSION

D-Glucose transport in diabetes

Since different membrane preparations were to be compared, an experimental protocol was set up which would measure the ability of the isolated membranes to accumulate D-glucose against a concentration gradient using a "standard" driving force. When either a NaCl or a NaSCN concentration gradient (medium concentration > intravesicular concentration) is provided as driving force, D-glucose uptake from the medium shows a typical "overshoot" (Fig. 1, upper curve). The overshooting part represents active D-glucose transport. It is transient because of the limited energy supply from the salt gradient existing at the beginning of the incubation and being dissipated with time. The ratio between the maximum of the "overshooting" uptake and the equilibrium uptake can be calculated and taken as a measure of the capacity to transport sugars actively.

The D-glucose transport was found to be qualitatively similar in normal and diabetic microvillous membranes. However, quantitative differences were apparent, as demonstrated in Table 1. Membranes from diabetic rats accumulated D-glucose better than those of controls with either a NaCl or a NaSCN gradient as driving force. Since the treatment during isolation was identical for control and diabetic membranes, this result suggests that acute diabetes affects the properties of the intestinal microvillous plasma membrane. That D-glucose transport of intact epithelial tissue is elevated under these conditions has been amply demonstrated (1-4, 7). It is noteworthy that the changes with respect to monosaccharide transport are similar in intact tissue and isolated plasma membrane, i.e., higher active transport in both preparations. This observation indicates that an alteration of membrane properties may form the basis for the increased overall absorption of sugars associated with acute diabetes.

In order to define the nature of the transport change more clearly, the isolated membranes from normal and diabetic rats were also compared with respect to a number of other parameters. This is especially necessary when looking at active D-glucose transport in isolated membranes, where transport is a function of vesicle size and "leakiness," glucose "carrier" concentration in the membrane, and driving force. The appearance of freeze-fractured preparations from control and diabetic animals was similar in the electron microscope, with both membranes forming vesicles of about the same size and shape. The specific activity of sucrase (EC 3.2.1.48; sucrase α-glucohydrolase), a marker of intestinal brush border, was likewise similar in control and diabetic membranes (Table 2).

In order to appraise differences in glucose "carrier" concentrations between the two membranes, the kinetics of D-glucose translocation were carefully investigated. In one series of four diabetic and five control membrane preparations, the rate of D-glucose uptake was measured in the absence of a driving force, except for the D-glucose gradient itself, but in the presence of Na⁺. This was achieved by preincubation

![Graph showing D-glucose uptake over time](image)
of the membranes with 0.1 M NaSCN. Under these experimental conditions, D-glucose equilibrates between the medium and the intravesicular space (Fig. 1, lower curve). With the assumption of first-order kinetics the time for half-maximal filling ($T_{1/2}$) of the membrane vesicles can be estimated by the method described in the legend to Table 3. $T_{1/2}$ was used as kinetic parameter to characterize and compare the transport of different membrane preparations. Table 3 presents the results obtained at 25 and 3°. Most of the glucose translocation appeared to be mediated by a "carrier," as 0.1 mM phlorizin increased $T_{1/2}$ by approximately 12 and 4 times at 25 and 3°, respectively. Table 3 also shows that the rates of D-glucose uptake were similar for control and diabetic membranes. In a second series of experiments (not shown), consisting of three diabetic and three control animals, the rates of D- and L-glucose uptake were measured at 3° in the presence of NaCl. In this second series no significant differences between control and diabetic membranes could be detected. The two series together indicate that the kinetics of D-glucose transport are similar in both membranes when the driving forces are equal.

It is noteworthy that the time for half-maximal filling was under 10 sec at 25° in the absence of a driving force, and thus much shorter than the incubation time necessary to achieve the maximum uptake (about 45 sec) in the presence of a NaSCN gradient. A comparison of these times suggests that the kinetics of D-glucose transport do not control the overshoot, i.e., the ratio of maximal intravesicular to extravesicular D-glucose concentration, but that the driving force is the determining factor, in either normal or diabetic membranes.

**Effect of ionophorous antibiotics**

Since the electrochemical gradient of Na+ forms the driving force for D-glucose transport (11), the overshooting D-glucose uptake may be an indicator of the Na+ gradient and its rate of dissipation. Under conditions of a 100-fold excess of Na+ over D-glucose (see Table 1) it seems likely that the glucose-independent Na+ permeability of the membrane, or more precisely the Na+ conductance, determines the dissipation of the electrochemical gradient of Na+, established as NaCl or NaSCN gradient between medium and intravesicular space. In order to demonstrate the effect of the glucose-independent Na+ conductance, D-glucose transport experiments were carried out in the presence of the ionophorous antibiotics monactin and gramicidin D. Both antibiotics have been shown to increase specifically the cation conductance, including that of Na+, of biological and lipid bilayer membranes (16-20). A typical result is shown in Fig. 1. At sufficiently high concentrations, monactin could abolish the overshooting D-glucose uptake induced by a NaSCN gradient. The antibiotics had the same qualitative effect on control and diabetic membranes. The minimal concentration of monactin that effected a lower-

![Fig. 2. Efficacy of monactin in lowering active D-glucose transport. D-Glucose uptake was measured as in the legend of Fig. 1 in the presence of a NaSCN gradient and varying concentrations of monactin. The ratio of maximum/equilibrium uptake was calculated for each uptake curve and plotted against the monactin concentration. The different uptake curves were obtained with aliquots of the same membrane preparation.](image)

**Table 2.** Sucrase activity of microvillous membrane

<table>
<thead>
<tr>
<th>State of animal</th>
<th>Sucrase* mean ± SD (n)</th>
</tr>
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<tbody>
<tr>
<td>Normal</td>
<td>1.7 ± 0.6 (4)</td>
</tr>
<tr>
<td>Diabetic</td>
<td>2.0 ± 0.7 (4)</td>
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* In μmol/min per mg of protein at 37° and pH 6.0. In parentheses, number of animals.

**Table 3.** Comparison of the kinetics of D-glucose uptake in normal and diabetic microvillous membranes

<table>
<thead>
<tr>
<th>Condition</th>
<th>Normal mean ± SD (n)</th>
<th>Diabetic mean ± SD (n)</th>
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</thead>
<tbody>
<tr>
<td>25°, no addition</td>
<td>9.7 ± 0.6 (4)</td>
<td>7.7 ± 1.6 (4)</td>
</tr>
<tr>
<td>25°, + phlorizin</td>
<td>113 ± 48 (5)</td>
<td>89 ± 10 (4)</td>
</tr>
<tr>
<td>3°, no addition</td>
<td>170 ± 73 (5)</td>
<td>111 ± 10 (3)</td>
</tr>
<tr>
<td>3°, + phlorizin</td>
<td>570 ± 90 (2)</td>
<td>653 ± 87 (3)</td>
</tr>
</tbody>
</table>

$T_{1/2}$ was determined as follows: Microvillous membranes were prepared as indicated in the legend of Table 1. The membranes were preincubated with 0.1 M NaSCN for 10 min to equilibrate the salt between medium and the intravesicular space. The membranes were then brought to the indicated incubation temperature, D-[1-14C]glucose was added, and the D-glucose uptake measured for 2.5 min (for time points, see Fig. 1). Subsequently, all membranes were incubated for another 10 min at 25° to insure equilibration of D-glucose, and several samples were taken to obtain the equilibrium uptake. $T_{1/2}$ was estimated from a plot of logarithm (equilibrium uptake minus uptake at a certain time point) against time. For D-glucose uptake at 25° in the absence of phlorizin, the 10-sec time point was used. For all other conditions the least-square method was used to fit a straight line through the origin and the first four time points up to 130 sec. First-order kinetics were assumed. Phlorizin, when present, was 0.1 mM.

**Fig. 2.** Efficacy of monactin in lowering active D-glucose transport. D-Glucose uptake was measured as in the legend of Fig. 1 in the presence of a NaSCN gradient and varying concentrations of monactin. The ratio of maximum/equilibrium uptake was calculated for each uptake curve and plotted against the monactin concentration. The different uptake curves were obtained with aliquots of the same membrane preparation.
words, the association of acute diabetes with lowered Na+-conductance of the intestinal microvillar membrane. Unfortunately, it is not possible to measure the Na+-conductance of the vesiculated membranes directly.

In principle, changes in the membrane conductance for anions could also account for changes in the dissipation time of the Na+-gradient. However, SCN- is a very lipophilic anion, and it appears unlikely that diabetes would grossly alter the lipid phase of membranes and thereby the permeability to SCN-.

It is worthwhile to consider whether the findings with the isolated membranes can explain the known transport changes in the small intestine. If in acute diabetes the glucose-independent Na+-conductance (or a combination of processes that appear as Na+-conductance) of the microvillar membrane is decreased and the Na+-transport out of the cell (e.g., via the Na+, K+-ATPase) is maintained, then the electrochemical gradient of Na+ across the microvillar membrane should increase (under otherwise equal conditions). This, in turn, represents a greater driving force for all Na+-dependent transport processes of nonelectrolytes. Thus, a change in the Na+-conductance, as indicated by the findings with the isolated membranes, provides an explanation why all Na+-dependent active transport activities, but not the Na+-independent ones, are elevated in diabetes (3).

Cyclic AMP levels in diabetes

It is likely that the Na+-permeability of the small intestine is regulated, at least in part, by cyclic adenosyl monophosphate (AMP). In many cases, where hormones or other agents influence the electrolyte transport in this tissue, increased intracellular cyclic AMP levels have been associated with decreased absorption (21-28). Therefore, it was of interest to measure the cyclic AMP levels for diabetic mucosa and compare them with normal levels. Control mucosa contained 7.9 ± 2.5 pmol of cyclic AMP per mg of protein (mean ± SD of 24 samples), while the mucosa of alloxan-diabetic rats 4-5 days after injection contained 12.3 ± 3.8 pmol/mg of protein (30 samples). The difference is significant at the 1% level. The 1.5-fold elevation compares well with other states accompanied by a decrease of the Na+-permeability of the small intestine (27, 28). It is not clear whether the elevation of cyclic AMP is due to a lack of insulin, a preponderance of other hormones, an elevation of blood sugar, or a greater secretion of other hormones under the stress of acute diabetes.

The association of elevated cyclic AMP levels with acute diabetes is merely suggestive of a possible involvement of this nucleotide in the pathogenesis of the increased intestinal transport. However, it indicates a possible route whereby information could be transmitted from the lateral-basal plasma membrane, the site of interaction with polypeptide hormones, to the luminal side of the epithelial cell. The possible involvement of cyclic AMP is consistent with the relatively short period (1 hr) in which the effects of experimental diabetes on intestinal transport have been demonstrated (7).

Taken together, the data of this report suggest as a possibility a sequence of biochemical events that would lead to higher driving forces for active intestinal transport, and thus provide a unifying concept for the simultaneous elevation of active transport of several nonrelated classes of molecules. The isolated membrane preparation has the advantage of giving information about a single specialized plasma membrane. However, uncertainties remain when extrapolations to properties of the intact tissue are made, due mainly to the complexity of intact epithelium whose transport properties are determined not only by the plasma membranes of the epithelial cell, but also by the intercellular adhesions (29-32). Thus, it is difficult to relate the observed change of the nutrient-independent Na+-conductance of the brush border membrane to reported elevated Na+-fluxes across the whole epithelium in diabetes, especially since these latter measurements were carried out in the presence of d-glucose (33, 34).

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FIG. 3. Effect of monactin and gramicidin D on the differential uptake of D- over L-glucose. The difference between D- and L-glucose uptake (lower curve) was measured in the presence of NaSCN, but absence of a gradient. For this purpose, membranes in the usual buffer were preincubated with 0.1 M NaSCN for 10 min, then ethanol (Δ), gramicidin D (○), or monactin (□) was added and the uptake of glucose initiated by the addition of 1 mM D-[1-3H]glucose and 1 mM L-[1-3H]glucose to the incubation medium. Uptake of both glucose isomers was determined as described (10), and the difference plotted. The concentration of both antibiotics was 20 μg/ml. In the control experiments (upper three curves), active D-glucose uptake was measured in other aliquots of the same membrane preparation. Experimental conditions correspond to those in Table I with NaSCN. Further additions: None (Δ), 11 μg/ml of gramicidin D (○), 7.5 μg/ml of monactin (□). Ethanol concentration (less than 1%) was similar in all experiments and did not affect D-glucose transport.