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Reduced expression of the liver/beta-cell glucose transporter isoform in glucose-insensitive pancreatic beta cells of diabetic rats

(type II diabetes/immunofluorescence/anti-peptide antibodies/streptozocin)

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ABSTRACT **Rats injected with a single dose of streptozocin at 2 days of age develop non-insulin-dependent diabetes 6 weeks later. The pancreatic beta islet cells of these diabetic rats display a loss of glucose-induced insulin secretion while maintaining sensitivity to other secretagogues such as arginine. We analyzed the level of expression of the liver/beta-cell glucose transporter isoform in diabetic islets by immunofluorescence staining of pancreas sections and by Western blotting of islet lysates. Islets from diabetic animals have a reduced expression of this beta-cell-specific glucose transporter isoform and the extent of reduction is correlated with the severity of hyperglycemia. In contrast, expression of this transporter isoform in liver is minimally modified by the diabetes. Thus a decreased expression of the liver/beta-cell glucose transporter isoform in beta cells is associated with the impaired glucose sensing characteristic of diabetic islets; our data suggest that this glucose transporter may be part of the beta-cell glucose sensor.**

The plasma membrane of pancreatic beta islet cells contains a particular glucose transporter isoform, the liver/beta-cell glucose transporter (1). This transporter is also present on the sinusoidal membrane of hepatocytes and on the basolateral membrane of absorptive intestinal and kidney epithelial cells (2, 3). It has a low affinity and a high K_m for glucose (17 mM) (4) and is present in insulin-secreting beta cells but not in other pancreatic islet endocrine cells (5). The main function of beta cells is insulin secretion, mainly in response to increases in the extracellular concentration of glucose. At the normal blood glucose concentration of about 5 mM, insulin is secreted at a low basal rate; secretion is near maximal at 15 mM glucose. For insulin secretion to occur, both glucose uptake and metabolism are required, and the rate of glycolysis must reflect the variation in extracellular glucose concentration (6). The beta-cell glucokinase has a high K_m (6 mM) (7) for glucose and is thought to be the rate-limiting step in glycolysis; it has been suggested to be the "glucose sensor" of beta cells (6). We have suggested that a high-K_m glucose transporter isoform in the plasma membrane of beta cells is required to allow the rate of glucose uptake to increase proportionately to the extracellular glucose concentration over the stimulatory range (5–15 mM); consequently, the simultaneous presence of a high-K_m glucose transporter and a high-K_m glucokinase is required for normal glucose sensing (1, 5, 8).

Non-insulin-dependent diabetes mellitus is characterized by a resistance of peripheral tissues to the action of insulin and by dysfunction of pancreatic beta cells. In particular, there is a disappearance of glucose-induced insulin secretion (9). This disease has a strong genetic component (10) and the molecular defects underlying insulin resistance and beta-cell dysfunction are mostly unknown. Mutations in the insulin receptor are the cause of some forms of severe insulin resistance such as those associated with leprechaunism or acanthosis nigricans (11–14). Also, the expression of the fat/muscle glucose transporter isoform in adipose tissue of rats is markedly reduced after experimentally induced insulin resistance (15–18), suggesting that a defect in expression of this transporter may also be a cause of insulin resistance.

Because of the critical role of the liver/beta-cell glucose transporter isoform in the normal functioning of beta cells, we studied its expression in islets from the neonatal streptozocin-treated rat model of non-insulin-dependent diabetes (19, 20). Newborns are injected at 2 days with a single dose of streptozocin, which provokes a transient hyperglycemia followed by a near normal glycemia. By 6 weeks the rats develop a hyperglycemia that does not require insulin treatment (19–22). A characteristic of the beta cells of these rats is a selective loss of glucose-induced insulin secretion; islets retain the capability to secrete insulin in response to other secretagogues such as arginine (20, 22). Here, we show by both immunofluorescence staining of pancreatic islets and Western blot analysis of islet lysates that the beta cells of these diabetic animals have a reduced expression of the liver/beta-cell glucose transporter and that this reduction is proportional to the severity of hyperglycemia. Strikingly, the expression of this transporter is only minimally altered in the livers of the same diabetic rats.

MATERIALS AND METHODS

Rats, Streptozocin Injections, and Plasma Glucose Determination. Pregnant Sprague–Dawley rats (Taconic Farms) with free access to chow and water were checked at the hours of 0900 and 1600 daily for delivery of pups. Day of birth was considered day 0. Two-day-old neonates were injected with streptozocin (90 mg/kg) and were subsequently bled on days 4 and 10, and once each following week until 8 weeks of age (19). Blood samples, taken between the hours of 0900 and 1100 in the fed state, were centrifuged in a microcentrifuge. Plasma glucose levels were measured using a Beckman glucose analyzer model II. Based on plasma glucose levels at 4 days of age, streptozocin-treated rats in some experiments were grouped according to high or low glucose levels. Those of the low glucose group had, as adults, plasma glucose levels only moderately elevated as compared to controls, usually 200–250 mg/dl; those of the high glucose group became more severely hyperglycemic by 6 weeks of age with plasma glucose levels >300 mg/dl and sometimes 400–500 mg/dl. Rats were used for experiments at 8 weeks of age.

Western Blot and Immunofluorescence Staining Analysis. For immunofluorescence studies, an affinity-purified antibody against a peptide corresponding to the C-terminal amino acids (residues 513–522) of the liver/beta-cell glucose transporter

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was prepared as described (1, 5); for Western blot analysis, another antibody against the peptide corresponding to amino acids 510–522 was prepared exactly as described (1).

For immunofluorescence staining of pancreatic islets, a pancreas was perfused with a paraformaldehyde/lysine/periodate fixative (23). Frozen tissue sections (5 μm) were prepared with a Reichert cryostat. The liver/beta-cell glucose transporter was detected by indirect immunofluorescence using the affinity-purified anti-glucose transporter antibody at 1 μg/ml and a fluorescein-conjugated goat anti-rabbit immunoglobulin antibody (Calbiochem) as the second reagent, as described (1–3). Photomicrographs were taken with Kodak TMAX films, using Neofluor objectives on a Zeiss Photomicroscope III.

For Western blot analysis of the liver/beta-cell glucose transporter isoform, islets were isolated as described (1) and lysed in a solution containing 5% (wt/vol) SDS, 80 mM Tris·HCl (pH 6.8), 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 0.2 mM *N*-ethylmaleimide. The lysates were sonicated for three 30-sec periods in the bath of a sonicator (model W-225; Heat Systems/Ultrasonics) operated at maximum power. Livers were homogenized in the same lysis buffer with the help of a Polytron (Brinkman Instruments) homogenizer. The protein content of the lysates was measured by the BCA protein assay (Pierce) using bovine serum albumin as standard. Proteins were separated by electrophoresis on 10% polyacrylamide gels containing SDS, transferred to nitrocellulose, and stained with Ponceau S (Sigma). The filters were then processed for detection of the glucose transporter by using the anti-peptide antibody and ^{125}I-labeled protein A (Amersham) exactly as described (1). The autoradiograms were quantified by scanning with a model 2202 Ultroscan LKB laser densitometer.

RESULTS

Rats treated with streptozocin 2 days after birth showed greater variations in plasma glucose levels as adults than reported (19). Table 1 summarizes the number of rats used for islet preparation in each experiment, their plasma glucose levels, and the relative content of the liver/beta-cell glucose transporter isoform in islets and liver as detected by Western blot analysis. In experiments 2 and 3, islets were pooled from streptozocin-treated rats that had only minimally elevated glucose levels (low glucose) as well as from more diabetic rats (high glucose). The number of rats used for immunofluorescence analysis of the liver/beta-cell glucose transporter in pancreatic islets and their plasma glucose levels are presented in Table 2.

Table 1. Level of expression of the beta-cell glucose transporter in islets and liver of control and neonatal streptozocin-treated rats

	n	Plasma glucose, mg/dl	Beta-cell GT, % of control	
			Islets	Liver
Experiment 1				
Control	1	158	100	
NSTZ	4	424 ± 108	7	
Experiment 2				
Control	3	179 ± 13	100	100
NSTZ (low glucose)	6	204 ± 25	68	133
NSTZ (high glucose)	6	513 ± 129	15	116
Experiment 3				
Control	1	151	100	100
NSTZ (low glucose)	1	157 ± 6	82	136
NSTZ (high glucose)	3	344 ± 95	18	97
Experiment 4				
Control	2	154 ± 3	100	100
NSTZ	3	536 ± 86	4	63

The beta-cell glucose transporter results are from densitometry scanning of the autoradiograms. Results for plasma glucose are expressed as mean ± SEM. *n*, Number of animals used for islet preparations and Western blots; NSTZ, neonatal streptozocin-treated.

For the immunofluorescence study, several dozen islets from each group of animals were observed and/or photographed. Representative immunofluorescence staining of the liver/beta-cell glucose transporter in islets of a control rat is shown in Fig. 1 *A* and *B*. Islets from mildly diabetic rats (plasma glucose at 154 mg/dl the morning of the sacrifice during week 8, but at 207 and 210 mg/dl during weeks 6 and 7) are presented in Fig. 1 *C* and *D*. Islets from a more hyperglycemic diabetic rat (plasma glucose at 301 mg/dl the morning of the sacrifice during week 8) are presented in Fig. 1 *E* and *F*. In the mildly diabetic rats, the size of the islets was near normal, as was the number of beta cells, as determined by the number of cells expressing the liver/beta-cell glucose transporter isoform (compare Fig. 1 *A* and *B* with *C* and *D*). In contrast, the intensity of immunofluorescence staining for the liver/beta-cell glucose transporter was considerably decreased (Fig. 1 *C* and *D*). The heterogeneity in staining intensity of individual beta cells in a single islet was consistently observed on all islets of all diabetic rats studied. Islets from a more diabetic rat (Fig. 1 *E* and *F*) had a reduced number of beta cells. In similarly diabetic rats, the number of beta cells per islet was about 75% of control (19). These islet beta cells showed a greater reduction in staining for the liver/beta-cell glucose transporter than those from mildly diabetic rats. In the pancreas of the most diabetic rats (see Table 2), the size of the islets was much reduced and the intensity of the immunofluorescence staining for the liver/beta-cell glucose transporter, although clearly visible, was greatly decreased and mostly apparent as dots on the plasma membrane (data not shown).

To obtain a more quantitative estimation of expression of the liver/beta-cell glucose transporter isoform in islets from diabetic rats, we performed Western blot analysis on total lysates of isolated islets. In each experiment, islets were isolated from several rats (see Table 1), pooled, and lysed; equivalent amounts of protein from control and diabetic islets were separated by gel electrophoresis. Western blot analysis therefore averages the levels of transporter present in several hundred islets per rat. Fig. 2 *Upper Left* shows the result of one such experiment (Table 1, experiment 2). The glucose transporter was reduced by 32% in islets from mildly diabetic rats (lane 3) and by 85% in islets from severely diabetic rats (lane 2) compared to the control (lane 1). In Fig. 2 *Upper Right*, staining of the filter with Ponceau S prior to immunodetection of the glucose transporter indicated that similar amounts of proteins were loaded on each lane and also that the protein composition of the diabetic islets was indistinguishable from the control. Immunodetection of the glucose

Table 2. Control and neonatal streptozocin-treated rats used for immunofluorescence study with their respective plasma glucose levels

	n	Plasma glucose, mg/dl
Experiment 1		
Control	1	151
NSTZ	2	459, 494
Experiment 3		
Control	2	165, 172
NSTZ	2	154, 301
Experiment 4		
Control	1	151
NSTZ	1	560

n, Number of rats; NSTZ, neonatal streptozocin-treated.

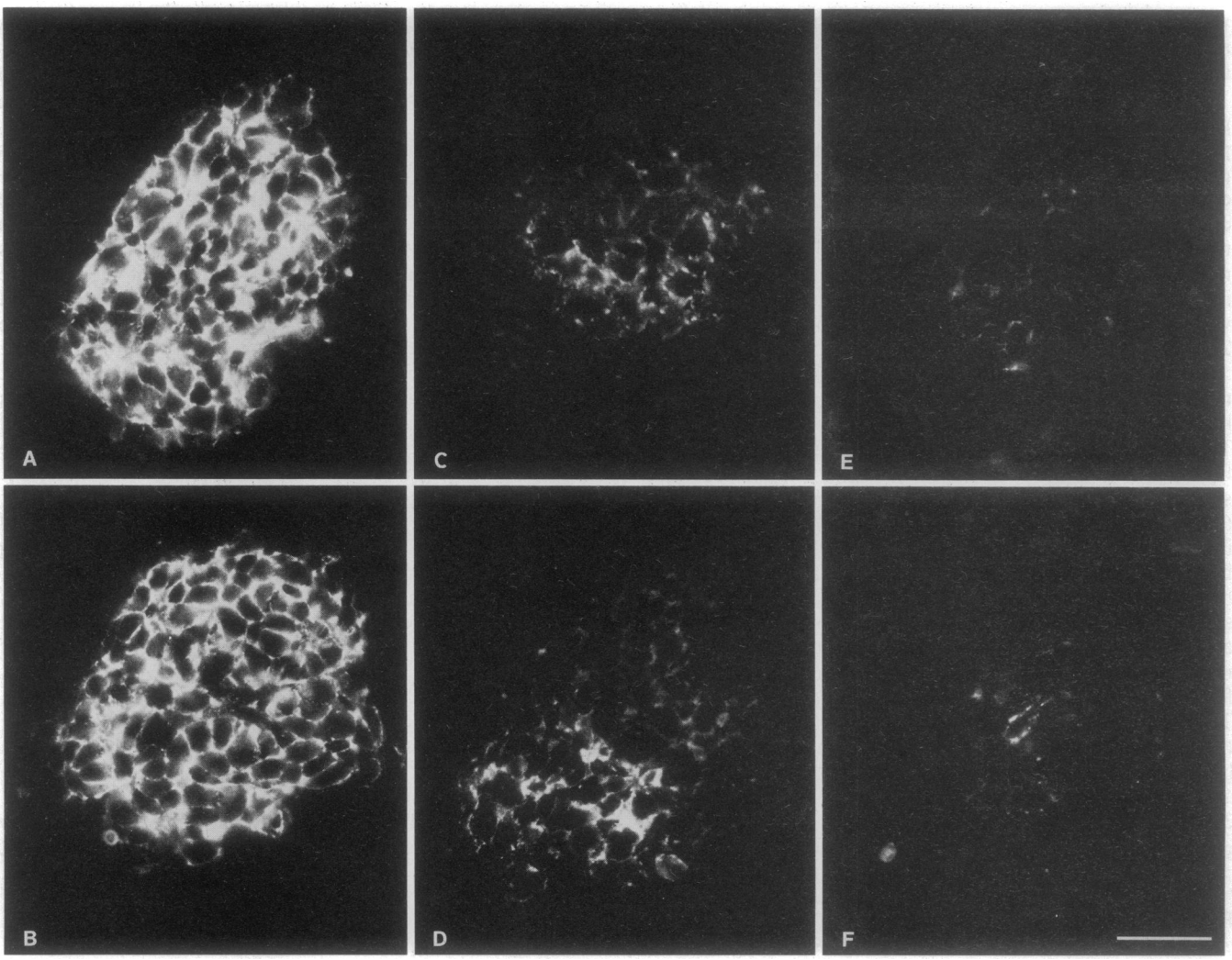

FIG. 1. Immunofluorescence detection of the liver/beta-cell glucose transporter in the plasma membrane pancreatic islet beta cells from control and diabetic rats. (*A* and *B*) Islets from a control rat. (*C* and *D*) Islets from mildly diabetic rats (plasma glucose at 154 mg/dl on the day of the sacrifice during week 8 but at 207 and 210 mg/dl during weeks 6 and 7. (*E* and *F*) Islets of strongly diabetic rats (plasma glucose at 301 mg/dl during week 8). All micrographs were photographed on the same type of film using identical exposure times. The level of immunofluorescence staining for the glucose transporter is reduced in mildly diabetic beta cells and much more in the more diabetic cells. Note the heterogenous staining of the mildly diabetic beta cells. (Bar = 48 μm.)

transporter in the lysate of pooled livers from the same control and diabetic rats (Fig. 2 *Lower Left*) showed that the level of transporter was essentially unchanged by the diabetes (Table 1); this result is similar to our previous observations on adult rats made diabetic by streptozocin treatment (24). Ponceau S staining of the filter again showed that equivalent amounts of proteins were analyzed (Fig. 2 *Lower Right*). Table 1 summarizes the results of expression of the glucose transporter in islets and livers of control and diabetic rats. Fig. 3 shows that the level of immunoreactive glucose transporter in islets was inversely proportional to the extent of glycemia.

DISCUSSION

Our results show that, in a rat model of non-insulin-dependent diabetes, the level of expression of the liver/beta-cell glucose transporter isoform is reduced in beta cells and that the extent of reduction is proportional to the hyperglycemia. In the same rats, the level of this transporter isoform in hepatocytes is essentially unchanged.

The neonatal streptozocin rats are characterized by glucose unresponsiveness of islet beta cells, a hallmark of type II diabetes (19–22). In isolated islets of these rats, there is also a modest decrease in the number of beta cells: in rats with plasma glucose at about 350 mg/dl, the number of beta cells, as a percentage of total islet cells, is reduced by about 25% (19). Our Western blot quantification of expression of the liver/beta-cell glucose transporter isoform shows that, in islets of similarly diabetic rats (see Table 1, experiment 3), the reduction in transporter expression is 82%. Thus our analysis shows that the reduction in transporter expression is much larger than the reduction in beta cells per islet. Therefore, individual beta cells express a much lower number of glucose transporters than do those in normal rats. These quantitative data are in agreement with our semiquantitative observations that immunofluorescence staining of this transporter in individual beta cells is markedly reduced during diabetes (Fig. 1). In islets of mildly diabetic rats (plasma glucose at 154 and 204 mg/dl), there may be an even lower reduction in the number of beta cells (although this has not been formally quantitated), but there is still a reduction of expression of the glucose transporter isoform assessed both by immunofluorescence staining of individual cells (Fig. 1 *C* and *D*) and by Western blot analysis of pooled islets (Table 1 and Fig. 2). We have consistently noted that heterogenous pattern of immunofluorescence staining of the beta-cell transporter in islets from mildly diabetic rats, with some cells displaying normal staining while others have an intermediate level or no staining at

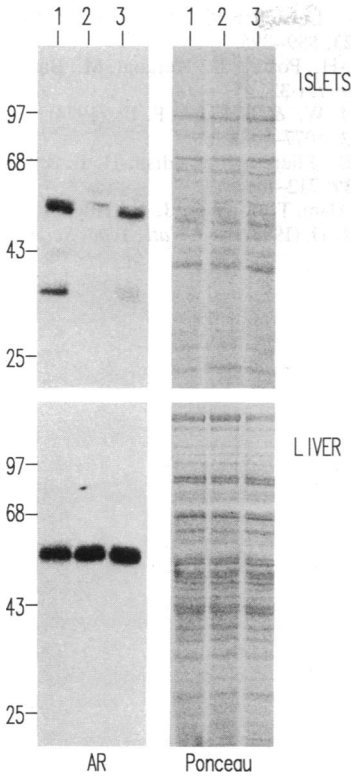

FIG. 2. Immunoblot analysis of the liver/beta-cell glucose transporter in islets (*Upper*) and liver (*Lower*) from control and diabetic rats. (*Upper Left*) Immunodetection of the glucose transporter in islets of control (lane 1) and diabetic rats with high (513 ± 129 mg/dl; lane 2) or low (204 ± 25 mg/dl; lane 3) plasma glucose. The bands migrating with a higher mobility than the 43-kDa marker are most probably degradation products of the glucose transporter and are observed in some experiments. (*Upper Right*) Ponceau S staining of the same blot prior to immunodetection of the glucose transporter. (*Lower Left*) Immunodetection of the glucose transporter in livers of the same rats. (*Lower Right*) Ponceau S staining of the filter on left prior to immunodetection of the glucose transporter. Molecular masses (in kDa) of marker proteins are on the left. AR, autoradiogram; Ponceau, Ponceau S staining of the filters.

all. The cause of this heterogenous expression pattern is not known, but it may indicate a differential sensitivity of beta-cell subpopulations either to the effect of streptozocin or to the ambient hyperglycemia.

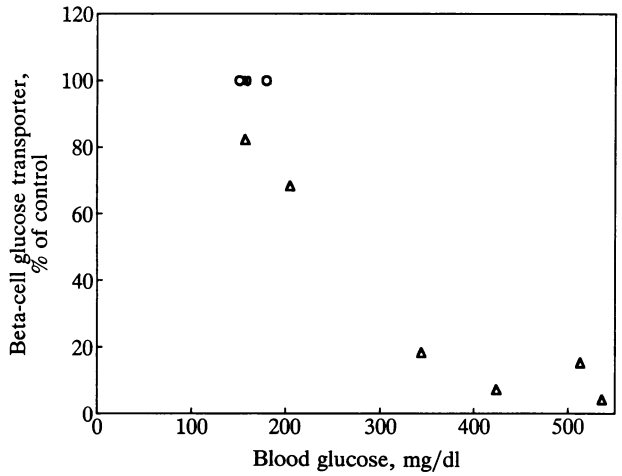

FIG. 3. Correlation between the level of immunodetectable liver/beta-cell glucose transporter in the islets of the control (open circles) and diabetic (open triangles) rats and plasma glucose levels. Data are from Table 1.

Glucose sensing by beta cells requires both glucose uptake and metabolism (6). In normal beta cells, the rate of glucose metabolism is controlled by glucokinase and glucose uptake is not limiting over the range of glucose concentrations stimulating insulin secretion (5–15 mM) because of the presence of a high-K_m glucose transporter (1, 4, 5, 8). We show here that a decrease in the level of this glucose transporter correlates with the diabetic state of the animals and the glucose unresponsiveness of islet beta cells. A decreased expression of the glucose transporter on the plasma membrane of beta cells will decrease the rate of uptake of glucose into the cell. This may limit the rate of access of glucose to glucokinase, thus preventing normal glucose sensing. Additional experiments will be required to measure directly the rates of glucose uptake and phosphorylation in islets from control and diabetic rats. However, Chen *et al.* (25) have shown that beta cells from rats maintained hypoglycemic by continuous infusion of insulin lose expression of the mRNA encoding the liver/beta-cell glucose transporter; these islets also lose high-K_m glucose transport (4) and display a strong reduction in glucose-induced insulin secretion.

Our studies show a strong correlation between the reduction of the expression of the liver/beta-cell glucose transporter isoform in diabetic islets and the hyperglycemic state of the animal. In the liver of the same rats, the expression of this transporter is minimally if at all modified. Thus these observations suggest that a specific decrease in expression of the liver/beta-cell glucose transporter in beta cells may be important in the development of the beta cell dysfunction characteristic of non-insulin-dependent type II diabetes.

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