The Loss of GLUT2 Expression by Glucose-unresponsive β Cells of db/db Mice Is Reversible and Is Induced by the Diabetic Environment

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Abstract

Glucose-induced insulin secretion by β cells of diabetic db/db mice was studied by a pancreas perfusion technique, and the levels of GLUT2 protein in pancreatic islets were assessed by immunofluorescence microscopy and protein blot analysis. β cells from diabetic mice had a high basal rate of insulin secretion; they did not respond to glucose stimulation but displayed a normal secretory response to arginine. At the same time, GLUT2 expression by db/db islets was lost whereas β cells from nondiabetic db/+ mice expressed high levels of this transporter. GLUT2 levels in liver or kidney of diabetic mice were, however, mostly unaltered. Transplanting islets from db/db mice under the kidney capsule of db/+ mice restored normal GLUT2 levels. Conversely, transplantation of db/+ islets into db/db mice induced the disappearance of GLUT2 expression. When islets from db/+ mice were transplanted under the kidney capsule of streptozocin-diabetic mice, the immunodetection of GLUT2 also disappeared. We conclude that: (*a*) GLUT2 expression is decreased in glucose-unresponsive β cells from db/db mice; (*b*) the decreased expression of GLUT2 is reversible; (*c*) the loss of GLUT2 expression is induced by the diabetic environment of db/db and streptozocin-induced diabetic mice. These observations together with previously published data suggest that a factor different from glucose or insulin regulates the β cell expression of GLUT2. (*J. Clin. Invest.* 1992. 90:77–85.) Key words: anti–peptide antibodies • immunofluorescence microscopy • non–insulin-dependent diabetes mellitus • pancreas perfusion • transplantation

Introduction

The major dysfunction of β cells associated with the preovert phase of type I diabetes or with type II diabetes is a decreased secretory response to glucose (1–8). Expression in the plasma membrane of β cells of the glucose transporter isoform GLUT2 (9, 10), which has a high K_m for glucose (17 mM) (11) may be required for the normal functioning of the β cell glucose sensor (9–12). Recently, several reports have shown the glucose-unresponsiveness to be correlated with a reduced expression of the β cell-specific glucose transporter GLUT2. For example, at the day of onset of diabetes in the BB/W rat, the β cells are no longer sensitive to variations in extracellular glucose concentrations but still secrete insulin in response to a challenge with arginine (2). This loss of glucose sensing is accompanied by a decreased rate of glucose uptake by islet cells to ~ 10% of the normal value. At the same time, about 50% of the β cells have lost GLUT2 expression, as assessed by immunocytochemical methods (13). In rat models of type II diabetes such as the neonatal streptozocin rat (14) and the diabetic Zucker rat (15, 16), GLUT2 expression is also reduced in glucose-unresponsive β cells and the extent of reduction is proportional to the severity of the hyperglycemia. In the diabetic Zucker rat, the decreased expression of GLUT2 correlates with a decreased rate of glucose uptake through the high K_m transporter (GLUT2) (15). Thus a decreased expression of GLUT2 is associated with the β cell dysfunction of rodent models of insulin-dependent diabetes mellitus and non–insulin-dependent diabetes mellitus and decreased GLUT2 levels may impair normal glucose uptake and metabolism thereby preventing glucose sensing (17).

Decreased GLUT2 expression is the first morphological marker for β cells from type II diabetic animals so far described. Therefore, the discovery of the factors regulating GLUT2 expression during the development of diabetes and whether the loss of GLUT2 expression precedes or follows the appearance of the β cell dysfunction may lead to a better understanding of the pathogenesis of diabetes. Several experiments suggested that the decreased expression of GLUT2 did not correlate with hyperglycemia. When Zucker rats are treated for several weeks with acarbose, an inhibitor of intestinal glucosidases, blood glucose levels are corrected and maintained close to normoglycemia. GLUT2 levels are however still decreased to values similar to that of diabetic rats (15, 16). Also, infusion of glucose to normal or partially pancreatectomized rats for several weeks does not result in the loss but rather in a small increase in GLUT2 expression (13). These experiments suggested that hyperglycemia per se was not the cause of GLUT2 reduction, and that a decreased expression of GLUT2 in diabetic rats may be at the origin of the β cells dysfunction leading to the diabetic syndrome.

In this article, we examine a mouse model of type II diabetes, the db/db mouse. We show that the β cells from these mice display the typical glucose-unresponsiveness associated with type II diabetes and that they have lost GLUT2 expression. To determine whether the decreased expression of GLUT2 was preceding or was the result of the diabetes, we performed transplantation experiments. Islets from diabetic mice were placed under the kidney capsule of normal mice or, conversely, islets from normal animals were transplanted into diabetic (types I and II) mice. We show that the decreased expression of GLUT2 characteristic of diabetic mice is reversible when the islets are placed in a nondiabetic environment, and that exposure of normal β cells to the diabetic environment of

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type I or II diabetes reduces GLUT2 levels. Taken with previous data, our results suggest that a factor different from glucose or insulin but present in both forms of diabetes may induce the decreased expression of GLUT2. This study also demonstrated that db/db mice can be used as a valuable model of type II diabetes in which the β cell-specific regulation of GLUT2 can be studied.

Methods

In situ perfused mouse pancreas. The methodology for the in vitro isolated pancreas perfusion has previously been described for the rat (18). The technique for the mouse was identical except for minor surgical modifications in terms of vessel cannulation. Blood for plasma glucose measurements was collected by tail snipping before anesthetizing with sodium amobarbital (150 mg/kg i.p.). The perfusate was a Krebs-Ringer bicarbonate buffer, pH 7.4, which contained 4% Dextran T70 (Sigma Chemical Co., St. Louis, MO), 5.5 mM glucose, 2 mM Ca^{++}, and bovine serum albumin fraction V (Sigma Chemical Co.). Perfusate containing the higher glucose concentration (16.7 mM) was stored in a second reservoir. Arginine (10 mM) was added by a sidearm syringe. A midline incision was made and the major vessels from the aorta except those of the pancreas were tied off. The aorta was cannulated with a blunted 26-gauge needle. Perfusate was infused at 1 ml/min and the portal vein was cannulated with PE50 polyethylene tubing (Clay Adams, Parsippany, NJ). After completion of the surgery, the body cavity was covered with gauge soaked in saline and maintained at 36–39°C by a heat lamp. The initial 10 min served as an equilibration period during which no samples were taken. Thereafter, minute samples were collected in chilled tubes containing 4 mg EDTA and kept on ice pending storage at −20°C. After perfusion shown in Fig. 1, the pancreas was perfused with 5 ml of paraformaldehyde/lysine/periodate fixative (19) over 5 min for assessment of GLUT2 by indirect immunofluorescence.

Analytical methods. Plasma glucose was measured with a Glucose Analyzer II (Beckman Instruments, Inc., Brea, CA). Insulin concentrations were determined by a radioimmunoassay which used charcoal separation (20) and rat insulin standards (Eli Lilly & Co., Indianapolis, IN).

Islet isolation and transplantation. After anesthesia with sodium amobarbital (150 mg/kg i.p.), the bile duct was cannulated with a 27-gauge needle and the pancreas was distended with 2 ml of a solution containing TC 199 medium (GIBCO/BRL, Gaithersburg, MD), 1% penicillin/streptomycin, and 2 mg/ml collagenase (Serva, Heidelberg, FRG). The pancreas was incubated in a tissue culture flask at 37°C for 21 min, washed, and vortexed. Large particles were removed with a mesh filter. Islets were obtained from the filtrate by gradient separation: a mixture of the filtrate from a single pancreas and 10 ml Histopaque (Sigma Chemical Co.) was placed in a 50-ml conical tube, then 10 ml of TC 199 was gently laid over it. After spinning at 900 *g* for 20 min, the islets were located at the interface. Islets were hand-picked and placed in a gelfoam-plugged 250-μl pipette tip. Under sodium amobarbital anesthesia, the left flank of the recipient mouse was shaved, a 1-cm incision was made, and the kidney was "popped out." A small cut was made in the membrane on the greater curvature, and the islets were gently layered under the capsule. 100 islets were transplanted in all experiments. 2 wk posttransplant, the graft was fixed with paraformaldehyde-lysine-periodate fixative (19). After anesthesia with sodium amobarbital, ties were placed around the right kidney vessels and around the aorta above the renal vessels. The aorta was cannulated below the left renal artery as previously described and fixative was infused at 1.5 ml/min for 5 min. The islet graft was excised and processed for GLUT2 immunofluorescence.

Antibodies. An antibody was raised against a peptide derived from the COOH terminus of mouse GLUT2 (amino acids 512–523) (21). The peptide was coupled with glutaraldehyde (22) to keyhole limpet hemocyanin, emulsified in complete Freund's adjuvant, and injected subcutaneously into rabbits. Additional boosts were performed by subcutaneous injection of the antigen emulsified in incomplete Freund's adjuvant. For immunofluorescence staining, antibodies were first immunopurified by passage over an Affigel 15 column (Bio-Rad Laboratories, Richmond, CA) to which was coupled a complex of the immunizing peptide cross-linked with glutaraldehyde to bovine serum albumin, as described (9, 23). The affinity-purified antibodies were subsequently stored in small aliquots at −20°C in the presence of 1 mg/ml bovine serum albumin.

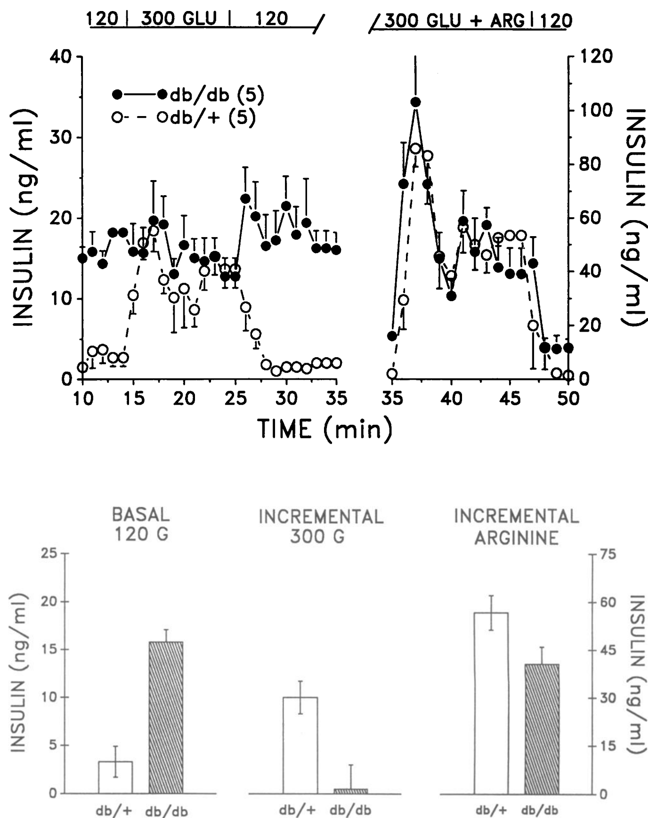

Figure 1. (*Top*) Insulin secretion from perfused pancreas. Pancreases from db/db or db/+ mice were perfused as described in Methods and the insulin content of the effluent was measured by radioimmunoassay. Islets from db/+ mice secrete insulin at a low level in the presence of a basal level of glucose (120 mg/dl) and the secretion is stimulated severalfold by a challenge with 300 mg/dl glucose. In contrast, islets from db/db mice have a high basal insulin secretion and do not respond to variations in glucose concentrations. However, they display a large secretory response to an arginine challenge, similar to the response of islets from control mice. (*Bottom*) Cumulative insulin secretion. This is expressed as the mean concentration of insulin in the perfusate during the entire time interval of each condition. The total amount of insulin secreted by db/db pancreas during the five minutes at the basal condition is fivefold higher than that from control mouse pancreases. The incremental secretion in response to glucose is not significant for the diabetic mouse pancreases whereas it is fourfold for the control mice. In contrast, incremental arginine secretion is however similar in both groups.

For immunofluorescence detection of GLUT2, pancreas or kidneys were perfused-fixed by a paraformaldehyde-lysine-periodate fixative (19). The tissues were embedded in OCT medium (Miles Scientific, Kenkakee, IL), and 4–5-μm sections were cut with a Reichert Jung cryostat (Reichert Scientific Instruments, Buffalo, NY). The detection of GLUT2 was performed with the affinity-purified antibody and a fluorescein-conjugated second antibody exactly as described (9, 23). Immunostaining was completely abolished when the incubation was performed in the presence of the immunizing peptide at a concentration of 10 μg/ml. Pictures were taken with an Axiophot photomicro-

scope (Carl Zeiss, Inc., Thornwood, NY) and TX MAX films (Eastman Kodak Co., Rochester, NY).

For Western blot detection of GLUT2, islet lysates were prepared by solubilizing the islets in a solution containing 5% sodium dodecyl sulfate, 80 mM Tris/HCl, pH 6.8, 5 mM EDTA, 0.5 mM phenylmethylsulfonylfluoride followed by sonication with three 30-s pulses at maximum power in a sonicator (Heat Systems, Inc., Farmingdale, NY). Liver and kidney were lysed in the same solution but with the help of a homogenizer (Polytron, Brinkmann Instruments, Inc., Westbury, NY). Protein concentrations were determined by the bicinchoninic acid protein assay (Pierce Chemical Co., Rockford, IL) using bovine serum albumin as standard. The lysates were separated by electrophoresis on 10% sodium dodecyl sulfate polyacrylamide gels, and the detection of GLUT2 by Western blotting was performed after electrotransfer to nitrocellulose membranes, using the antiserum to GLUT2 diluted 1:200, exactly as described (9). Addition of the immunizing peptide at 10 μg/ml during immunoblotting abolished the detection of the GLUT2 signal.

Streptozocin-induced diabetic mice. C57Bl/6 mice were made diabetic by a single injection of streptozocin at a dose of 200 mg/kg body weight. Transplantation of db/+ islets was performed 12 d after streptozocin injection and the islets were retrieved 2 wk later. Blood glucose of the three mice was 444, 444, and 386 mg/dl 6 d after streptozocin injection and 404, 474, and 303 mg/dl the day before the termination of the experiment. The mice did not receive any treatment during the course of the experiment.

Results

Insulin secretion by the perfused pancreas. The pattern of secretion by pancreatic islet β cells from db/db and db/+ mice was studied with an isolated perfused pancreas method. Six db/db mice (body weight 41.7±0.3 g, blood glucose 517±24 mg/dl) and six db/+ mice (body weight 22.7±0.4 g, blood glucose 168±10 mg/dl) were used. β cells from nondiabetic db/+ mice secreted low levels of insulin in the presence of 5 mM glucose (3.3±1.6 ng/ml) and the secretion rate was increased to 13.3±1.4 ng/ml in response to a change of glucose concentration to 16.7 mM; a further increase in the rate of insulin secretion was achieved when 10 mM arginine was added to the high glucose concentration (Fig. 1). In contrast, insulin secretion by db/db islets showed a high basal level of insulin secretion, similar in magnitude to the maximum of secretion induced by 16.7 mM glucose in control mice, and was not further increased by a rise in glucose concentration in the perfusate. This high basal rate of insulin secretion may result in part from β cells hyperplasia (24) in diabetic mice but also from a hypersecretion of insulin per β cells. The respective contributions of hyperplasia and hypersecretion to the high basal insulin secretion rate is, however, not known. Challenge of db/db pancreas with arginine in the presence of 16.7 mM glucose induced a strong secretory response similar in magnitude to the response of the control pancreas (Fig. 1, *top*). In Fig. 1 (*bottom*), the cumulative secretion of insulin from pancreases of diabetic or control mice in the different stimulatory conditions is presented.

Loss of GLUT2 expression by β cells from db/db mice. The expression of the β cell-specific glucose transporter GLUT2 was studied by immunofluorescence microscopy and by Western blot analysis. The immunostaining for GLUT2 in β cells from db/+ mice and db/db mice is shown in Fig. 2 (*upper panels*) and demonstrates a complete lack of GLUT2 detection in beta cells from diabetic mice, although we occasionally detected a few (< 5%) lightly GLUT2-positive β cells in sections of db/db islets. In contrast, each β cell from the db/+ islets displayed a strong staining for GLUT2. Fig. 2 (*lower left*) shows that GLUT1 was not induced in db/db islets. The staining for GLUT1 outside of the β cell area was on α cells and represented a nonspecific sticking of the antibody to these cells: by immunofluorescence microscopy using ultracryomicrotome sections the staining was clearly shown to be associated with secretory granules and not with the plasma membrane; by immunoelectron microscopy analysis, gold particles revealed the GLUT1 antibody to be mostly associated with the core of the secretory vesicles and not with any membrane structures (B. Thorens and D. Brown, unpublished observations). The β cells from db/db mice had, however, a normal pattern of staining for insulin (Fig. 2, *lower right*). Therefore, the disappearance of GLUT2 from glucose-unresponsive β cells of db/db mice was similar to observations made in other rodent models of type II diabetes (13–15).

The disappearance of GLUT2 expression was measured by Western blot analysis of lysates of islets isolated from diabetic and control mice (Fig. 3). GLUT2 was present in islets from db/+ mice but not in islets from db/db mice. The appearance of GLUT2 as a doublet in freshly isolated islets was observed consistently and represented proteolytic degradation of GLUT2 occurring during the isolation procedure and which could not be prevented by addition of protease inhibitors to the collagenase solution (B. Thorens, unpublished observation). The detection of both bands could be blocked with the peptide antigen. GLUT2 levels in lysates from liver and kidney were not markedly altered by the diabetes of the animals (Fig. 3). A change in the electrophoretic mobility of GLUT2 in the liver of diabetic mice was observed (Fig. 3); the cause for this change in apparent molecular weight is not known but may result from a change in the oligosaccharide side chain. The almost complete loss of GLUT2 expression in diabetic islets in face of unchanged expression in liver and kidney therefore indicated the presence of β cell-specific control of GLUT2 expression associated with diabetes.

GLUT2 decreased expression is the result of the diabetic environment. Decreased expression of GLUT2 is a consistent finding in islets from diabetic rodents and represents a valuable marker of the diabetic state. To determine whether GLUT2 regulation in diabetic mouse islets was a result of the diabetic environment, we performed transplantation experiments. Islets isolated from db/db mice were surgically implanted under the kidney capsule of nondiabetic db/+ mice. 2 wk later, the kidneys were removed and GLUT2 expression was assessed by immunofluorescence microscopy on frozen sections. GLUT2 expression in transplanted db/db islets (Fig. 4, *left and middle*) was restored to a level indistinguishable from GLUT2 in islets from db/+ mice transplanted into db/+ mice (compare Fig. 4, *middle and right*). When islets from db/db mice were transplanted under the kidney capsule of db/db mice, no restoration of GLUT2 expression could be observed (Fig. 5, *top*). The presence of β cells in the tissue section was confirmed by subsequent staining of the section for insulin (Fig. 5, *bottom*). The transplantation of islets from db/+ mice under the kidney capsule of db/db mice resulted in the disappearance of GLUT2 (Fig. 6, *top*); the presence of β cells was also shown by subsequent staining of the same section for insulin (Fig. 6, *bottom*). These data indicated that the decreased expression of GLUT2 in db/db mice was reversible and was the result of the diabetic environment. The data in Fig. 6 also showed strikingly the differential regulation of GLUT2 in β cells as compared to its

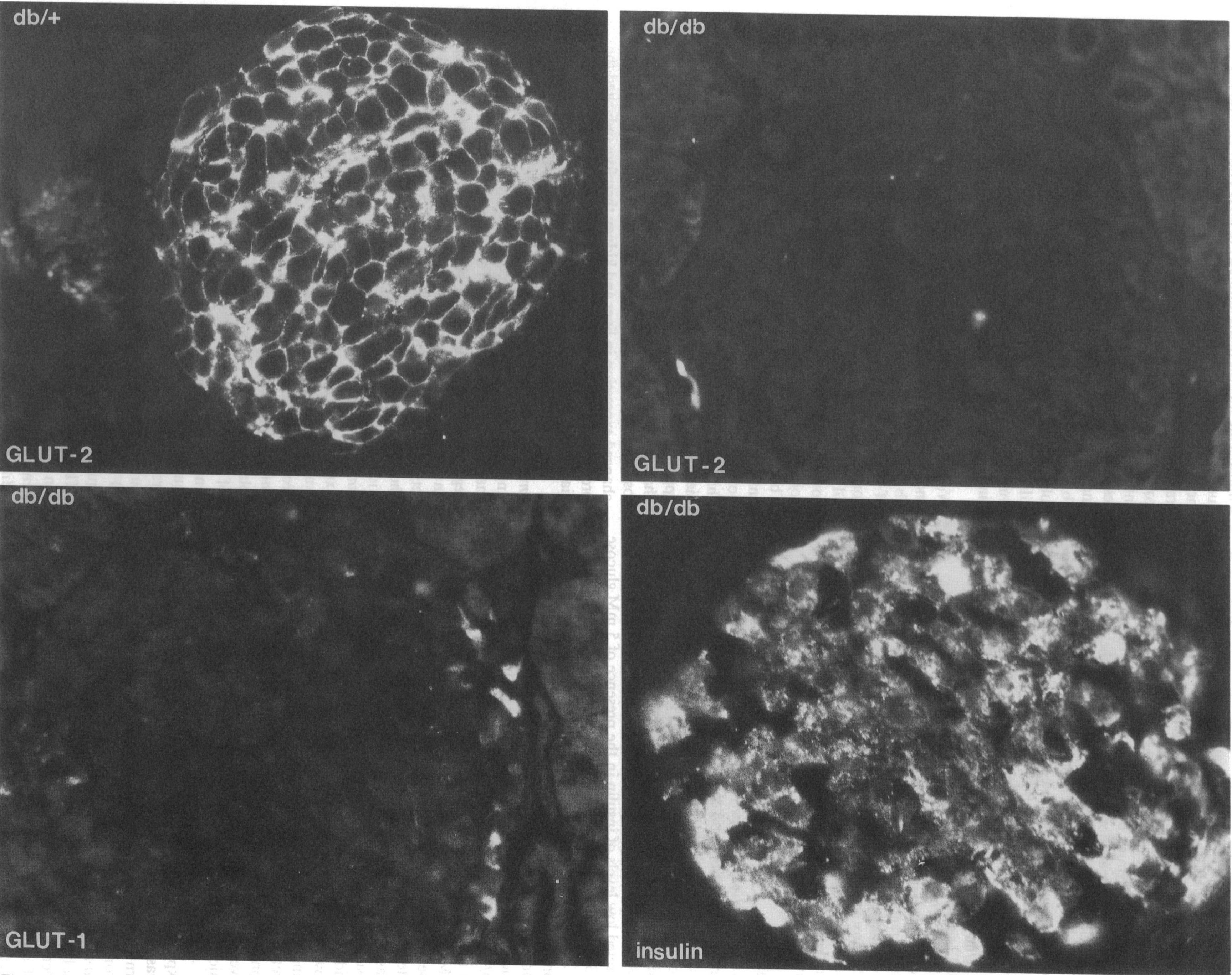

Figure 2. Loss of GLUT2 immunodetection in db/db islets. GLUT2 immunostaining in islets from db/+ mice is on every β cell although β cells from db/db mice do not show any staining. GLUT1 is not induced in diabetic islets but these islets stain normally for insulin. ×370.

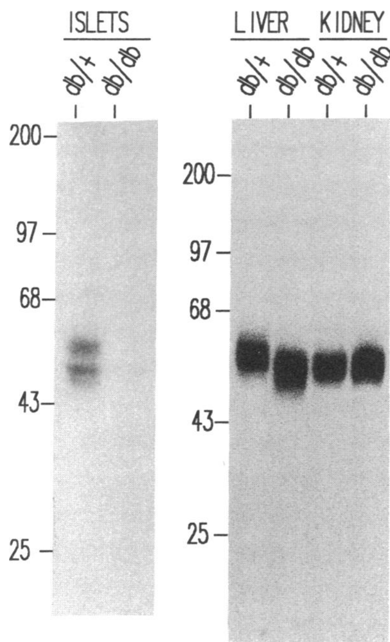

Figure 3. β cell-specific decreased expression of GLUT2. GLUT2 protein levels were determined by immunoblotting of the total cellular lysates of islets, liver, and kidney. The lysates corresponding to 50 islets were loaded on each islet lane and 50 μg of total protein for liver and kidney lysates was loaded. Note the appearance of GLUT2 as a doublet in the db/+ lane; this results from partial proteolytic degradation of the transporter during the islet isolation procedure before cell lysis. No GLUT2 could be detected in db/db islets. The level of GLUT2 expression is mostly unaltered in liver and kidney of diabetic mice. The mobility of the liver GLUT2 is however modified in db/db mice. Liver and kidney proteins obtained from homogenate of organ fragments pooled from three animals. This experiment has been repeated with another set of three animals with identical results.

regulation in kidney: GLUT2 which was normally expressed at a high level in normal islets from db/+ mice has disappeared in islets transplanted into the kidney of diabetic mice whereas the expression of GLUT2 by cells of the proximal convoluted tubule of the same kidney was normal (25). We further tested the effect of transplanting normal islets under the kidney capsule of mice made diabetic by streptozocin treatment. Islets were transplanted in low number (50–100 per mice) to avoid correcting the diabetes of the recipient mice. After 2 wk of transplantation, GLUT2 levels were much decreased whereas insulin staining was normal (Fig. 7). Therefore, the diabetic environment of streptozocin induced type I diabetes also lead to decreased GLUT2 expression.

Discussion

In this study we show that the β cells from db/db mice hypersecrete insulin in the basal state, do not respond to a glucose challenge, but maintain a normal response to arginine. We also show that these β cells have lost GLUT2 expression. By using transplantation experiments, we show that the decreased expression of GLUT2 is reversible and depends on the diabetic environment, and that types I and II diabetes may provide the same factor(s) inducing the decreased expression of GLUT2.

The glucose unresponsiveness of β cells from patients in the preovert phase of type I diabetes or from type II diabetic patients or animal models is an established fact (1–8, 13–16). Diabetes in the db/db mouse develops according to a well-studied pattern: hyperphagia and hyperinsulinemia appear

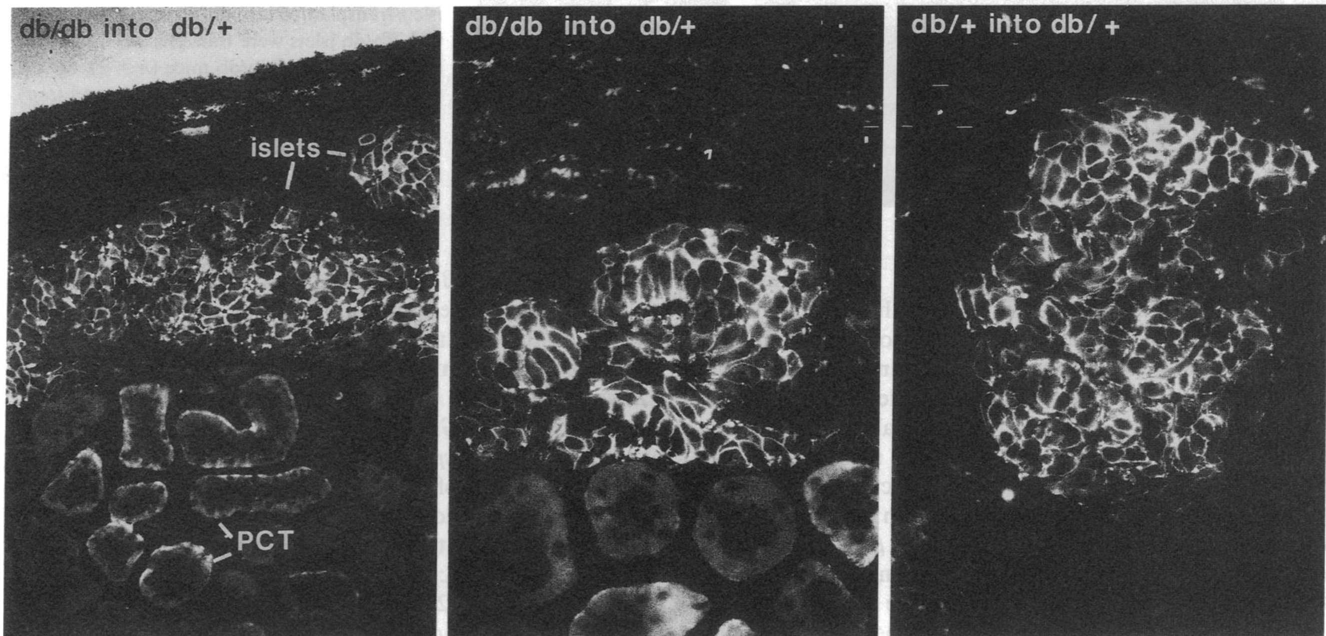

Figure 4. Reexpression of GLUT2 in db/db islets transplanted under the kidney capsule of db/+ mice. Islets from diabetic mice were transplanted under the kidney capsule of normal mice (n = 3); the kidney was removed 2 wk later. The picture in the left panel shows part of a section through the kidney with the capsule at the top, the transplanted islets below and the cortex with some proximal convoluted tubules (*PCT*) in the lower part. Both the islets and the PCT cells show strong staining for GLUT2. The middle and right panels show at the same magnification that the staining for GLUT2 is similar for db/db or db/+ islets transplanted in the kidney of control mice (n = 2 for db/+ into db/+). *Left:* ×148. *Middle and right:* ×237.

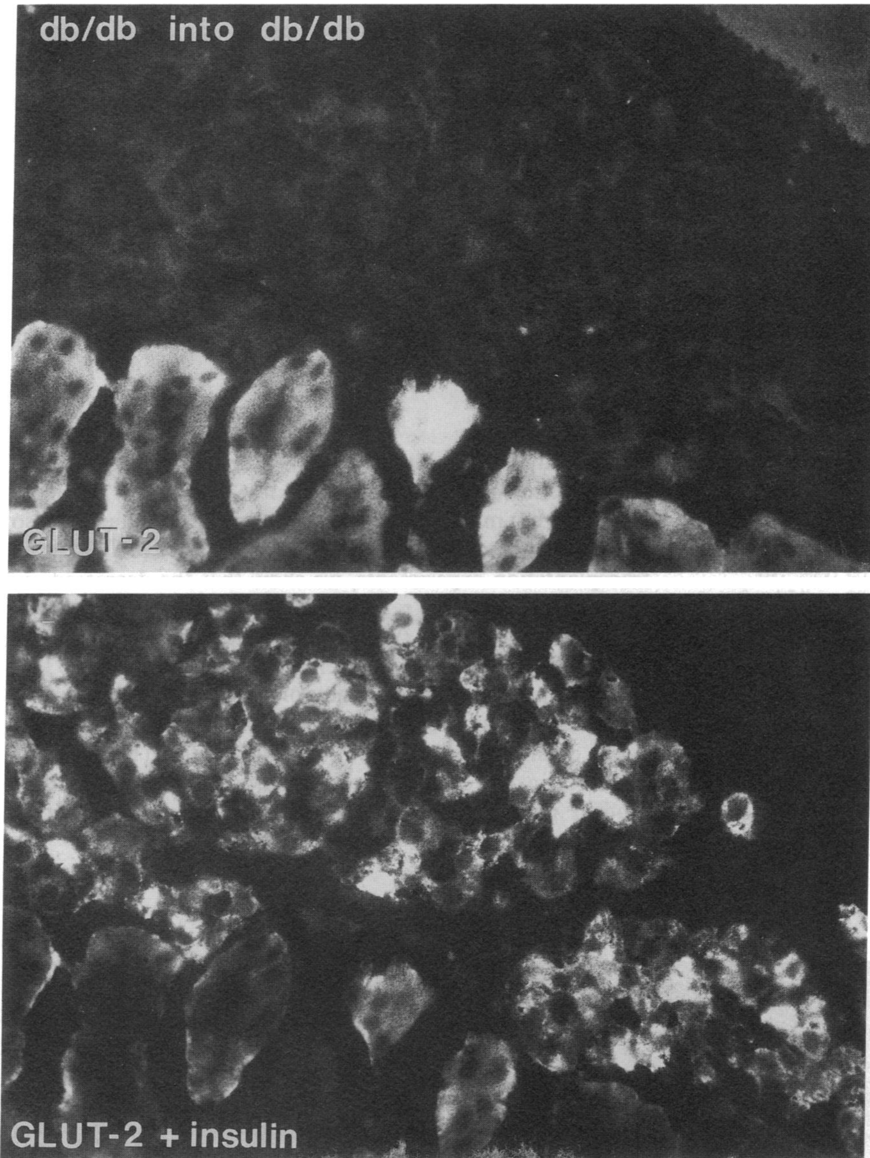

Figure 5. No reexpression of GLUT2 in db/db islets transplanted into db/db mice. As a control, db/db islets were transplanted under the kidney capsule of db/db mice ($n = 2$), no GLUT2 reexpression could be observed (*top*). After being first photographed for GLUT2 staining, the sections were stained for insulin and photographed again. The β cells were positive for insulin (*bottom*). ×312.

during the first month of life, during the second and third month hyperglycemia develops together with marked hyperinsulinemia and weight gain. Later on, in association with atrophy of β cell mass, resulting from nonimmunologic causes, the hyperglycemia still progresses although the levels of insulin decrease and the mice lose weight and eventually die (26). In the present study we used mice between the second and third month of life at a time when the pancreatic islets presented a normal morphological structure and insulin immunostaining. At that age these mice displayed the usual β cell dysfunction associated with type II diabetes: glucose unresponsiveness in face of normal sensitivity to the nonglucose secretagogue arginine. In addition, in agreement with other models of type I or II diabetes (see Introduction in reference 17), the β-cell glucose unresponsiveness was associated with an almost complete loss of GLUT2 expression. This further establishes decreased expression of GLUT2 as a valuable marker for diabetic β cells. It is interesting to note that GLUT2 expression is only minimally altered in the liver and kidney of the same animal, indicating the existence of a β cell-specific control of its expression, an observation consistent with previous reports. Also, expression of the insulin-regulated glucose transporter GLUT4 is unaltered in different muscles and in adipose tissue of the db/db mouse (27).

What controls GLUT2 expression in β cells of db/db mice and what is the respective role of glycemia and insulinemia? The development of diabetes is controlled by the db locus on chromosome 4, which has a complete penetrance in the homozygous mouse, but the severity of the diabetes varies with the genetic background: it is more severe in the C57Bl/Ks than in the C57Bl/6 mice (26). The gene at the db locus which causes the disease is not known. It is, however, distinct from the genes for GLUT1, GLUT2, GLUT3, and GLUT4, which map to different chromosomal sites in the mouse (28). Therefore, an abnormality in the GLUT2 gene can be excluded as a cause of its underexpression in β cells of diabetic mice.

Our transplantation experiments show that the decreased expression of GLUT2 in db/db islets is reversible in that a

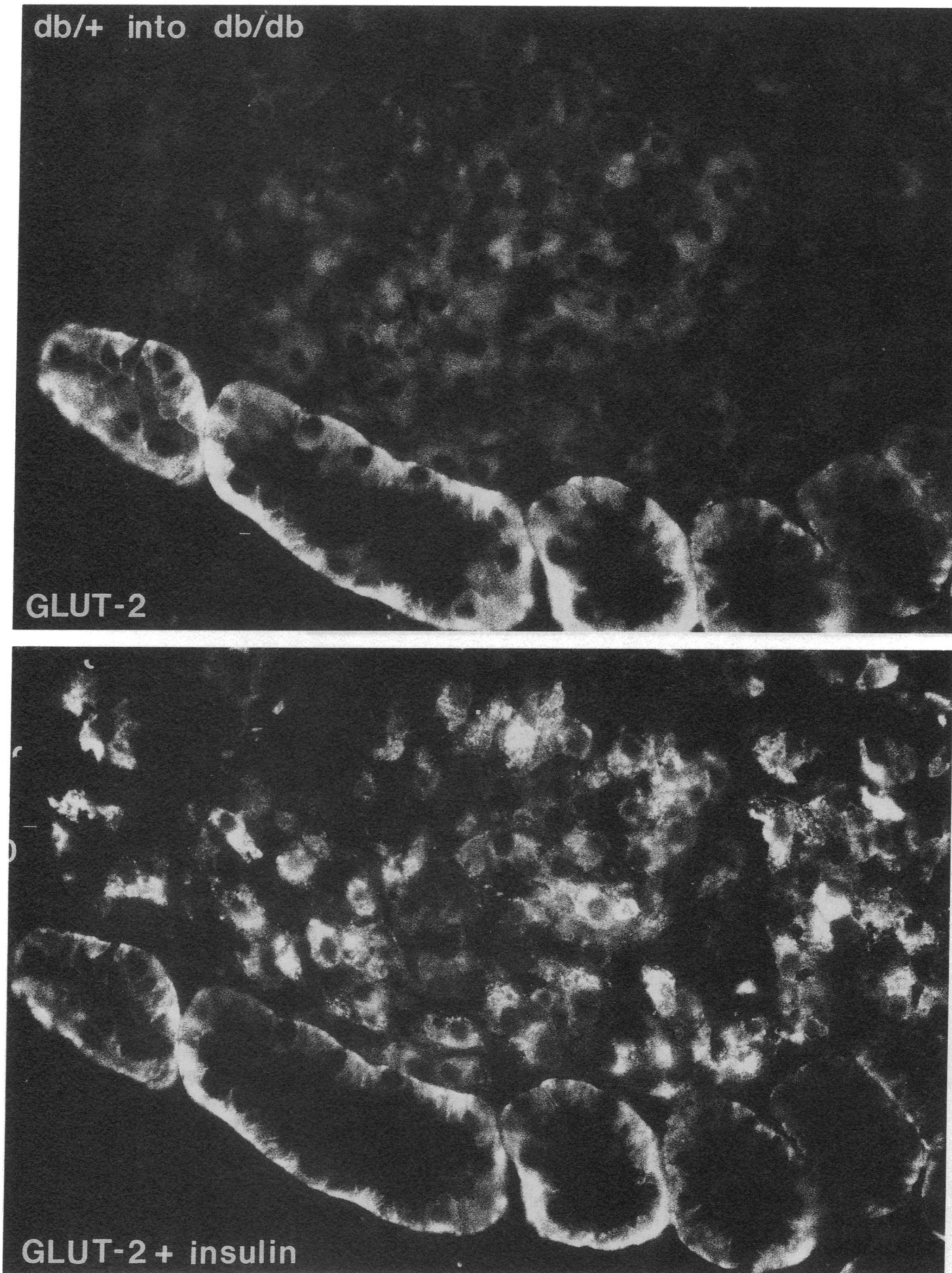

Figure 6. The diabetic environment of db/db mice induces the loss of GLUT2 expression. Islets from db/+ mice were transplanted under the kidney capsule of db/db mice and sections of the kidney were stained for GLUT2 2 wk later. No staining for GLUT2 could be detected in the transplanted islets (*top*) although these cells express normal levels of insulin (*bottom*). Note the strong staining for GLUT2 in cells from the proximal convoluted tubules while no staining is detected in the transplanted control islets, thereby further illustrating the β cell-specific regulation of GLUT2. ×375.

nondiabetic environment can induce the normal reexpression of that transporter. Conversely, GLUT2 expression in normal β cells can be suppressed when islets are exposed to the diabetic environment of db/db or of streptozocin-induced diabetic mice. The primary cause of GLUT2 down-regulation may be the high insulin or glucose levels or another as yet unidentified factor.

Insulinemia. The presently studied diabetic mice are both hyperinsulinemic and hyperglycemic. Other genetic models of diabetes such as the Zucker diabetic and the Wistar Kyoto rat are also hyperinsulinemic and hyperglycemic and have reduced GLUT2 levels (15, 16). However, neonatal streptozocin rats (13) and GK diabetic rats, which are both nonobese and have slightly reduced or normal insulin levels, also have reduced GLUT2 expression (16), whereas female Zucker rats, which are hyperinsulinemic and do not become diabetic, express normal levels of GLUT2 (15). Therefore, there appear to be no direct correlation between GLUT2 levels and ambient insulinemia. Also, the transplantation of nondiabetic db/+ islets into streptozocin diabetic mice induces a decreased detection of GLUT2, indicating that insulin is not the primary regulator of GLUT2 expression.

Glycemia. A similar lack of correlation between hyperglycemia and GLUT2 down-regulation is also apparent. GLUT2 is

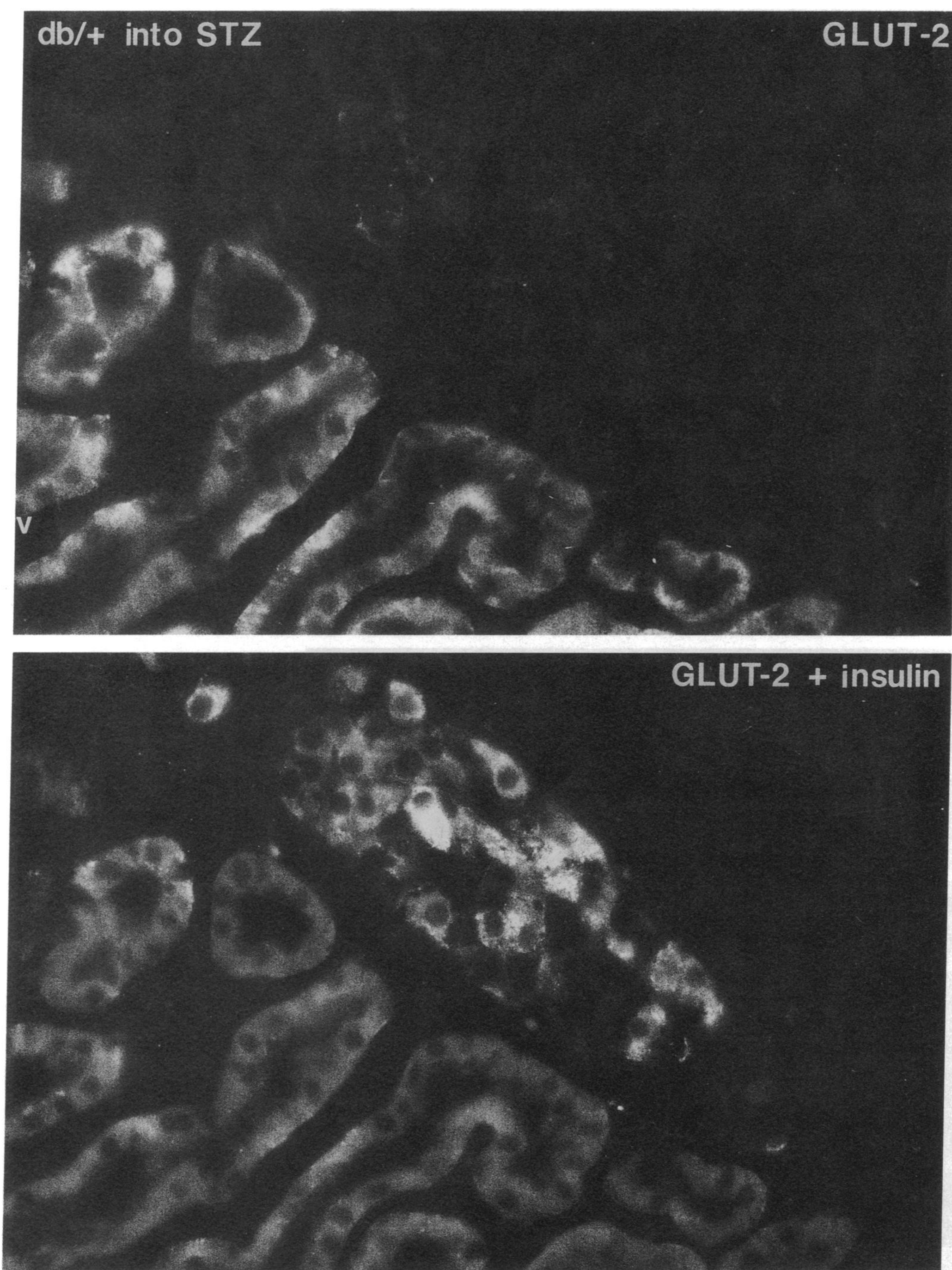

Figure 7. Loss of GLUT2 staining in db/+ islets transplanted into streptozocin-induced diabetic mice. Islets from db/+ mice were transplanted under the kidney capsule of mice made diabetic by streptozocin treatment ($n = 2$) and stained 2 wk later for GLUT2. Note the disappearance of GLUT2 immunostaining (*top*) whereas the β cells are clearly present as evidenced by their staining for insulin (*bottom*). ×375.

reduced in the db/db mouse β cells and in nondiabetic db/+ cells transplanted into db/db or streptozocin mice and in β cells from the other models of type I or II diabetes studied (cf. Introduction and above). However, GLUT2 expression is not modified in β cells of rats partially pancreatectomized and infused with a 50% glucose solution for 2 wk so as to maintain a glycemia of ~250 mg/dl (13). GLUT2 mRNA is increased in glucose-infused nonpancreatectomized rats (29) and labeling with immunogold particles is also increased in β cells kept for 1–5 wk in the presence of 33.4 mM glucose (16). The above observations indicate that there is also no direct correlation between ambient glycemia and GLUT2 increased or decreased expression. Also there is no correlation between the simultaneous presence of high glucose and high insulin and decreased GLUT2 levels because the db/db mouse, the Zucker diabetic rat, or the Wistar Kyoto rat are all hyperglycemic and hyperinsulinemic and have low GLUT2 whereas the glucose-infused rats with or without partial pancreatectomy are also hyperglycemic and hyperinsulinemic and express normal or elevated levels of GLUT2.

What then is the primary regulator of GLUT2 expression in β cells? Our transplantation experiments suggest that it is a circulating factor or set of factors. In the db/db mouse, diabetes is the result of a genetic mutation present at the db locus and is

thought to affect first the function of the hypothalamus (26). This hypothalamic dysfunction may thus lead to the overexpression of factors which may induce β cell dysfunction and ultimately diabetes. We do not yet know whether GLUT2 down-regulation parallels, precedes, or follows, the development of the β cell secretory defect. If GLUT2 loss of expression would correlate with the development of the hyperinsulinemia, then the study of its expression in islets in vivo during the progression of the disease or in vitro after culture in the presence of different factors, may be an extremely valuable marker to try to characterize the factors which initiate the development of the disease. Whether the same factor is induced in the streptozocin rat, as a result of the destruction of the β cells or as a result of streptozocin action on another target organ, is also not known.

In summary, our data confirm that a loss of GLUT2 expression is a characteristic of glucose-unresponsive β cells from diabetic animals. We further show that this decreased expression is reversible and is the result of the diabetic environment. However, our data taken together with previously published observations suggest that neither glucose nor insulin by themselves are the regulators of GLUT2 expression. Therefore, we propose that an as yet uncharacterized factor or conjugation of different factors may regulate GLUT2 in β cells independently of hyperglycemia or hyperinsulinemia. The decreased expression of GLUT2 is a marker of and may be part of the cause of the glucose unresponsiveness of the β cells. Because it is a marker of β cell dysfunction, understanding GLUT2 regulated (decreased) expression may help in the characterization of the primary causes (factors) that lead to the development of diabetes. In that respect, the presently studied db/db mouse offers a valuable model for these studies.

Acknowledgments

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