Virchows Arch (1996) 428:177–185

ORIGINAL ARTICLE

Anne Jörns · Markus Tiedge · Eckart Sickel
Sigurd Lenzen

Loss of GLUT2 glucose transporter expression in pancreatic beta cells from diabetic Chinese hamsters

Received: 25 January 1996 / Accepted: 4 March 1996

Abstract The diabetic Chinese hamster is a well-established animal model for NIDDM with a defective glucose-induced insulin secretory response. In the pancreas of nondiabetic hamsters, the GLUT2 glucose transporter was localized in the plasma membrane of insulin-positive beta cells. At variance with the rat, immunoreactivity was also detected in the cytoplasm. Other islet cell types were not GLUT2 positive. GLUT2 immunoreactivity was already significantly reduced in beta cells from mildly diabetic animals in spite of a normal insulin immunoreactivity. In severely diabetic animals the majority of the beta cells had lost GLUT2 immunostaining. This observation was confirmed in a Western blot analysis of the GLUT2 protein in isolated pancreatic islets. Only beta cells that were densely immunostained for insulin were still GLUT2 positive. However, around 40% of the beta cells devoid of GLUT2 immunoreactivity were still insulin immunoreactive. Thus, the loss of GLUT2 immunoreactivity, which is an important component of the glucose recognition apparatus of the pancreatic beta cell, is an early indicator of beta cell dysfunction before the development of degenerative lesions or the loss of insulin immunoreactivity. GLUT2 loss may be important in the deterioration of glucose-induced insulin secretion in the diabetic Chinese hamster.

Key words Diabetic Chinese hamster · Pancreatic beta cell · Immunocytochemistry · Insulin · GLUT2 glucose transporter

A. Jörns (✉)
Department of Anatomy I, Hannover Medical School, D-30623 Hannover, Germany

M. Tiedge · S. Lenzen
Institute of Clinical Biochemistry, Hannover Medical School, D-30623 Hannover, Germany

E. Sickel
Central Institute for Laboratory Animal Breeding, Hannover, Germany

Introduction

The Chinese hamster is an excellent animal model for NIDDM [8]. In previous morphological studies the changes in the pancreatic islets and the destruction of the beta cells during the development of diabetes have been documented [7, 9, 18, 19]. The degenerative beta cell lesions are accompanied by a deterioration of glucose-induced insulin secretion [6].

Together with glucokinase, the low-affinity plasma membrane GLUT2 glucose transporter in the pancreatic beta cell is responsible for recognition of glucose as the signal for glucose-induced insulin secretion [16, 17, 20]. Rat pancreatic beta cells display a dense immunostaining for GLUT2 in the cell membrane [12, 29] with a preferential localization on microvillous domains [23]. In pancreatic beta cells from various rat and mouse models of type I and type II diabetes a reduction or even a complete loss of GLUT2 expression has been described [13, 21, 22, 24, 25, 27, 30, 31]. No immunocytochemical studies on GLUT2 expression in beta cells in Chinese hamsters have been performed.

In the present study, GLUT2 glucose transporter expression has been studied by the use of immunocytochemical methods in semithin sections of pancreatic beta cells from normal and diabetic Chinese hamsters and in Western blots. We show that the development of diabetes in Chinese hamsters is accompanied by a concomitant reduction of GLUT2 immunoreactivity, which precedes the loss of insulin immunoreactivity and ultrastructurally, the development of the degenerative lesions in the affected beta cells.

Materials and methods

Animals

Chinese hamsters aged 5–7 months and of both sexes (25–37 g body weight), bred in the Central Institute for Laboratory Animal Breeding in Hannover/Germany, were used. Animals were kept under standard laboratory conditions and had free access to food

and drinking water. Measurements of nonfasting blood glucose values at weekly intervals and, in addition, glucose tolerance tests at intervals of 6 weeks to characterize the changes in the metabolic state of the animals showed that Chinese hamsters can be subdivided into controls and mildly diabetic and severely diabetic animals [15]. Animals were diabetic for 3–4 months before the experiments. Pancreata and (for reference purposes) liver, kidney and small intestine were isolated from control Chinese hamsters (CHIA/Han subline; blood glucose 7.3±0.9 mM; n=9), from mildly diabetic (CHIG/Han subline; blood glucose 9.3±0.4 mM; n=12) and severely diabetic Chinese hamsters (CHIG/Han subline; blood glucose 26.4±7.6 mM; n=8). Nonfasting blood glucose concentrations were measured at sacrifice with the glucose oxidase method.

Light microscopy

Small pancreatic tissue specimens from the splenic part or corpus were quenched in isopentane precooled in liquid nitrogen, freeze-dried (–35°C for 72 h) and fixed by vapor-phase p-formaldehyde. In addition, small and larger pieces of pancreatic and hepatic, renal and small intestinal tissue were fixed in Bouin's solution. The small specimens were embedded in Araldite and the larger pieces in paraffin. The specimens embedded in Araldite were used for demonstration of the quantitative changes in the immunoreactivities for insulin and GLUT2 glucose transporter.

Electron microscopy

Small pancreatic specimens from the splenic part or corpus were fixed in a solution of 4% p-formaldehyde and 0.5% or 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3. Most of the specimens were postfixed in 1% OsO_4 for 1 h. Finally, all specimens were embedded in Epon. Thin sections (50–80 nm) were cut on an ultramicrotome. The sections were contrast-stained with saturated solutions of lead citrate and uranyl acetate and viewed in an electron microscope.

Immunohistochemical protocol

Serial semithin sections (0.5–1.0 μm) were stained using the avidin–biotin complex (ABC) method [11]. Following the removal of the resin and incubation with the first antibody overnight a biotinylated goat anti-rabbit IgG (1:100; 30 min) and a streptavidin–biotin–peroxidase complex (1:1,000; 30 min), both obtained from Jackson Immuno Research, West Grove, USA, were used as second and third layers. The demonstration of the peroxidase was performed with 0.7 mM diaminobenzidine and 0.002% H_2O_2 in 0.05 M Tris HCl buffer, pH 7.6.

Antisera and specificity controls

Polyclonal antisera against insulin (Novo, Bagsvaerd, Denmark), diluted 1:7,000, glucagon (Milab, Malmö, Sweden), diluted 1:30,000 and somatostatin (Dr. Etzrodt, Ulm, Germany), diluted 1:8,000 were used in the present study. In addition a polyclonal antiserum against the rat GLUT2 glucose transporter (WAK-Chemie, Bad Homburg, Germany) was used in a dilution of 1:10,000–1:30,000. The antisera against islet hormones have been used successfully in previous studies, in which they have been examined for method and antibody specificity [3, 10]. The antiserum against the GLUT2 glucose transporter has also been characterized biochemically and immunocytochemically [29]. In the present study this antiserum was tested for antibody specificity by preadsorption with the GLUT2 peptide (WAK-Chemie) and peptides with unrelated specificities (insulin from Novo, Bagsvaerd, Denmark and glucagon from Serva, Heidelberg, Germany in concentrations of 6.25–100 μg/ml). Upon preadsorption with the homologous antigen GLUT2 at concentrations as low as 6.25 μg/ml the GLUT2 immunostaining disappeared completely. In particular, both the cell membrane and cytoplasmic staining of the GLUT2 glucose transporter were absent after preadsorption with GLUT2 peptide in the endocrine pancreas of the nondiabetic and diabetic Chinese hamsters. Control experiments performed in the rat pancreas also showed the familar GLUT2 staining of the cell membrane, which was similarly abolished after preadsorption. In addition, larger pancreatic specimens were studied to confirm these results in a greater area of the pancreas.

Morphometrical analysis

The serial semithin sections were immunostained sequentially for the established islet hormones or only for insulin and the GLUT2 glucose transporter. Serial sections immunostained for the islet hormones were used for the study of the histology of the islets in nondiabetic animals and the changes within the islets in the diabetic state. This allows a correlation between the reduced immunoreactivities for insulin and GLUT2 in the beta cells during diabetes development and changes in the histology of the islet.

In 5–8 animals in each experimental group the volume fraction of all beta cells was determined in 5–10 sections of islets immunostained for insulin by measuring the cross-sectional area of beta cells (>80% with nucleus) within the islet and dividing by the total area of the islet. In control experiments it was confirmed that the total area for insulin, glucagon and somatostatin immunoreactivies was the same as the total islet area. To document the development of diabetes, intact beta cells and beta cells with degenerative lesions within 3–5 islets in 4 animals in each experimental group were counted in thin sections. Using the consecutive semithin/thin section sequence, beta cells identified on the basis of the degree of their GLUT2 or insulin immunoreactivity in one of the semithin sections were examined ultrastructurally in the next thin section. To verify the changes in the immunoreactivities of GLUT2 after the onset of diabetes the immunoreactivities were densitometrically determined by a computer-assisted system, the Interactive Image Analysis System (IBAS, Zeiss-Kontron, Munich, Germany) using a program for densitometric analysis as described previously [1, 5, 10]. The images of whole islets or beta cells were recorded with a CCD/89D video camera (Sony, Tokyo, Japan) after the illumination value (area without section) had been constantly set to a grey value of 170 (Light Control Unit LCU 2; Kontron, Munich, Germany). The densities of the immunoreactivities were transferred into grey values given as arbitrary units (1=black, 255=white). After background subtraction the grey values of the GLUT2 immunoreactivities ranged between 95 and 160 and the mean values of GLUT2 immunoreactivities within single cells between 120 and 160. The distribution of grey values in a defined area was plotted in a histogram and expressed as integrated grey values/μm^2. The total amount of GLUT2 immunoreactivity (integrated grey values/μm^2) within the islets was measured by setting a threshold of 160 to avoid overlapping with unspecific staining in 10–15 islets from control animals and from mildly and severely diabetic animals. This makes it possible to exclude areas without specific staining, i.e. areas that exhibit background staining only, such as alpha cells and other non-beta cells and beta cell nuclei. In parallel with this procedure, the same islets were analysed densitometrically by measuring the GLUT2 immunoreactivity in the immunostained areas of each beta cell. Then the beta cells were subdivided into cells densely (121–140) and faintly (141–160) immunostained for GLUT2. The results are given as mean values±SEM, and were tested for statistical significance with Student's t-test. In addition, insulin and GLUT2 immunoreactivities of the beta cells from pancreata of diabetic hamsters were determined densitometrically to compare GLUT2 immunoreactivity with their corresponding insulin immunoreactivity.

Western blot analysis

Pancreatic islets were isolated by collagenase digestion, washed in Krebs-Ringer bicarbonate buffer and stored in liquid nitrogen.

Batches of 300 islets were sonicated in a buffer containing 3% SDS, 80 mM Tris-HCl (pH 6.8), 5 mM EDTA and 10% glycerol. Insoluble material was pelleted by centrifugation at 14,000 *g* and 4°C for 15 min. The protein content of the supernatant was determined by a BCA assay (Pierce, Rockford, USA) using bovine albumin as standard. In each lane 30 µg protein was resolved on 10% SDS polyacrylamide gels and transferred to PVDF membranes by semi-dry electroblotting. Equal loading of the gel was confirmed by staining membranes with Ponceau S (Sigma, St. Louis, Mo.). Membranes were blocked for 60 min at 37° C in PBS containing 0.05% Tween-20 (PBS-T) and 5% non-fat dry milk. The polyclonal rabbit anti-rat GLUT2 antibody (WAK-Chemie) was diluted 1:5,000 in PBS-T and incubated for 2 h at room temperature. After two washes in PBS-T for 15 min at room temperature, membranes were incubated in PBS-T for 1 h at room temperature with sheep anti-rabbit Ig horseradish peroxidase-labelled antibody (Sigma) diluted 1:10,000. The membranes were finally washed twice for 15 min in PBS-T, and detection was performed by the ECL chemiluminescence system (Amersham, Braunschweig, Germany) on light-sensitive Hyperfilm MP (Amersham). The autoradiograms were quantified by densitometry using the Image 1.52 analysis program (NIH, Bethesda, Md.).

Results

Histology of the islets of Langerhans

Islets of Langerhans in pancreata from Chinese hamsters varied markedly in their maximal diameters. The endocrine cells within the islets showed a typical arrangement. The islet periphery, which is called its mantle, was mainly composed of glucagon (alpha) and somatostatin (delta) cells, whereas the beta cells were mostly localized in the centre of the islet. This confirms earlier observations in this species [9, 18, 19]. In parallel with the development of diabetes in Chinese hamsters, the morphology of the islet changed. The volume fraction of beta cells in the islets was significantly reduced ($P<0.05$) to 56±3% in the pancreata of mildly diabetic hamsters ($n=8$) and to 38±4% in the pancreas of severely diabetic hamsters ($n=8$), as against 79±4% in the pancreata of control animals ($n=5$). In many islets with a reduced number of beta cells the alpha and delta cells were also detected in the centre of the islets.

Immunocytochemical characterization of GLUT2 and insulin in pancreatic beta cells

Nondiabetic Chinese hamsters

Beta cells in islets and in extra-islet positions mostly displayed dense immunoreactivity for insulin (Fig. 1a) and GLUT2 (Fig. 1b). Immunoreactivity for GLUT2 was detected only in beta cells (Fig. 1b). Other islet cell types were not GLUT2 positive. As shown in Fig. 2a at a higher magnification, immunoreactivity was localized both in the cell membrane and in the cytoplasm. The densities for GLUT2 immunoreactivity varied markedly among beta cells within the islets, but there was no relationship with the extent of insulin immunoreactivity. Dense GLUT2 immunoreactivity was detected both in beta cells with strong insulin immunoreativity and in hyperactive degranulated beta cells. The hepatocytes, the tubular cells of the kidney and the enterocytes of the small intestine showed GLUT2 immunoreactivity in the cell membrane but not intracellularly (data not shown).

Mildly diabetic Chinese hamsters

During the initial stages of the development of diabetes 38% of the islets showed degenerative lesions. Beta cells in islets without degenerative changes exhibited the same immunostaining for insulin and GLUT2 as beta cells in nondiabetic Chinese hamsters. Beta cells in islets with degenerative lesions displayed a normally dense insulin immunoreactivity (Fig. 1c) but a reduced immunoreactivity for GLUT2 (Figs. 1d, 2b). Quantitative analysis revealed that the number of densely GLUT2 immunostained beta cells decreased significantly ($P<0.05$), from 67±5% in the islets from control animals ($n=4$) to 50±4% in the islets from mildly diabetic hamsters ($n=4$). The total GLUT2 immunoreactivity decreased significantly ($P<0.05$), from 439±97 grey values/µm^2 in the islets from control animals ($n=4$) to 253±56 grey values/µm^2 in the islets from mildly diabetic hamsters ($n=4$).

Severely diabetic Chinese hamsters

The endocrine pancreas showed a marked decrease in the number and size of the islets, and also a significant numerical reduction in beta cells, resulting in the appearance of vacuoles in the islet (Fig. 1e,f); in addition, vacuolization of variable extent was also evident in the cytoplasm of the beta cells – a possible sign of degeneration (Fig. 1e,f). The vacuoles did not contain fat or hyalin (Fig. 5c). In comparison with the dense and mostly homogeneous immunostaining for insulin in the nondiabetic state, the beta cells from severely diabetic hamsters exhibited a heterogeneous and mostly faint immunostaining for insulin (Fig. 1e). Only a very few beta cells still showed a dense immunoreactivity for GLUT2 (Figs. 1f, 2c). Quantitative analysis revealed that the number of densely GLUT2-immunostained beta cells decreased significantly ($P<0.01$), from 67±4% in the islets from control animals ($n=4$) to 15±4% in the islets from severely diabetic hamsters ($n=4$). The total GLUT2 immunoreactivity decreased significantly ($P<0.01$), from 439±97 grey values/µm^2 in the islets from control animals ($n=4$) to 124±21 grey values/µm^2 in the islets from severely diabetic hamsters ($n=4$).

The beta cells still densely immunostained for insulin also displayed dense GLUT2 immunoreactivity both in the cell membrane and in the cytoplasm (Fig. 1e,f). There was a significant correlation ($P<0.05$) between GLUT2 and insulin immunoreactivity in beta cells from the severely diabetic hamster pancreata, with a correlation coefficient (r) of 0.94 (Fig. 3). However, most re-

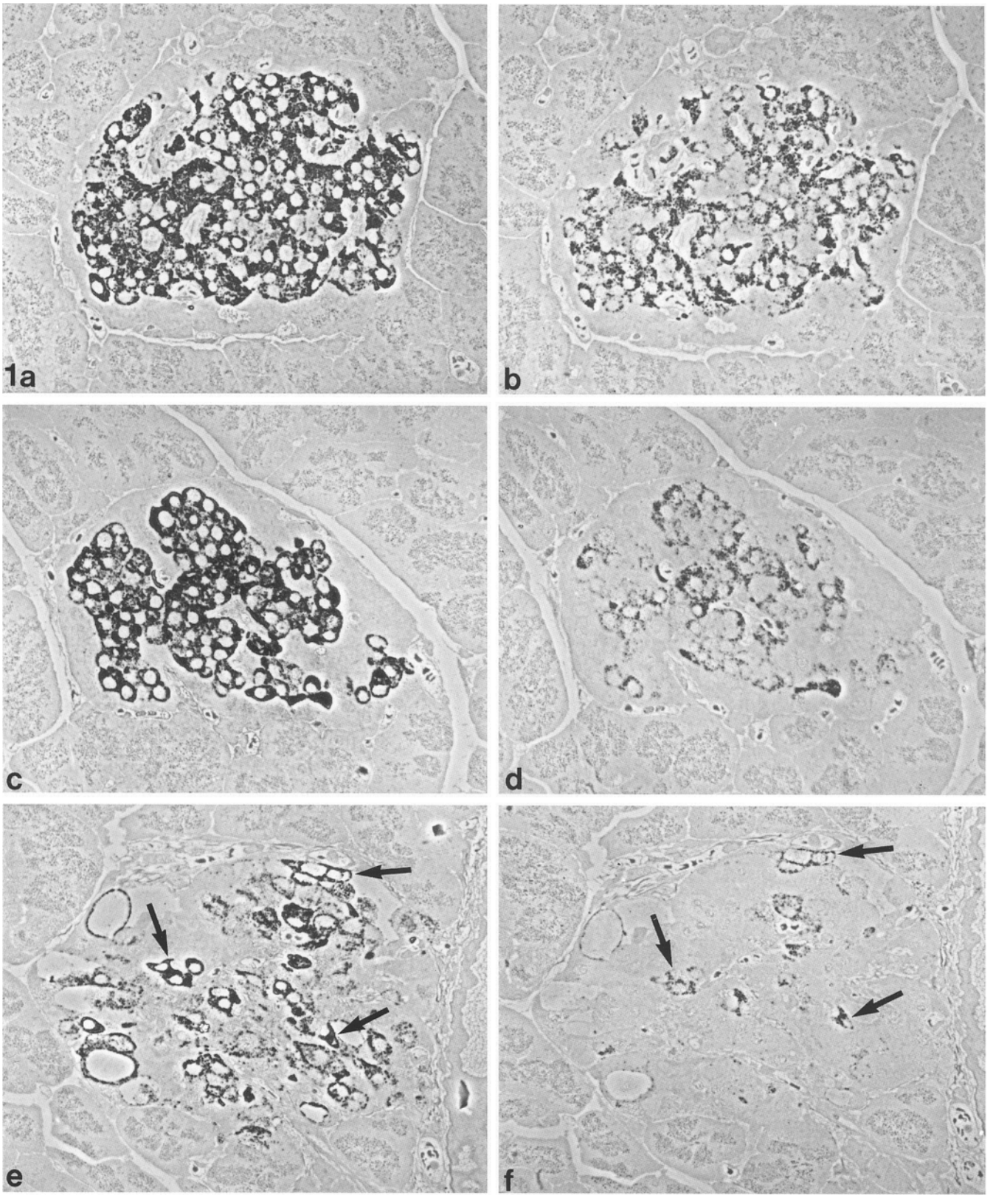


Fig. 1a–f Changes in insulin and GLUT2 immunoreactivities in Chinese hamster pancreatic beta cells during development of diabetes. Serial semithin sections of the hamster pancreas from controls (**a, b**) mildly diabetic (**c, d**) and severely diabetic animals (**e, f**) were immunostained for insulin (**a, c, e**) and GLUT2 (**b, d, f**). Pancreatic beta cells exhibit immunoreactivities for GLUT2 in the cell membrane as well as in the cytoplasm. GLUT2 immunoreactivity is reduced in most of the beta cells during the mildly diabetic state (**d**). Insulin immunoreactivity is reduced only in beta cells from severely diabetic animals (**e**). In pancreatic islets from diabetic hamsters only beta cells densely immunostained for insulin (*arrows*) also display an immunoreactivity for GLUT2 (*arrows*). ×350

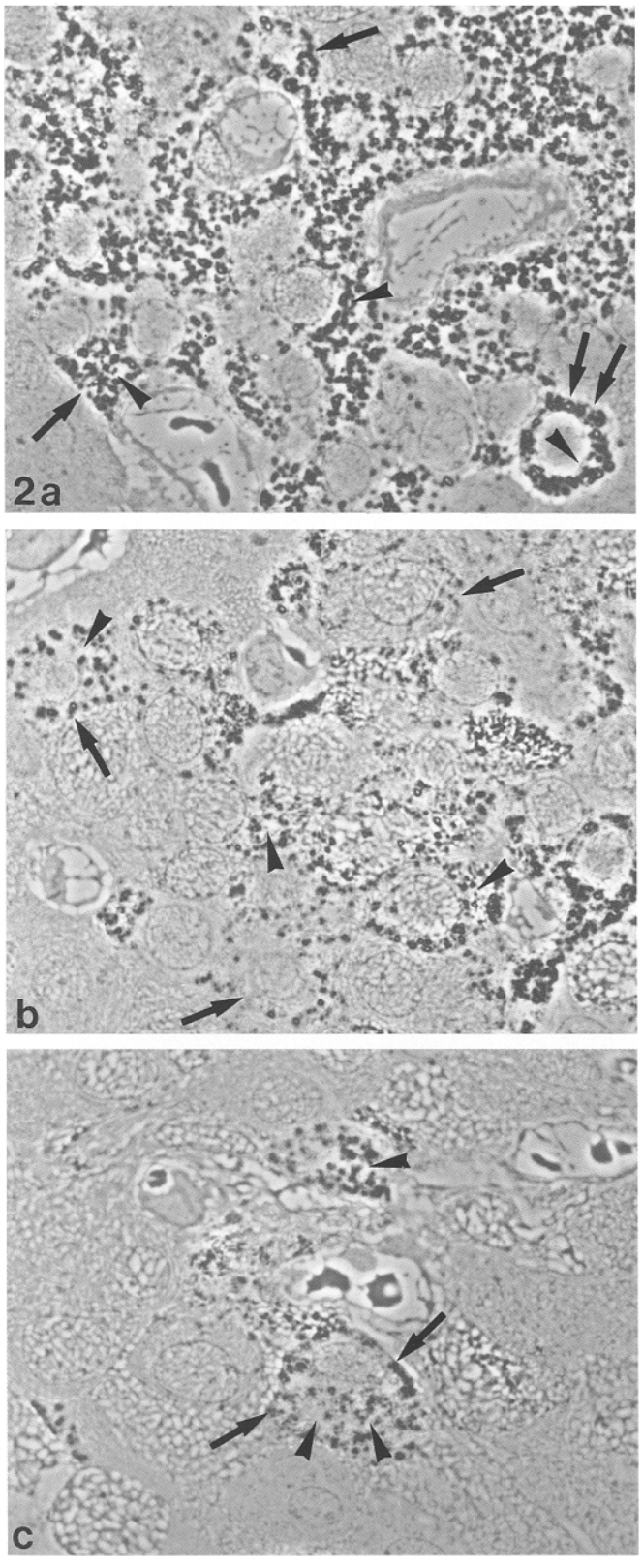


Fig. 2a–c Changes in GLUT2 immunoreactivity in Chinese hamster pancreatic beta cells during development of diabetes. Serial semithin sections of the pancreas from **a** a control, **b** a mildly diabetic, and **c** a severely diabetic animal were immunostained for GLUT2. Pancreatic beta cells exhibit immunoreactivities for GLUT2 in the cell membrane (*arrows*) as well as in the cytoplasm (*arrowheads*). GLUT2 immunoreactivity is reduced during development of diabetes in both localizations. ×1,400

markably, around 40% of the beta cells devoid of GLUT2 immunoreactivity were still insulin immunoreactive (Fig. 3). This shows that the beta cells lost GLUT2 immunoreactivity prior to insulin immunoreactivity. In addition, beta cells at extraislet positions exhibited a dense insulin and GLUT2 immunoreactivity independent of the diabetic state, and remained thus unchanged in comparison with those from control animals.

Irrespective of the reduction of the GLUT2 immunoreactivity in pancreatic beta cells from diabetic Chinese

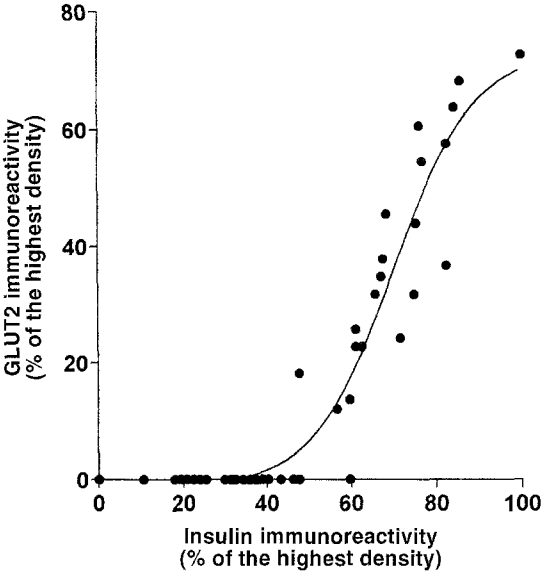


Fig. 3 Correlation between GLUT2 and insulin immunoreactivity in pancreatic beta cells from the severely diabetic Chinese hamsters. Immunoreactivities were quantified densitometrically and expressed as percentages of the highest density

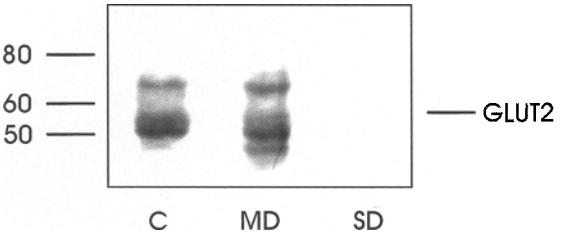
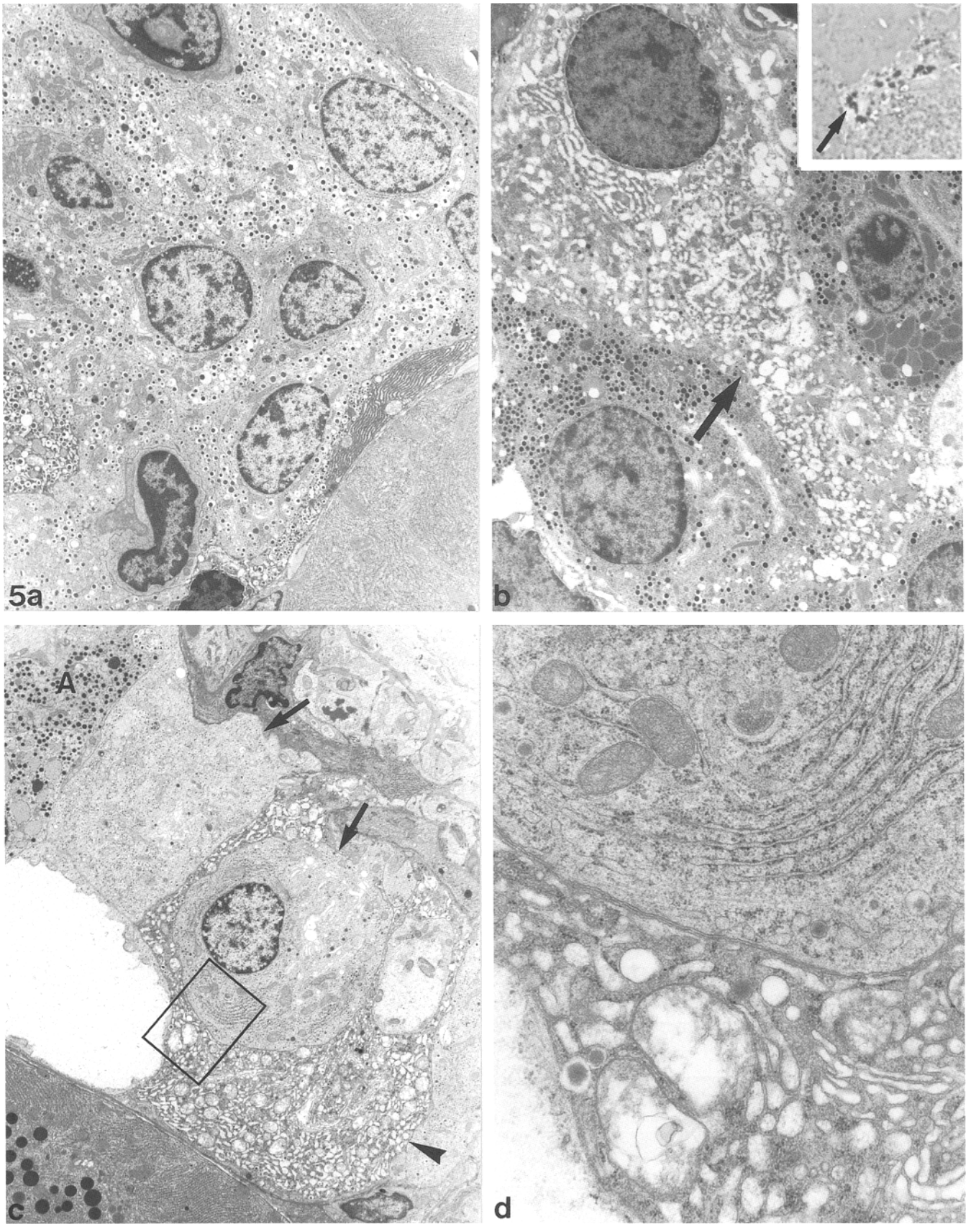


Fig. 4 Western blot analysis of GLUT2 protein in pancreatic islets from nondiabetic control Chinese hamsters (*C*), and animals with mild diabetes (*MD*) and with severe diabetes (*SD*). Protein (30 μg) from islet homogenates was loaded on each line and probed with a polyclonal rabbit anti-rat GLUT2 antibody. Molecular weights are indicated on the *left*. Blot shown is representative for four independent experiments


5a
b
A
c
d

hamsters, the hepatocytes, the tubular cells of the kidney and the enterocytes of the small intestine showed a normal and unchanged GLUT2 immunoreactivity (data not shown).

Western blot analysis of GLUT2 in isolated pancreatic islets

GLUT2 protein expression was determined in islets isolated from nondiabetic control Chinese hamsters and from hamsters with mild and with severe diabetes through Western blot analysis. GLUT2 was expressed in hamster islets as a major 56-kDa band, which corresponds to GLUT2 protein expression in rat islets (Fig. 4). The higher molecular weight band in the GLUT2 hamster islet Western blot may be a higher molecular weight precursor protein (Fig. 4). Compared with pancreatic islets from nondiabetic control hamsters, GLUT2 protein was slightly (but significantly, $P<0.05$) decreased in islets from mildly diabetic animals (82±2% of control; $n=4$) (Fig. 4), while GLUT2 expression in islets from severely diabetic animals was virtually undetectable (5±4% of control; $n=4$).

Electron microscopy of pancreatic beta cells

In the pancreata from nondiabetic Chinese hamsters, the three main endocrine cell types could be identified on the basis of their typical secretory granule morphology. It was found that 96±2% of the beta cells showed no signs of degenerative changes and were mostly well granulated, with a variable number of secretory granules (Fig. 5a). In mildly diabetic animals a small number of beta cells showed signs of pyknosis of the nuclei and degenerative changes such as swollen mitochondria and dilated cisternae of the Golgi complex and the rough endoplasmic reticulum, while 80±3% of the beta cells were intact. Comparable changes were observed in severely diabetic animals (Fig. 5b–d), but the portion of damaged beta cells was considerably higher. Only 50±2% of the detectable beta cells were still intact. Most of these intact beta cells showed signs of high functional activity, such as a well-developed Golgi complex and rough endoplasmic reticulum. They contained only a small number of secretory granules (Fig. 5c,d). A minority of these intact beta cells was well granulated without signs of high functional activity. An analysis of consecutive semithin/thin sections showed that these are also the GLUT2 beta-positive cells in these severely diabetic animals (Fig. 5b). Thus both in mildly ($P<0.05$) and severely ($P<0.01$) diabetic animals there was a significant reduction in the number of intact beta cells in the pancreas.

Fig. 5 Ultrastructure of the endocrine pancreas from a control (**a**) and a severely diabetic Chinese hamster (**b–d**). Beta cells from control animals are well preserved (**a** ×5,220). In consecutive semithin (**b** *inset*; ×800) and thin (**b** ×5,220) sections from a severely diabetic Chinese hamster all the GLUT2-immunoreactive cells can be identified as intact beta cells with secretory granules (*arrow* in **b** points to the beta cell shown at lower magnification in the *inset*). A well-preserved alpha cell and beta cells displaying a heterogeneous picture are shown in (**c**). Well-preserved beta cells (*arrows*) show signs of high functional activity and contain a low number of secretory granules, while the beta cells with degenerative lesions (*arrowheads*) contain dilated cisternae of the rough endoplasmic reticulum and swollen mitochondria at both magnifications shown (**c** ×5,220; **d** ×24,000)

Discussion

In the Chinese hamster the GLUT2 glucose transporter was detected immunocytochemically in the plasma membrane of pancreatic beta cells and also in hepatocytes, tubular cells of the kidney and enterocytes of the small intestine. Only in the pancreatic beta cells was GLUT2 immunoreactivity also present in the cytoplasm. This is identical with the situation found in mouse pancreatic beta cells [31], while in beta cells from the rat pancreas GLUT2 staining is not normally present in the cytoplasm [12, 23, 29].

The deterioration of glucose tolerance and glucose-induced insulin secretion [8] was paralleled by a reduction in GLUT2 immunoreactivity in pancreatic beta cells of diabetic Chinese hamsters. The loss of GLUT2 immunoreactivity in beta cells preceded the loss of insulin immunoreactivity and the development of the degenerative lesions. Western blot analysis of GLUT2 protein expression in isolated pancreatic islets confirmed the results of the immunocytochemical studies, even though the loss of GLUT2 in mildly diabetic hamsters was less pronounced than was observed in the immunocytochemical studies. This can be explained as a result of the collagenase isolation procedure, in which the better preserved islets are likely to be preferentially isolated.

The loss of GLUT2 immunoreactivity in pancreatic beta cells of the diabetic Chinese hamster is in agreement with similar observations made during the development of the diabetic state in pancreatic beta cells of streptozotocin diabetic Sprague-Dawley rats [30], Zucker rats [23], Wistar and BB rats [25], Wistar and Zucker rats made diabetic by dexamethasone treatment [13, 21, 22], and db/db mice [31].

Within pancreatic beta cells of severely diabetic Chinese hamsters heterogeneities became evident. In the islets only a few intact beta cells with a dense insulin immunoreactivity exhibited GLUT2 immunoreactivity, and it appears that this small subpopulation stores rather than releases insulin. This was also true for beta cells at extra-islet positions. The majority of the intact beta cells represents the group of the functionally active beta cells that have lost GLUT2 immunoreactivity. Confirming previous studies [9, 18, 19], these intact beta cells showed a well-developed Golgi complex and a large amount of rough endoplasmic reticulum with a marked reduction of secretory granules at the light- and electron-microscopical levels, as well as morphological signs of high insulin biosynthesis and secretory activity. Thus, expression of

GLUT2 alone is not a sufficient indicator of glucose responsiveness, as was previously considered in animal models for diabetes [32]. This is in accordance with the observation that a recovery of glucose-induced insulin secretion in streptozotocin-diabetic rats after insulin treatment was not accompanied by increased GLUT2 immunoreactivity [4]. The third group in these Chinese hamsters are the beta cells with degenerative lesions, which also lack GLUT2 immunoreactivity. Thus, during the development of diabetes in Chinese hamsters the pancreatic beta cells exhibit heterogeneity with respect to their functional activity and structural integrity.

Heterogeneous insulin immunoreactivity among beta cells is not only a physiological feature [14, 28], but also a characteristic of the diabetic state. The endocrine pancreata of patients with type I and type II diabetes exhibit a small number of beta cells with dense insulin immunoreactivity [26]. The small group of functionally inactive beta cells in these diabetic Chinese hamsters that remain immunocytochemically and ultrastructurally intact also appear to be cells that are not susceptible to degeneration leading to necrosis. The high insulin content of this population of beta cells might be an indicator of functional inactivity, and they may represent newly formed beta cells as proposed elsewhere [2], probably originating from exocrine duct cells expressing the GLUT2 glucose transporter [33]. There appears to exist heterogeneity with respect to the sensitivity of Chinese hamsters, beta cells to toxic damage. The ability to distinguish subpopulations of pancreatic beta cells with variable susceptibility is an interesting feature of diabetes development, which has not been reported in other experimental animal models for diabetes. Thus, loss of GLUT2 immunoreactivity is an early indicator of beta cell dysfunction and may be an element of importance for the deterioration of glucose-induced insulin secretion in diabetic Chinese hamsters.

Acknowledgements The skilful technical assistance of S. Fischer, D. von Mayersbach, H. Peesel, S. Gudat and D. Lischke is gratefully acknowledged. This study has been supported by a grant from the Gesellschaft der Freunde der Medizinischen Hochschule Hannover.

References

1. Bargsten G, Grube D (1992) Serotonin storage and chromogranins: an experimental study in rat gastric endocrine cells. J Histochem Cytochem 40:1147–1155
2. Bonner-Weir S, Smith FE (1994) Islet cell growth and the growth factors involved. Trends Endocrinol Metab 5:60–64
3. Cetin Y, Aunis D, Bader M-F, Galindo E, Jörns A, Bargsten G, Grube D (1993) Chromostatin, a chromogranin A-derived bioactive peptide, is present in human pancreatic insulin (beta) cells. Proc Natl Acad Sci USA 90:2860–2864
4. Chen C, Thorens B, Bonner-Weir S, Weir GC, Leahy JL (1992) Recovery of glucose-induced insulin secretion in a rat model of NIDDM is not accompanied by return of the B-cell GLUT2 glucose transporter. Diabetes 41:1320–1327
5. Ehrhart M, Jörns A, Grube D, Gratzl M (1988) Cellular distribution and amount of chromogranin A in bovine endocrine pancreas. J Histochem Cytochem 36:467–472
6. Frankel BJ, Heldt AM, Grodsky GM (1982) Insulin and glucagon release in the diabetic Chinese hamster: differences among inbred sublines. Diabetologia 22:292–295
7. Frankel BJ, Cajander S, Boquist L (1987) Islet morphology in young, genetically diabetic Chinese hamsters during the hyperinsulinemic phase. Pancreas 2:625–631
8. Gerritsen GC (1982) The Chinese hamster as a model for the study of diabetes mellitus. Diabetes 31:14–21
9. Gerritsen GC, Dulin WE (1967) Characterization of diabetes in the Chinese hamster. Diabetologia 3:74–84
10. Grube D, Jörns A (1991) The endocrine pancreas of glucagon- and somatostatin-immunized rabbits. I. Light microscopy. Cell Tissue Res 265:251–260
11. Hsu S-M, Raine L, Fanger H (1981) Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled PAP procedures. J Histochem Cytochem 27:577–580
12. Jetton TL, Magnuson MA (1992) Heterogeneous expression of glucokinase among pancreatic beta cells. Proc Natl Acad Sci USA 89:2619–2623
13. Johnson JH, Ogawa A, Chen L, Orci L, Newgard CB, Alam T, Unger RH (1990 Underexpression of beta cell high K_m glucose transporters in non-insulin-dependent diabetes. Science 250:546–549
14. Jörns A (1994) Immunocytochemical and ultrastructural heterogeneities of normal and glibenclamide stimulated pancreatic beta cells in the rat. Virchows Arch 425:305–313
15. Kohnert KD, Hemke B, Besch W, Keßler J, Klöting I (1995) Impaired β-cell function in the Chinese hamster CHIG/Han subline. Exp Clin Endocrinol 103:66–70
16. Lenzen S (1992) Glucokinase: signal recognition enzyme in pancreatic B-cells for glucose-induced insulin secretion. In: Flatt PR (ed) Nutrient regulation of insulin secretion. Portland Press, London Chapel Hill, pp 101–125
17. Lenzen S, Tiedge M (1994) Molecular mechanisms of insulin secretion and pancreatic B-cell dysfunction. Biochem Soc Trans 22:1–6
18. Like AA, Gerritsen GC, Dulin WE, Gaudreau P (1974) Studies in the diabetic Chinese hamster: light microscopy and autoradiography of pancreatic islets. Diabetologia 10:501–508
19. Like AA, Gerritsen GC, Dulin WE, Gaudreau P (1974) Studies in the diabetic Chinese hamster: electron microscopy of pancreatic islets. Diabetologia 10:509–520
20. Matschinsky F, Liang Y, Kesavan P, Wang L, Froguel P, Velho G, Cohen D, Permutt MA, Tanizawa Y, Jetton TL, Niswender K, Magnuson MA (1993) Glucokinase as pancreatic beta cell glucose sensor and diabetes gene. J Clin Invest 92:2092–2098
21. Ogawa A, Johnson JH, Ohneda M, McAllister C, Inman L, Alam T, Unger RH (1992) Role of insulin resistance and beta-cell dysfunction in dexamethasone-induced diabetes. J Clin Invest 90:497–504
22. Ohneda M, Johnson JH, Imman LR, Unger RH (1993) GLUT2-function in glucose-unresponsive beta cells of dexamethasone-induced diabetes in rats. J Clin Invest 92: 1950–1956
23. Orci L, Thorens B, Ravazolla M, Lodish HF (1989) Localization of the pancreatic beta cell glucose transporter to specific plasma membrane domains. Science 245:295–297
24. Orci L, Ravazolla M, Baetens D, Inman L, Amherdt M, Peterson RG, Newgard CB, Johnson JH, Unger RH (1990) Evidence that the down-regulation of beta-cell glucose transporters in non-insulin dependent diabetes may be the cause of diabetic hyperglycemia. Proc Natl Acad Sci USA 87:9953–9957
25. Orci L, Unger RH, Ravazolla M, Ogawa A, Komiya I, Baetens D, Lodish HF, Thorens B (1990) Reduced beta cell glucose transporter in new onset BB rats. J Clin Invest 86:1615–1622
26. Pipeleers D, Ling Z (1992) Pancreatic beta cells in insulin-dependent diabetes. Diabetes Metabolism Rev 8:209–227
27. Portha B, Serradas P, Bailbe D, Suzuki K-I, Goto Y, Giroix M-H (1991) Beta cell insensitivity to glucose in the GK rat, spontaneous nonobese model for type II diabetes. Diabetes 40: 486–491

28. Stefan Y, Meda P, Neufeld M, Orci L (1987) Stimulation of insulin secretion reveals heterogeneity of pancreatic β-cells in vivo. J Clin Invest 80:175–183
29. Thorens B, Sarkar HK, Kaback HR, Lodish HF (1988) Cloning and functional expression in bacteria of a novel glucose transporter present in liver, intestine, kidney, and beta-pancreatic islets cell. Cell 55:281–290
30. Thorens B, Weir GC, Leahy JL, Lodish HF, Bonner-Weir S (1990) Reduced expression of the liver/beta cell glucose transporter isoform in glucose-insensitive pancreatic beta cells of diabetic rats. Proc Natl Acad Sci USA 87:6492–6496
31. Thorens B, Sarkar HK, Kaback HR, Lodish HF (1992) The loss of GLUT2 expression by glucose-unresponsive beta cells of db/db mice is reversible and is induced by the diabetic environment. J Clin Invest 90:77–85
32. Thorens B, Gerard N, Deriaz N (1993) GLUT2 surface expression and intracellular transport via the constitutive pathway in pancreatic beta cells and insulinoma: evidence for a block in trans-Golgi-network exit by brefeldin A. J Cell Biol 123: 1687–1694
33. Wang RN, Klöppel G, Bouwens L (1995) Duct- to islet-cell differentiation and islet growth in the pancreas of duct-ligated adult rats. Diabetologia 38:1405–1411