Reduced insulin, GLUT2, and IDX-1 in β-cells after partial pancreatectomy

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Reduction of GLUT2 is associated with loss of glucose-induced insulin secretion in genetic and chemical diabetes and in transplanted islets exposed to chronic hyperglycemia. To examine the mechanisms for this loss of GLUT2 in normal islets exposed to hyperglycemia, we performed studies on Sprague Dawley rats 4 weeks after a 90% partial pancreatectomy (Px), a well-characterized model of hyperglycemia. GLUT2 immunofluorescence in the β-cell of Px rats was greatly reduced. Western blot analysis of homogenates of isolated Px islets also showed a reduction in GLUT2 protein; densitometry measurements were 36 ± 3% of values from islets of sham-operated controls. Insulin protein levels were decreased to a similar extent. Islet GLUT2 and insulin mRNA were measured with quantitative reverse transcriptase–polymerase chain reaction. The level of GLUT2 mRNA from Px islets was 24 ± 4% of that of islets from sham-operated controls; similar results were obtained for insulin. Because both these β-cell-specific messages were reduced, we analyzed the Px islets for the pancreas-duodenum–specific transcription factor IDX-1 (IPF-1, STF-1, PDX-1) protein. It was markedly reduced (~80%) in islets from the Px rats. These data suggest that 1) the loss of GLUT2 protein associated with hyperglycemia is at least partially explained by reduced levels of the GLUT2 gene transcripts; 2) the reduction of β-cell insulin content during chronic hyperglycemia may not be completely due to degranulation (reduced levels of gene transcripts may play a role); and 3) the reduction in the transcription factor IDX-1 raises the possibility that dysregulation of transcription factors may contribute to the abnormal β-cell function found in states of chronic hyperglycemia. Diabetes 46:258–264, 1997

C hronic hyperglycemia is associated with a selective loss of glucose-induced insulin secretion in both NIDDM and early IDDM (1–4). The molecular mechanisms responsible for this phenomenon have not been identified. The preserved insulin responses to nonglucose secretagogues, such as arginine (5,6), have led investigators to focus on glucose-dependent components of the metabolic pathways that lead to insulin secretion.

In rodent models of diabetes, such as Zucker Diabetic Fatty (ZDF) rats (7), neonatal streptozocin rats (8), BB rats (9), and db/db mice (10), the loss of glucose-induced insulin secretion is accompanied by a marked reduction of the glucose transporter GLUT2 protein in β-cells. A similar association was found when mouse islets were transplanted into a hyperglycemic environment (11). Although the reduction of GLUT2 may not limit glucose entry into β-cells enough to account for the impairment of insulin secretion (12,13), the marked change in the expression of this protein serves as a marker for β-cell adaptation to the diabetic state. The mechanism for this loss of GLUT2 is unclear, and data about gene expression are conflicting. On one hand, GLUT2 mRNA in islets was found to be reduced in 12-week-old ZDF rats (7,14). On the other hand, cultured islets exposed to hyperglycemia (30 mmol/l) for up to 7 days have been shown to have higher GLUT2 mRNA (15).

To examine the influence of the chronic hyperglycemic milieu on islets in genetically normal rats, the model of 90% partial pancreatectomy (Px) (16) was used. At 28 days after Px, the islets have been exposed to prolonged hyperglycemia. Although active regeneration occurred in the remnant during the 2 weeks after Px (17,18) the islets were relatively stable thereafter, except for their adaptation to the diabetic state. Not only were GLUT2 protein and message reduced, but insulin protein and message were also reduced to a similar degree. The amount of the homeobox transcription factor IDX-1 (IPF-1, STF-1, PDX-1) (19–21) protein was also found to be markedly reduced in these islets. IDX-1 is expressed in pancreatic β-cells and, through knockout experiments (IPF-1/PDX-1), has been found to be essential for the full development of both the exocrine and endocrine pancreas (22,23). Moreover, IDX-1 has been found to bind to a glucose-response element of the insulin gene promoter, have a response element on the promoter of GLUT2 (24,25), and be reduced in HIT cells chronically exposed to hyperglycemia (26). The reduction of IDX-1 in the Px islets, a nongenetic animal model of hyperglycemia, raises questions about whether the deranged β-cell function found in diabetic states is related to reduced expression of key transcription factors.
Animals. A 90% partial Px was performed on 4–5 week old Sprague Dawley rats (Taconic, Germantown, NY) as previously described (16). Removal of most of the pancreas was done by gentle abrasion with cotton applicators, leaving the major blood vessels supplying other organs intact. The remnant (residual pancreas) was anatomically well defined, being the tissue within 1–2 mm of the common pancreatic duct, and extending into the two different ducts. The RNA levels were determined by comparing ethidium bromide gel band intensities between each pair of bands (29); the upper band in each lane was the amplification product of the fixed amount of specific competitor cDNA. The lower band was the amplification product of a known dilution of the tissue’s total RNA equivalent. When these bands were equal, it was assumed that this dilution of tissue sample contained the same amount of the cDNA sequence as the known amount of competitor (See Fig. 5 for example).

Insulin gene expression by Northern blot analysis. Because the insulin message in islets is fairly abundant and can be detected even in very small amounts of total islet RNA, a Northern blot was performed to confirm the results obtained by the quantitative PCR. Aliquots of 0.1 μg of total islet RNA from the same RNA samples used as quantitative PCR were fractionated on formaldehyde 1% agarose gels and transferred to nylon membranes (Gene-screen, DuPont, Boston, MA). RNA bound to the membrane was hybridized overnight at 65 °C with a random primed (30) fluorescein-d12 UTP labeled cDNA (31) probe for rat insulin 1 gene. Membranes were washed, dried, and then developed for chemiluminescence as previously described (32).

Insulin protein expression. Islets isolated from pancreatic remnants were lysed in SDS; a 5-μg islet protein was then assayed for insulin by radioimmunoassay using rat insulin standards (27).

RESULTS

As previously described (16), after several days of no weight gain, Px animals gained weight at a similar rate as the sham control rats. The difference in body weight between the two groups was not greater than 10% (Fig. 1A). Fed blood glucose levels were already significantly elevated (>160 mg/dl) when first measured at 7 days after Px, and continued to rise gradually to about 250 mg/dl at 28 days. A representative sample is shown in Fig. 1B.

Care was taken to use handpicked islets of similar size for comparative studies; the islets selected from the sham and Px rats were 100–300 μm in diameter. The visual similarity in islet size was confirmed by measurements of islet protein. The protein yield per islet (islets obtained from samples used for the IDX-1 Western blots) was 0.95 ± 0.08 μg for shams islets (n = 5) and 0.94 ± 0.09 μg for Px islets (n = 5). Moreover, the total RNA yield per 100 islets (islets obtained from samples used for quantitative PCR) was 1.62 ± 0.14 μg for the Px islets (n = 4) and 1.97 ± 0.41 μg for the Px islets (n = 4).

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DECREASED IDX-1 IN β-CHELs

FIG. 1. Weight gain and blood glucose levels after 90% pancreatectomy (Px). A: the weight gain during the first 4 weeks after Px is slightly lower in the Px group, though not significantly different than in the sham group. B: fed blood glucose levels are significantly increased in the Px group as early as day 7 after Px (first post-Px glucose testing). Data are means ± SE.

of 72.9%. Because similar islets were used for Western blots, radioimmunoassay (RIA), immunostaining, and mRNA assays, the β-cell contribution from the Px and control islets should have been virtually identical.

FIG. 2. Western blot for GLUT2 in pancreatectomy (Px) and sham islets. Islets isolated from pancreatic remnants 28 days after Px or from pancreas of sham operated or unoperated rats were lysed in SDS and sonicated. Ten micrograms of protein from each sample (individual animals) was fractionated with electrophoresis and stained for GLUT2 protein (55kDa—indicated by arrow) with Western blotting. The identity of the fainter higher molecular weight band is unknown, but it may represent a glycosylated form of GLUT2. Islets from pancreatectomized animals (Px) with chronic hyperglycemia (n = 7) had much lower levels of GLUT2 protein than those of sham operated (S; n = 3) or unoperated control (+C; n = 3) rat islets.

In these hyperglycemic Px rats, GLUT2 protein levels, as determined by Western blot analysis on islets isolated 28 days after Px, were markedly decreased when compared with those from sham controls (Fig. 2). Densitometric readings expressed as a percentage of control values revealed GLUT2 protein levels of Px islets to be reduced to 36.1 ± 3.3% (Fig. 3). Insulin protein levels of the same islets measured by RIA were decreased by the same magnitude, to 22.5 ± 7.0%. Sham insulin protein levels were 53.8 ± 16.8 ng/μg islet protein compared with only 12.1 ± 3.8 ng/μg islet protein in the Px islets (Fig. 3). Twenty-eight days after Px, the GLUT2 staining on the β-cell plasma membrane had almost completely disappeared in the Px islets, in contrast to the clear staining of the β-cells of the sham controls (Fig. 4).

To determine if this reduction in GLUT2 and insulin protein was related to changes in mRNA, specific mRNA levels for the two genes were measured by quantitative reverse transcriptase–polymerase chain reaction (RT-PCR). As the insulin message is much more abundant (~1,000 times GLUT2), we were able to confirm the insulin mRNA levels by Northern blot

FIG. 3. Insulin, GLUT2, and IDX-1 protein levels 28 days after pancreatectomy (Px). A: in protein extracts from islets isolated from pancreatic remnants 28 days after Px, insulin protein levels were determined by radioimmunoassay. Islets from Px animals with chronic hyperglycemia had significantly (*P < 0.025) lower insulin levels compared with sham controls. B: GLUT2 protein levels from the same islets studied for insulin were obtained by computerized densitometry from the Western blot in Fig. 2. Protein levels are presented as percentage of sham, with the mean sham value considered as 100%. GLUT2 protein was significantly reduced (**P < 0.005) in the Px group. These reductions in insulin and GLUT2 protein are parallel in magnitude. C: quantitation of IDX-1 protein levels from the same islets was performed as for GLUT2. The IDX-1 Western blot is shown in Fig. 7; IDX-1 levels are markedly decreased (**P < 0.005) in Px group. Data are means ± SE.
analysis (Fig. 5C). An example of the determination of insulin mRNA by quantitative RT-PCR is shown in Fig. 5B. The level of insulin message in the Px islets was reduced to 27.3 ± 11.1% of that found in the control islets (Figs. 5 and 6). A similar marked decline in GLUT2 mRNA—23.7 ± 3.4% of control values—was also found in the Px islets (Fig. 6).

The similarly decreased message levels of the two β-cell specific genes suggest that these changes may have resulted from a common mechanism. To explore this possibility, levels of the pancreatic homeobox protein IDX-1, which has response elements on both the insulin and GLUT2 promoters (24,25), were quantified in both Px and sham islets. Using Western blot analysis, a profound reduction was found in the IDX-1 protein levels of islets 28 days after Px (Fig. 7). Densitometry of this Western blot revealed that IDX-1 band intensities of the Px islets were reduced to 14.1 ± 2.3% of those found in sham control islets (Fig. 3). With immunochemistry on the light microscope level (Fig. 8), clear IDX-1 nuclear immunostaining was found in control islets but, in contrast, was markedly reduced in the Px islets, and in some cells not even discernible.

DISCUSSION

The selective loss of glucose-induced insulin secretion found in human diabetes is closely mimicked by similar secretory profiles in animal models of diabetes, particularly in many rodent models (3). The molecular basis of this defect has
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FIG. 6. Insulin and GLUT2 mRNA levels 28 days after pancreatectomy (Px). A: insulin message levels in islets isolated from pancreatic remnants 28 days after 90% pancreatectomy determined by quantitative PCR. Insulin mRNA levels in sham islets were, on average, 3.5 times that of the Px islets. B: GLUT2 message levels quantitated in the same manner showed a similar pattern. The mean GLUT2 message in sham islets was 23.9 pg/µg of total RNA, compared with only 5.7 pg/µg of total RNA in Px islets. Data are means ± SE. Significance: *P < 0.025, **P < 0.005.

not been elucidated. There has been considerable interest in GLUT2, the expression of which is markedly reduced in β-cells of virtually all rodent models of chronic hyperglycemia in which it has been studied (7–10,34,35). In the present study, a similar reduction in GLUT2 protein by immunofluorescence and Western blot analysis was found in the Px model. This model is valuable because it is a relatively pure way to expose islets to the milieu of the diabetic state without the confounding variables of gene defects, β-cell toxins, or whatever abnormalities might be induced by islet transplantation. Although marked regeneration occurs in 1–2 weeks following Px, hyperglycemia ensues. The changes that occur during hyperglycemia with these islets may resemble the islet adaptation that takes place in all forms of diabetes. Although we suspect that chronic hyperglycemia is the major force responsible for these changes, the contributions of increased free fatty acids or other factors must be considered.

With regard to whether this reduction in GLUT2 could contribute to the secretory impairment of diabetes, some reduction of the transport capacity for glucose has been demonstrated (7,36), but the change does not seem sufficient to allow transport to become rate limiting for glucose metabolism. Furthermore, it has recently been found that human β-cells have very little GLUT2 but instead have abundant GLUT1 (37), which raises questions about whether GLUT2 has any special role in the abnormal insulin secretion of either human or rodent diabetes. However, some provocative data suggest that GLUT2 may somehow interact with glucokinase to increase glucose phosphorylation and thereby enhance insulin secretion (38–41). Despite these questions, GLUT2 is an interesting marker of normal β-cell differentiation at least in rodents, and its loss of expression is closely linked to secretory malfunction.

The molecular basis for the loss of GLUT2 protein is uncertain, but reduced GLUT2 mRNA has been found in ZDF islets (7,14). On the other hand, islets cultured for variable periods less than 10 days in a high-glucose concentration were found to have either increased or unchanged levels of GLUT2 message in their islets (15,42–45). Because of the short time period and the unphysiological nature of tissue culture conditions, these results probably have little rel-
evance to the situation in diabetes. In the present study of Px rats, a clear reduction in GLUT2 mRNA was found in the islets. This finding is consistent with the concept that the loss of GLUT2 protein is at least partially caused by reduced availability of its message because of either reduced transcription or decreased mRNA stability.

A loss of insulin content in the Px islets was not unexpected, because degranulation is often found in hyperglycemic states, but the reduction in insulin mRNA in a genetically normal animal model was remarkable. In an earlier study with Px rats, reduced levels of pancreatic proinsulin mRNA were found in a group of rats with relatively severe hyperglycemia (46), levels similar to those of the present study. The proinsulin mRNA reduction of our study cannot be explained on the basis of a reduced relative volume of β-cells in islets that were studied, because, if anything, the proportion of β-cells in the islets of these Px rats were slightly increased. The finding of comparable reductions in GLUT2 and insulin mRNA in the islets of the Px rats is reminiscent of similar changes in the islets of ZDF rats (14). Interestingly, the ZDF rat islets also had reductions in islet glucokinase, mitochondrial glyceral-3-phosphate dehydrogenase, and voltage-dependent calcium channel and potassium channel mRNA. These similar findings in Px and ZDF rats raise questions about whether the diabetic state somehow leads to altered expression of multiple β-cell genes through a common mechanism.

The recently discovered pancreatic transcription factor IDX-1/IPF-1/STF-1/PDX-1 is involved in pancreas development and regeneration (22–23,47–48). It is expressed in the pancreatic β-cells of adult islets and is thought to be an important transcription factor for glucose activation of the insulin gene (20,24,49). MacFarlane et al. (50) showed that IUF-1 (immunologically similar to IPF-1 in an electrophoretic mobility shift assay and with Western blot analysis) binding activity to the insulin gene was decreased in isolated islets cultured at low glucose and returned to basal levels with reexposure to high glucose. In addition, HIT cells chronically exposed to 11.2 mM glucose had decreased transcription of the insulin gene, decreased STF-1 (IDX-1) protein levels and decreased STF-1 binding to the insulin promoter (26,51). In this study, we showed for the first time that this transcription factor is markedly reduced in normal islets exposed to chronic hyperglycemia in vivo. These changes are unlikely to be related to pancreatic regeneration, because most of this activity occurs during the first week after Px (18). Although growth factors were enhanced, as characterized for insulin-like growth factor I and Reg/pancreatic stone protein during this regeneration, their mRNA levels returned to basal levels by 14 days after Px surgery (52,53). Furthermore, during the first week after Px, IDX-1 levels in the remnant were increased rather than decreased (48). Therefore, the changes found in β-cells studied 28 days after Px were probably not related to the perturbation of the first week after surgery, and were likely to be an adaptation to the milieu of the chronic diabetic state.

IDX-1 binds to CT boxes on the human insulin promoter that are homologous to the FLAT and P elements of the rodent insulin promoter (17,24); similar response elements exist on the GLUT2 and glucokinase gene promoters and on other pancreatic genes promoters (e.g., somatostatin and rat islet amyloid polypeptide [24,54,55]). IDX-1 is not the only transcriptional regulatory protein for insulin nor probably for GLUT2 (56), but its normal absence in liver and kidney could account for why GLUT2 expression in these tissues is not reduced in association with hyperglycemia (10). The reduction in IDX-1 found in β-cells exposed to chronic hyperglycemia raises the possibility that this, and perhaps other, transcription factors play a major role in the β-cell dysfunction of diabetes. IDX-1 may be a key factor in maintaining the expression of the unique genes that characterize β-cells—those that produce the array of proteins required for optimal insulin secretory capacity. Downregulation of IDX-1 in diabetes could lead to change in the expression of various genes affecting critical metabolic pathways sufficient enough to cause the observed profound reduction in glucose-induced insulin secretion.

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