Upregulated Hexokinase Activity in Isolated Islets from Diabetic 90% Pancreatectomized Rats

Hitoshi Hosokawa, Yuka Amino Hosokawa, and Jack L. Leahy

Glucokinase is the β-cell glucose sensor, i.e., the site in glucose metabolism that determines the glucose set-point (sensitivity) for insulin secretion. Hexokinase is also present, but it normally contributes little to glucose metabolism because of end-product inhibition by glucose 6-phosphate. There is a lowered glucose set-point for insulin secretion in 90% pancreatectomized (Px) diabetic rats. We investigated the mechanism by measuring hexokinase and glucokinase activity in islet extracts. Glucokinase activity was minimally raised in Px islets (V_{max} 125% of sham-operated control rats). In contrast, hexokinase V_{max} was 250% of the control value, suggesting that the increased hexokinase activity caused the β-cell glucose hypersensitivity. Additional evidence was obtained with a 40-h fast that was performed because of a previous observation that the inhibitory effect of fasting on insulin secretion was impaired in Px rats. Glucokinase activity fell normally in the Px rats (32 ± 4% reduction in sham vs. 37 ± 4% in Px rats) as opposed to hexokinase activity, which was unaffected in either group. In summary, a feature of hyperglycemia is upregulated islet hexokinase activity. The result is that hexokinase assumes partial control over the glucose set-point for insulin secretion. As such, regulatory effects on insulin secretion, such as fasting, that are mediated through glucokinase activity may be altered. *Diabetes* 44:1328–1333, 1995

The high K_m glucose phosphorylation enzyme glucokinase is the β-cell glucose sensor, i.e., the site that determines the glucose sensitivity (set-point) for insulin secretion (1). Studies in cultured islets have shown that high glucose concentrations upregulate both the β-cell content and catalytic activity of this enzyme, resulting in a lowered glucose set-point for insulin secretion (2,3). Other modulatory factors for the catalytic activity of glucokinase may exist, because this pathway was upregulated in two nondiabetic rat models (4,5). Consistent with the in vitro results, islet glucokinase V_{max} was increased in several rodent models of non-insulin-dependent diabetes mellitus (NIDDM) (6) although the biochemical basis in terms of cellular content versus catalytic activity was not investigated. We studied islets from markedly hyperglycemic (nonfasting plasma glucose, 18 mmol/l), glucose-infused rats and found that glucokinase content and catalytic activity were both increased (4). At variance with the in vitro studies, hexokinase V_{max} also was increased. The relevance of these findings for NIDDM is unclear because of the short-term nature of the model (48-h infusions) and the marked hyperglycemia. A more useful model may be 90% pancreatectomized (Px) rats, which are usually studied after several weeks of modest hyperglycemia and are known to have a lowered glucose set-point for insulin secretion (7).

Fasting suppresses glucose phosphorylation by glucokinase (8,9) and glucose-induced insulin secretion (10). The reported mechanism is a lowered activity of glucokinase with no change in its cellular content (11). How fasting would affect glucokinase in hyperglycemic states is not known. This question is important because of the recent report that a 60-h fast paradoxically enhanced the insulin response to oral glucose in people with NIDDM (12). We investigated this finding in 90% Px rats and identified a similar aberrant effect of fasting on incretin-induced insulin secretion. Specifically, the insulin response to glucagon-like peptide I–high glucose increased after a 40-h fast, whereas there was a 60% lowering in normal rats (13). The role played by aberrant effects of fasting on glucokinase activity and/or cellular level is not known.

The current study characterized hexokinase and glucokinase activity and cellular level in islets from 90% Px rats under basal conditions and after a 40-h fast.

RESEARCH DESIGN AND METHODS

The 90% pancreatectomy rat model and islet isolation. We performed 90% pancreatectomies on 100-g male Sprague-Dawley rats (Taconic, Germantown, NY) using the method of Bonner-Weir et al. (14). During pentobarbital sodium anesthesia (100 mg/kg, i.p.), a midline abdominal incision was made and the pancreas was mobilized by gently breaking mesenteric connections with the stomach, bowel, and retroperitoneum. Cotton applicators were used to abrade pancreatic tissue away from the major blood vessels. The pancreas was removed in toto except for the portion bordered by the bile duct and the duodenum. Sham-operated control rats underwent laparotomy and mobilization of the pancreas. Postoperatively, all rats were given standard rat chow and tap water ad libitum. Rats were studied 4–6 weeks after surgery. The fasting protocol was 40 h (6:00 P.M. to 10:00 A.M. on day 2), with nonfasted rats studied in parallel. Islets were isolated using an adaption of the method of Gotoh et al. (15): pancreatic duct infiltration with 2 mg/ml collagenase (Serva, Heidelberg, Germany), Histopaque gradient separation (Sigma, St. Louis, MO), and hand picking. The method for the Px and control rats was identical except for the volume of collagenase solution (6 ml in sham rats and 3 ml in Px rats) and the digestion time at 37°C (18 min in fasted Px rats; 18 min, 30 sec in fasted/nonfasted sham rats; and 19 min in nonfasted Px rats). Islet yield was 500–600 in sham rats and 100–150 in Px rats. The insulin secretion experiment used islets from three Px rats and one control rat. Otherwise, islets from one sham rat and pooled islets from four Px rats were used, with a portion going to the measurement of glucose phosphorylation and the remainder being

From the Division of Endocrinology, Diabetes, Metabolism, and Molecular Medicine, New England Medical Center, Tufts University School of Medicine, Boston, Massachusetts.

Address correspondence and reprint requests to Dr. Jack Leahy, New England Medical Center #268, 750 Washington St., Boston, MA 02111.

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BSA, bovine serum albumin; NIDDM; non-insulin-dependent diabetes mellitus; Px, pancreatectomized; STZ, streptozotocin.

frozen on dry ice and stored at −70°C pending Western blot measurement of glucokinase protein level.

Insulin secretion. After isolation, islets were preincubated 90 min at 37°C in Krebs-Ringer bicarbonate buffer, 10 mmol/l HEPES, 2.8 mmol/l glucose, and 0.5% bovine serum albumin (BSA). Triplicate batches of 10 islets were placed in glass vials containing 1 ml Krebs-Ringer bicarbonate buffer, 10 mmol/l HEPES, 0.5% BSA, and glucose (2.8, 5.5, 8.3, 11.1, and 16.7 mmol/l) and were incubated 60 min in a 37°C shaking water bath. The medium was separated from the islets by gentle centrifugation (500–750 rpm for 5 min at 10°C) and stored at −20°C pending insulin measurements by radioimmunoassay (16).

Glucose phosphorylation. Glucose phosphorylation was measured in islet extracts using the method of Liang et al. (17) that measures conversion of NAD^+ to NADH by exogenous glucose-6-phosphate dehydrogenase. After a 90-min preincubation at 37°C in RPMI-1640, 10 mmol/l HEPES, 2.8 mmol/l glucose, and 0.5% BSA, 300 islets were homogenized on ice in 300 μl buffer (1 mmol/l EDTA, 20 mmol/l K_2HPO_4, 110 mmol/l KCl, and 5 mmol/l dithiothreitol) by 25 strokes of a machine-driven Teflon pestle in a Kontes glass homogenizer (0.004–0.006 in). Aliquots (10 μl × 3) were stored at −70°C for DNA content (18). After a 10-min centrifugation at 12,000*g* to remove mitochondrial-bound hexokinase (19), 5-μl aliquots were added to 100 μl of reaction buffer that consisted of 50 mmol/l HEPES/HCl, pH 7.6, 5 mmol/l ATP, 100 mmol/l KCl, 7.4 mmol/l $MgCl_2$, 15 mmol/l β-mercaptoethanol, 0.5 mmol/l NAD^+, 0.05% BSA, glucose (0.03, 0.06, 0.125, 0.25, 0.5, 6, 12, 24, 60, and 100 mmol/l), and 0.7 U/ml glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* (Boehringer, Indianapolis, IN). After 90 min at 30°C, the reaction was stopped with 1 ml of 500 mmol/l $NaHCO_3$, pH 9.4. Triplicate samples were performed at each glucose concentration in parallel with reagent blanks (no homogenate) and a tissue blank (islet homogenate in reaction buffer that contained 0 mmol/l glucose). The standard curve was glucose-6-phosphate standards (0.3–3 nmol) in a reaction buffer that contained 100 mmol/l glucose. From the replicate results for each glucose concentration, the mean was calculated to give a single data point. Glucokinase and hexokinase V_{max} and K_m were calculated by linear regression from an Eadie-Scatchard plot after extrapolating the data to 37°C assuming a Q_{10} of 2 (17,20), followed by 10 cycles of the method of Spears et al. (21) to separate hexokinase and glucokinase activity.

Glucokinase Western blot. Islets were lysed in 5% sodium dodecyl sulfate, 80 mmol/l Tris/HCl, pH 6.8, 1 mmol/l phenylmethylsulfonyl fluoride, 5 mmol/l EDTA, 10 μg of DNase, and 0.2 mmol/l *N*-ethylmaleimide. Protein content was measured by the BCA Protein Assay (Pierce, Rockford, IL) with BSA as standard. Aliquots (20 μl) were resolved by electrophoresis on a 0.75-mm 10% polyacrylamide gel containing sodium dodecyl sulfate and electroblotted onto nitrocellulose (Schleicher & Schuell, Keene, NH) (22). Filters were blocked overnight at 4°C in 5% nonfat dry milk, 0.01% Tween 20, and 20 mmol/l Tris/HCl, pH 7.4 and then incubated at room temperature with sheep antiserum raised against an *Escherichia coli*-derived B1 isoform of rat glucokinase (gift from Dr. Mark Magnuson, Vanderbilt University) at 1,500 dilution for 3 h, followed by rabbit anti-sheep IgG (Sigma) at 1:1,500 dilution for 1 h. Bound antibody was detected with ^{125}I-conjugated protein A (ICN, Costa Mesa, CA). Band intensity was quantified by densitometry using IMAGE 1.4 software (National Institutes of Health, Bethesda, MD). Each gel contained extracts from one group of fasted and nonfasted sham and Px rats. The densitometry results were expressed in relative terms by assigning a value of 100% to the sham nonfasted band.

Analytical methods. Plasma glucose was measured at the end of the fasting/nonfasting period with a Beckman Glucose Analyzer II (Beckman, Fullerton, CA) in blood obtained by tail snippings from nonanesthetized rats.

Data presentation and statistical methods. All data are expressed as means ± SE. The *n* values for all data are the number of experiments performed. Statistical significance was determined by one-way analysis of variance, except for the Western blots, in which a one-way Student's *t* test was used.

RESULTS

Glucose-induced insulin secretion in islets from 90% Px and control rats. Fig. 1 shows glucose concentration and insulin secretion curves in islets from nonfasted 90% Px and control rats. In the control rats, the expected sigmoidal shaped curve was seen, with the steep part in the glucose range of 8–15 mmol/l glucose, and an apparent ED_{50} value of ~10 mmol/l glucose. The curve in the Px rats was shifted to the left, with the maximal response being reached by 11.1 rather than 16.7 mmol/l glucose in the control rats, and had an ED_{50} value of 7.7 ± 0.8 mmol/l glucose.

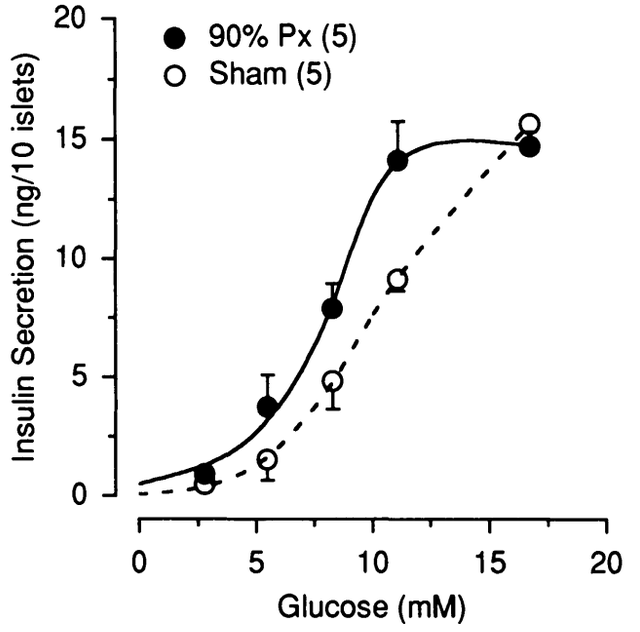

FIG. 1. Glucose concentration and insulin secretion curves in isolated islets from 90% Px and sham-operated control rats.

Effect of 40-h fast on body weight and plasma glucose values in Px and sham rats. Nonfasted Px rats were modestly hyperglycemic (10.3 ± 0.3 mmol/l nonfasted Px vs. 8.1 ± 0.2 mmol/l nonfasted shams, $P < 0.001$) and had a normal body weight. Fasting lowered the body weight equally in Px and sham rats. Plasma glucose values also fell with fasting in both groups to the same level of hypoglycemia (5.8 ± 0.1 mmol/l in Px vs. 5.4 ± 0.1 in sham rats, NS) (Table 1).

Islet glucose phosphorylation. The kinetic parameters for glucokinase and hexokinase, plus islet DNA measurements, are listed in Table 2. DNA values were equal in all four groups, although there appeared to be a nonsignificant trend for a small reduction with fasting. Under basal (nonfasting) conditions, hexokinase V_{max} in islets from Px rats was more than twice that of islets from sham rats ($P < 0.001$), with no difference in K_m. Fasting had no effect on these measures in either group. Glucokinase V_{max} was also increased in islets from nonfasted Px rats versus those from nonfasted sham

TABLE 1
General characteristics of 40-h fasted 90% Px and sham-operated control rats

Animal group	*n*	Postfast weight (g)	Weight loss during fast (g)	Plasma glucose (mmol/l)
Sham nonfasted	7	322 ± 13	ND	8.1 ± 0.2
Sham fasted	7	279 ± 16*	33 ± 2	5.4 ± 0.1*
90% Px nonfasted	21	333 ± 5	ND	10.3 ± 0.3†
90% Px fasted	22	285 ± 6*	34 ± 1	5.8 ± 0.1*

Data are means ± SE. Statistical significance was determined by ANOVA. Weight loss during fast was calculated for each rat by comparing pre- and postfast weights. *$P < 0.001$ between fasted and nonfasted rats. †$P < 0.001$ between nonfasted shams and nonfasted Px rats. ND, not done.

TABLE 2
Kinetic parameters for glucokinase and hexokinase in 40-h fasted 90% Px and sham-operated control rats

	Hexokinase		Glucokinase		
Animal group (n)	V_{max} (mol glucose · kg DNA^{-1} · 60 min^{-1})	K_m (mmol/l glucose)	V_{max} (mol glucose · kg DNA^{-1} · 60 min^{-1})	K_m (mmol/l glucose)	Islet DNA content (ng)
Sham nonfasted (5)	2.66 ± 0.32*	0.13 ± 0.03	5.09 ± 0.14†	15.8 ± 1.2	25 ± 2
Sham fasted (5)	2.93 ± 0.68	0.09 ± 0.02	3.45 ± 0.23‡	14.8 ± 1.6	21 ± 2
90% Px nonfasted (5)	6.46 ± 0.60	0.06 ± 0.01	6.45 ± 0.45	13.4 ± 1.1	29 ± 4
90% Px fasted (5)	5.95 ± 1.81	0.06 ± 0.01	4.05 ± 0.27‡	16.0 ± 3.1	22 ± 1

Data are means ± SE. Statistical significance was determined by ANOVA. *$P < 0.001$ between sham nonfasted and Px nonfasted rats. †$P < 0.019$ between sham nonfasted and Px nonfasted rats. ‡$P < 0.002$ between fasted and nonfasted rats.

rats ($P < 0.019$), with no difference in K_m. However, the increase (25%) in V_{max} for glucokinase was much less than for hexokinase. Fasting lowered glucokinase V_{max} equally in both groups: 32 ± 4% reduction in sham vs. 37 ± 4% in Px rats (NS) (Fig. 2).

Glucokinase Western blot. The mechanism of the fasting effect on glucokinase V_{max} was investigated by quantifying glucokinase protein level by Western blot. A representative blot is shown in Fig. 3 and combined data from five experiments in Table 3. The glucokinase level was the same in islets from Px and sham rats under basal conditions (Px level 110 ± 6% of sham, NS). With fasting, the level fell in both groups: the sham fasted result was 83 ± 5% of sham nonfasted ($P < 0.032$) and the Px fasted result was 69 ± 6% of Px nonfasted ($P < 0.006$), which did not differ statistically. It should be noted that these results are virtually identical to the fractional reductions in glucokinase V_{max} from the previous experiment, which means that the effect of fasting on glucokinase was mediated through a lowered cellular content of the enzyme.

DISCUSSION

This study, in combination with a previous report in glucose-infused rats (4), investigated the upregulatory effects of hyperglycemia on the kinetics of glucose phosphorylation in β-cells. The results of the studies vary, presumably because of the differences in glycemia. In mildly hyperglycemic Px rats, the increase in glucokinase V_{max} was one-tenth (25 vs. 250%) that in the markedly hyperglycemic glucose-infused rats. Also, only the activity of the enzyme was increased, whereas in glucose-infused rats, the cellular content and activity of glucokinase were both upregulated. Hexokinase V_{max} was also increased in the Px rats, which confirms the same finding in the glucose-infused rats (4) and reports in diabetic Zucker rats (23) and neonatally streptozotocin (STZ)-injected rats (24). Unlike glucokinase, the increases in hexokinase activity in the Px and glucose-infused rats were identical (250% of the control value). Thus, our results show upregulated islet hexokinase and glucokinase activity with hyperglycemia. Also, graded effects of hyperglycemia occur for glucokinase but not for hexokinase.

These results add to the growing literature that glucose phosphorylation is a regulatory site for adaptive changes in β-cell function. Our studies to date have focused on the high K_m isoform glucokinase because of the purported role for this enzyme in controlling the glucose sensitivity for insulin secretion (1). Proof of this hypothesis has recently come from studies of maturity-onset diabetes of the young patients with genetic mutations in one of their glucokinase alleles (25) and transgenic mice in which glucokinase ribozyme was

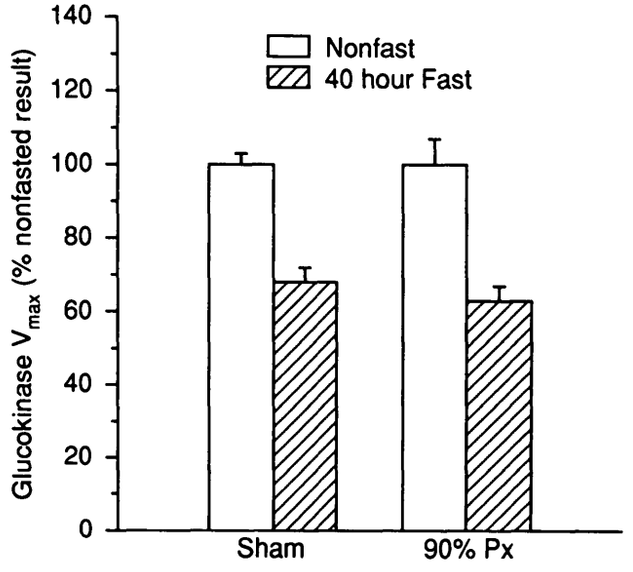

FIG. 2. Effect of a 40-h fast on glucokinase activity in islet extracts from 90% Px and sham-operated control rats. Data are expressed as a percentage of the nonfasted value for each group of rats.

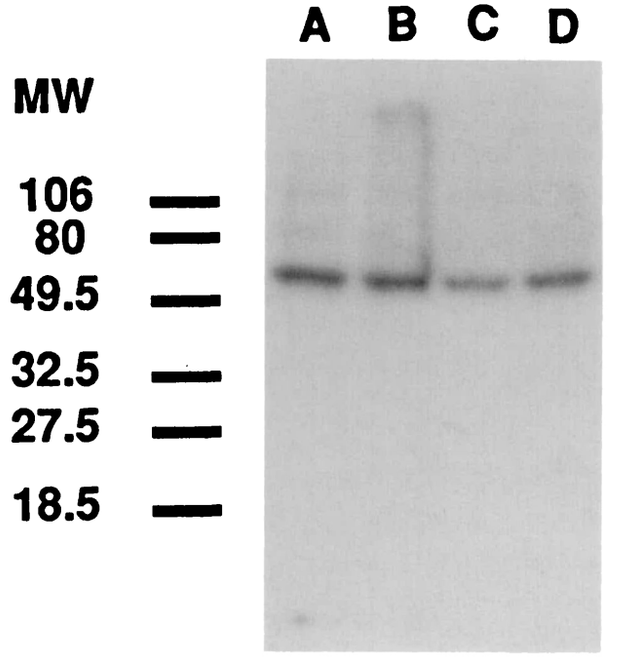

FIG. 3. Western blot for islet glucokinase in 40-h fasted and nonfasted 90% Px and sham-operated control rats. Islets from single sham-operated rats and pooled islets from four Px rats were studied. *A*: sham nonfasted. *B*: Px nonfasted. *C*: sham fasted. *D*: Px fasted.

TABLE 3
Glucokinase Western blot measurements in islets from 40-h fasted 90% Px and sham-operated control rats

Animal group	Glucokinase protein level (% sham nonfasted)
Sham nonfasted	100
Sham fasted	83 ± 5*
Px nonfasted	110 ± 6
Px fasted	75 ± 7†

Data are means ± SE. $n = 5$ for all. Statistical significance was determined by ANOVA. *$P < 0.032$ between fasted and nonfasted sham rats. †$P < 0.006$ between fasted and nonfasted Px rats.

used to reduce the β-cell expression of glucokinase (26); in both studies, the glucose set-point for insulin secretion was increased. We identified a novel regulatory mechanism for glucokinase in β-cells that entails variable intrinsic activity (3). Upregulation of this system proved to be the mechanism for the adaptive hyperinsulinemia in nondiabetic glucose-infused rats (4) and nondiabetic insulin-resistant spontaneously hypertensive rats (5), and the result was a lowered glucose set-point for insulin secretion. Thus, substantial evidence suggests that variable β-cell glucokinase activity is an important regulatory mechanism for prevention of hyperglycemia. Stated another way, a shifted glucose set-point probably explains the hallmark clinical finding in insulin resistance of hyperinsulinemia and normoglycemia.

Diabetic rats are also known to have a lowered ED_{50} for glucose-induced insulin secretion, as shown by the current results in the 90% Px rats and reports from other models (4,27–29). Based on the in vitro data showing upregulatory effects of glucose on glucokinase activity and cellular content (2,3), plus our previous results in markedly hyperglycemic glucose-infused rats showing a similar finding (4), we expected glucokinase activity and/or content to be increased in the Px islets. Instead, the increase was minimal (25%), which is not sufficient to explain the lowered ED_{50} for glucose-induced insulin secretion. Rather, we presume that the increased activity of the low K_m isoform, hexokinase, was the cause. This idea is counter to the general belief that hexokinase has virtually no regulatory role over β-cell glucose metabolism or the kinetics of glucose-induced insulin secretion because of end-product inhibition by glucose 6-phosphate (30). Our conclusion presupposes that the inhibition is incomplete so that when hexokinase is overexpressed, the low K_m isoform now contributes measurably to overall β-cell glucose phosphorylation. Only a small component of hexokinase activity is needed to lower the glucose set-point for insulin secretion because of the very low K_m of this enzyme. Supporting our conclusion are recent in vitro and in vivo studies showing that overexpression of hexokinase in β-cells lowers the glucose set-point for insulin secretion (31–33). Particularly germane are the results of Becker (32), in which hexokinase I was overexpressed in normal β-cells by adenovirus transfection: despite a multifold increase in β-cell hexokinase expression, glucose utilization increased only twofold, meaning that a site distal to glucose phosphorylation limited the rise in glucose metabolism. This doubling of the effective hexokinase activity that is of the same magnitude as the 90% Px islets caused a lowered ED_{50} for glucose-induced insulin secretion. Two important caveats to our interpretation need to be pointed out. In this study, islet hexokinase activity was only measured in the cytoplasmic fraction. Our conclusion assumes that the more active, mitochondrial-bound fraction is also upregulated in the Px islets, which is what was found by Milburn et al. (23) in diabetic Zucker rats. Second, our study and all of the cited studies were performed with islets, not purified β-cells. It remains to be determined that β-cell hexokinase activity is increased in the Px islets.

Consistent with the increased hexokinase activity in the Px islets, an upregulated islet hexokinase V_{max} has been observed in three other diabetic rodent models—markedly hyperglycemic glucose-infused rats (4), Zucker rats (23), and neonatally STZ-injected rats (24)—which suggests that this effect generally occurs with hyperglycemia. An increased glucokinase activity has also been reported in islets from several hyperglycemic diabetic rodent models (4,6). What then is the role of glucokinase versus hexokinase in the heightened β-cell sensitivity for glucose that occurs in islets of diabetic rodents (4,27–29)? Glucose-induced insulin secretion is known to directly reflect the β-cell glucose metabolism rate. As such, the ED_{50} for glucose-induced insulin secretion should be determined by how much the activities of hexokinase and/or glucokinase are increased, taking into account the different K_m values and susceptibility to end-product inhibition. Close perusal of the shapes of the glucose concentration-insulin secretion curves from islets with known combinations of increased glucokinase and/or hexokinase activity is consistent with this idea. In the mildly hyperglycemic Px rats, hexokinase causes virtually all of the increase in glucose phosphorylation (thus, glucose metabolism and insulin secretion). In vitro the enzyme activity is more than doubled, but in vivo the increase will be much less because of end-product inhibition, explaining why the secretion curve is shifted modestly to the left. In the more hyperglycemic glucose-infused rats, the same increase in hexokinase activity is paired with a nearly threefold increase in glucokinase activity, and the secretion curve is shifted more to the left, with maximum output occurring at 8.3 mmol/l glucose (4) vs. the 11.1 mmol/l glucose noted in this study for the Px rats. In euglycemic hyperinsulinemic glucose-infused rats (4) and spontaneously hypertensive rats (5), there is a greater increase in glucokinase activity than in the Px rats but no increase in hexokinase; the shift in the secretion curve is less than in Px rats, reflecting the high K_m of glucokinase. Thus, we speculate that how much the ED_{50} for glucose-induced insulin secretion is changed in different states of β-cell adaption depends on the proportional increases in the phosphorylation isoforms and their unique characteristics.

The statement that a lowered glucose set-point for insulin secretion is generally found with hyperglycemia seems at odds with the near total impairment of glucose-induced insulin secretion that typifies hyperglycemia, the so-called glucose toxicity (34). We investigated this paradox in Px rats by perfusing the pancreas in vitro for 40 min with 0 mmol/l glucose to reverse the glucose toxicity and found the predicted reduced ED_{50} value for glucose-induced insulin secretion (7). Virtually identical results were found in neonatal STZ-induced diabetic rats (35). In addition, glucose toxicity reverses for the most part during islet isolation. As stated previously, a lowered ED_{50} for glucose-induced insulin secretion has been noted in islets from several diabetic rodent models (4,27–29). Thus, a lowered glucose set-point for insulin secretion is commonly found with hyperglycemia.

However, it generally has gone unrecognized because of the superimposed glucose toxicity effect, which impairs glucose-induced insulin secretion.

The fasting part of this study was performed to determine whether the inhibitory effect on glucokinase activity was altered in the Px rats. The basis for this question was our finding that insulin secretion to high glucose/glucagon-like peptide I failed to suppress in Px rats with a 40-h fast (13). A similar result was obtained in human NIDDM patients using oral glucose and a 60-h fast (12). In the sham rats, glucokinase V_{max} fell 32%, which agrees with the 30% reduction reported with a 72-h fast (9). The results in the Px rats were identical, eliminating an aberrant effect of fasting on glucokinase activity. However, the major finding in this study of a different control for the glucose set-point in Px and sham rats provides a partial explanation for the impaired suppression of insulin secretion. Glucokinase is the dominant control in the normal rats; the glucose-potentiated insulin response to glucagon-like peptide I falls during fasting because of the reduced glucokinase activity. In Px rats, hexokinase also regulates β-cell glucose metabolism. The activity of this enzyme is unaffected by fasting (note the results in this study); thus, an impaired fall in the insulin response occurs.

An unexpected finding pertains to the mechanism of the fasting effect on glucokinase. It is generally believed that the mechanism of the reduced glucokinase activity with fasting is a lowered intrinsic activity of the enzyme because of a study that found no change in islet glucokinase protein or mRNA levels after a 72-h fast (11). Our results showed the opposite; the inhibitory effect was secondary to a reduced level of glucokinase protein. The plasma glucose level is known to modulate the β-cell content of glucokinase (2), and our result is more in keeping with the hypoglycemia that occurs when rats are fasted (note the current study). The discrepancy may represent different sensitivities of the antibodies in terms of identifying small changes in glucokinase protein level. Additional support for our result is via two studies that noted reductions in glucokinase islet mRNA level with fasting (36,37) rather than the lack of change reported in the cited study (11).

In summary, our results suggest that a feature of hyperglycemia is an upregulated hexokinase activity in β-cells. Of importance, the lack of the same effect in islets cultured at high glucose conditions (2,3) suggests that the cause is some factor other than hyperglycemia. The result is that hexokinase assumes partial control over the glucose set-point for insulin secretion, and regulatory effects on insulin secretion, such as fasting, that are mediated through glucokinase activity are altered.

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