Mechanism of Impaired Glucose-potentiated Insulin Secretion in Diabetic 90% Pancreatectomy Rats

Study Using Glucagonlike Peptide–1 (7–37)

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Abstract

Chronic hyperglycemia causes a near-total disappearance of glucose-induced insulin secretion. To determine if glucose potentiation of nonglucose secretagogues is impaired, insulin responses to 10^{-9} M glucagonlike peptide–1 (GLP-1) (7–37) were measured at 2.8, 8.3, and 16.7 mM glucose with the in vitro perfused pancreas in rats 4–6 wk after 90% pancreatectomy (Px) and sham-operated controls. In the controls, insulin output to GLP-1 was > 100-fold greater at 16.7 mM glucose versus 2.8 mM glucose. In contrast, the increase was less than threefold in Px, reaching an insulin response at 16.7 mM glucose that was 10±2% of the controls, well below the predicted 35–40% fractional β-cell mass in these rats. Px and control rats then underwent a 40-h fast followed by pancreas perfusion using a protocol of 20 min at 16.7 mM glucose followed by 15 min at 16.7 mM glucose/ 10^{-9} M GLP-1. In control rats, fasting suppressed insulin release to high glucose (by 90%) and to GLP-1 (by 60%) without changing the pancreatic insulin content. In contrast, in Px the insulin response to GLP-1 tripled in association with a threefold increase of the insulin content, both now being twice normal when stratified for the fractional β-cell mass. The mechanism of the increased pancreas insulin content was investigated by assessing islet glucose metabolism and proinsulin biosynthesis. In controls with fasting, both fell 30–50%. In Px, the degree of suppression with fasting was similar, but the attained levels both exceeded those of the controls because of higher baseline (nonfasted) values.

In summary, chronic hyperglycemia is associated with a fasting-induced paradoxical increase in glucose-potentiated insulin secretion. In Px rats, the mechanism is an increase in the β-cell insulin stores, which suggests a causative role for a lowered β-cell insulin content in the impaired glucose-potentiation of insulin secretion. (*J. Clin. Invest.* 1996. 97:180–186.) Key words: animal models non–insulin-dependent diabetes mellitus • fasting • proinsulin biosynthesis • islet of Langerhans glucose metabolism • insulin content

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Introduction

Insulin secretion is impaired in states of chronic hyperglycemia such as non–insulin-dependent diabetes mellitus (NIDDM)[1] (1, 2). The near-total disappearance of glucose-stimulated insulin release is best known. Studies of diabetic rodents have suggested that the mechanism is a direct effect of the high glucose environment impairing β-cell intermediary metabolism or of a second messenger (3). A second type of glucoregulation for insulin release is to modulate the β-cell responsiveness to the large number of insulinotropic hormones, nutrients, and neurotransmitters, so-called glucose potentiation of nonglucose secretagogues (4). Glucose potentiation is impaired in NIDDM (5). Virtually nothing is known about the pathogenesis of this defect. We monitored high glucose/arginine–induced insulin secretion in rats made diabetic by a 90% pancreatectomy (Px). A sequence of events was identified in which the β-cell glucose sensitivity increased, thereby augmenting basal insulin secretion (6), followed a couple of weeks later by impaired glucose potentiation of the arginine response (7). Diazoxide (an inhibitor of insulin secretion) raised the pancreas insulin content 50% and prevented the later defect (7). Thus, in contrast to an inhibitory effect of hyperglycemia on the β-cell, like that proposed for the impaired glucose-induced insulin secretion, our results suggest that the defect in glucose potentiation is mediated by a hyperstimulated insulin secretion, the so-called "overworked β-cell" hypothesis (8). The mechanism proposed by us and others is a depleted pool of releasable insulin (7, 9). Importantly, it was reported two decades ago that diazoxide increased insulin secretion to glucagon and tolbutamide in NIDDM (10), suggesting that the same process occurs in human diabetes.

In contrast to the wealth of information regarding impaired stimulation of insulin secretion in hyperglycemic states, little is known about the ability of β-cells to downregulate insulin secretion. Investigation of this question is necessary for a complete understanding of the β-cell dysfunction that occurs with chronic hyperglycemia. Fasting is a well-known inhibitor of β-cell function. Insulin secretion (11–13) and proinsulin biosynthesis (14, 15) decrease in parallel so that insulin content is unchanged. It was recently reported that a 4-d fast paradoxically increased insulin secretion to oral glucose and to glucagon in persons with NIDDM (16). This result is reminiscent of the diazoxide results in Px rats cited above. We thus predicted that the Px model would reproduce the aberrant fasting effect on insulin secretion, and allow investigation of the cellular mechanism. The current study tested this idea using as the

1. *Abbreviations used in this paper:* GLP-1, glucagonlike peptide–1; NIDDM, non–insulin-dependent diabetes mellitus; Px, 90% pancreatectomy rat.

secretagogue glucagonlike peptide–1 (GLP-1), a potent incretin (17, 18) that is known to have a glucose-dependent insulin stimulatory effect (19).

Methods

90% pancreatectomy rat model. 90% pancreatectomies were performed on 100-g male Sprague-Dawley rats (Taconic Farms Inc., Germantown, NY) using the method of Bonner-Weir et al. (20). During pentobarbital sodium anesthesia (100 mg/kg ip), 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. Postoperatively, rats were given standard rat chow and tap water ad lib. Studies were conducted 4–6 wk after surgery, using age-matched nonoperated rats (pancreas perfusion studies) or sham-operated rats (isolated islet studies) as controls. The fasting protocol was 40 h (6:00 p.m.–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. (21): pancreatic duct infiltration with collagenase (Serva, Heidelberg, Germany), Histopaque® gradient separation (Sigma Chemical Co., St. Louis, MO), and hand picking. Islet yield was 100–150 in Px and 500 in controls so that each experiment used pooled islets from two Px rats (both fasted and nonfasted groups) as opposed to one of each control group.

In vitro perfused pancreas and insulin content. The perfused pancreas technique has been described elsewhere (22). The perfusate was a KRB buffer, pH 7.4, plus 4% dextran T_{70} and 0.2% BSA fraction V (Sigma Chemical Co.). GLP-1 (7–37) was a gift of Scios Nova, Mountain View, CA. It was dissolved in perfusate and infused by a sidearm syringe to a final concentration of 10^{-9} M which added 0.2 ml/min to the usual flow rate of 3.5 ml/min. After the 17-min equilibration period, 1-min samples were collected in tubes containing 8 mg EDTA and kept on ice pending storage at −20°C. After perfusion, pancreases were excised, blotted, weighed, and stored at −20°C in acid ethanol. They were later homogenized and assayed by insulin RIA, which entailed charcoal separation (23) and rat insulin standards (Eli Lilly and Co., Indianapolis, IN). The sensitivity was 30 pM.

Islet glucose utilization. Islet glucose utilization was measured by the method of Ashcroft et al. (24). Following a 90-min preincubation in KRB/2.8 mM glucose, triplicate groups of 20 islets were incubated 90 min at 37°C in 100 μl KRB, glucose (2.8, 8.3, or 27.7 mM), 1.3 μCi D-[5-^{3}H] glucose (Amersham Corp., Arlington Heights, IL). The reaction was carried out in a 1-ml cup contained in a rubber-stoppered 20-ml scintillation vial that had 500 μl of distilled water surrounding the cup. Glucose metabolism was stopped by injecting 100 μl 1 M HCl through the stopper into the cup. An overnight incubation at 37°C was carried out to allow equilibration of the $[^3H]H_2O$ in the reaction cup and the distilled water, followed by liquid scintillation counting of the distilled water.

Islet proinsulin biosynthesis. Proinsulin biosynthesis was measured by [^{35}S]methionine radiolabeling using the method of Alarcón et al. (25, 26). Batches of 100 islets were preincubated 40 min at 37°C in 100 μl modified KRB, 20 mM Hepes, 0.1% BSA, 2.8 or 16.7 mM glucose, then radiolabeled 30 min with 100 μl incubation media that contained 0.25 mCi [^{35}S]methionine (New England Nuclear, Boston, MA). Islets were resuspended and sonicated in 250 μl lysis buffer (25) and centrifuged 2 min at 10,000 *g*. The immunoprecipitation consisted of the following steps: incubate supernatants 2 h at room temperature with 25 μl Cowan's strain *Staphylococcus aureus* cells (Sigma Chemical Co.), repeat the centrifugation, incubate supernatants with 25 μl insulin antiserum (Sigma Chemical Co.) for 18 h at 4°C, precipitate antigen/antibody complex with 50 μl Pansorbin (Calbiochem Corp., San Diego, CA) for 2 h at room temperature, repeat centrifugation. The immunoprecipitated proteins were eluted from the Pansorbin with 300 μl 0.1% HCl followed by alkaline-urea PAGE and fluorography (25). Band intensity was quantified by densitometry using IMAGE 1.4 software (National Institutes of Health, Bethesda, MD).

Islet proinsulin Northern blot. Total RNA extracted from isolated islets using RNeasy™ (QIAGEN Inc., Chatsworth, CA) underwent electrophoresis in 1% agarose/6% formaldehyde. The RNA was capillary transferred onto a nylon membrane and fixed by long wave ultraviolet irradiation and baking at 80°C for 2 h. After prehybridization for 3 h at 42°C (50% formamide, 5× SSPE, 2× Denhardt's solution, 0.1 mg/ml denatured salmon sperm DNA, 0.1% SDS, 10% dextran sulfate), filters were hybridized overnight at 42°C with a [^{32}P]CTP radiolabeled riboprobe for rat proinsulin I. The riboprobe was prepared from a plasmid that was a gift from L. G. Moss, New England Medical Center, Boston, MA, using the Riboprobe Gemini II Core System™ (Promega Corp., Madison, WI) and SP6 RNA polymerase (Pharmacia LKB Biotechnology Inc., Piscataway, NJ). Filters were washed with high stringency conditions (2× SSC, 0.1% SDS once at room temperature for 15 min and three times at 65°C for 10 min), and the hybridization was visualized by autoradiography and quantified by densitometry. Gels contained samples from a unique set of the four rat groups (fasted and nonfasted Px and sham-operated control rats), with the data expressed in relative terms by assigning the sham nonfasted result a value of 100%. A 72-h fast is known to lower rat islet RNA by half (15). We thus conducted five experiments; three with loading equal amounts of RNA (2.5 μg) in each lane, and two in which the total RNA yield from the same number of islets (200) was loaded in each lane. The results were similar using the two methods and are reported together.

Data presentation and statistical methods. All data are expressed as mean±SEM. The values for insulin secretion in the text are the mean insulin concentration of all samples collected during the designated perfusate condition. For all protocols, the listed *n* value is the number of separate experiments performed. Statistical significance was determined by one-way ANOVA. The one-way *t* test was used for the Northern blot results.

Results

Insulin secretion to GLP-1 in 90% Px rats. The overworked β-cell hypothesis predicts that glucose-potentiated insulin responses are generally impaired with chronic hyperglycemia. To date, we had studied only arginine in the Px rats (7). The first part of this study tested GLP-1 in nonfasted Px and control rats using a perfusion protocol of 10-min infusions of 10^{-9} M GLP-1 at 2.8, 8.3, and 16.7 mM glucose (Fig. 1). The two well-known regulatory effects of glucose on insulin secretion were evident in the controls: the direct effect to raise insulin output as shown by the sample preceding each GLP-1 infusion, and the potentiating effect (note the increased incremental area of the GLP-1 curve as the glucose level rose). Two differences were noted in the Px rats. The glucose set point was lowered as shown by the presence of a clear insulin response to GLP-1 at 2.8 mM glucose versus no stimulatory effect in the controls. Also, glucose potentiation was impaired as shown by the insulin response to GLP-1 at 16.7 mM glucose being 10±2% of the controls (0.87±0.17 nM in Px vs 8.57±0.79 nM in controls, $P < 0.001$). At first, the 10% result seems appropriate for a 90% Px. In reality, substantial β-cell regeneration follows a 90% Px such that by 8 wk the β-cell mass of the remnant has grown from the original 10% to 42% of age-matched normal rats (20). The majority of the regeneration occurs during the first 3 wk (27). As such, the β-cell mass of the remnant at 4–6 wk should be 35–40% of normal which fits with the 38% published value for islet mass in rats 4 wk after 90% Px (28). Thus, the 10% result is well below the fractional β-cell mass.

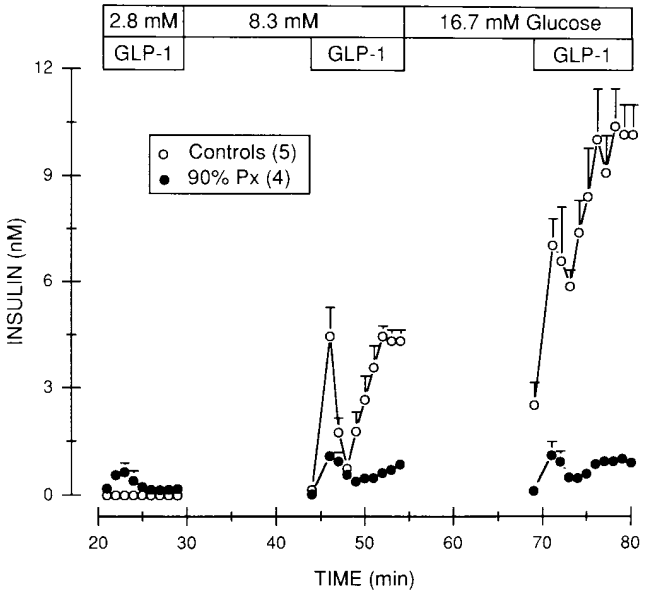

Figure 1. Insulin secretion to 10^{-9} M GLP-1 (7–37) in rats 4–6 wk after 90% pancreatectomy and age-matched controls assessed by the in vitro perfused pancreas. The first sample shown for each infusion period was collected before the GLP-1 was started.

The cause of the lowered insulin response was a combination of two effects: reduced insulin secretion to 16.7 mM glucose alone (sample which preceded the GLP-1 infusion was 6±3% of the controls), and a reduced GLP-1 response (incremental insulin response to GLP-1 was 12±3% of the controls).

GLP-1 insulin secretion in 40-h fasted 90% Px rats. A 40-h fast was performed to determine if insulin secretion to GLP-1 paradoxically increased in Px. Body weight and plasma glucose values are shown in Table I. Nonfasted Px rats had a normal body weight and were hyperglycemic compared to nonfasted controls (10.2±0.3 mM in Px vs 8.8±0.5 mM in controls, $P < 0.03$). Fasting caused a slightly greater fall in body weight in the Px rats (38±1 g in Px vs 30±2 g in controls, $P < 0.008$), but the final weights were not different. Plasma glucose fell to the same subnormal level in Px and controls with fasting (6.3±0.4 mM in Px vs 5.9±0.4 mM in controls).

The perfusion protocol was a baseline 5.5 mM glucose followed by 20 min at 16.7 mM glucose, then 15 min at 16.7 mM glucose/GLP-1 (Fig. 2). Nonfasted control rats had the expected large biphasic insulin response to 16.7 mM glucose, and a threefold additional increase to GLP-1. Fasting suppressed both insulin responses: 16.7 mM glucose by 90%, and GLP-1 by 60%. Nonfasted Px rats had the near-total suppression of insulin secretion to high glucose that occurs with chronic hyperglycemia. The GLP-1 response was 13±2% of the control rats (close agreement with the previous experiment), with all of the decrease being found in the second phase. Fasting affected insulin secretion in Px rats differently than the controls. There was no decrease of the insulin response to 16.7 mM glucose; the 102±36 pM value in nonfasted Px was four times the sensitivity of the insulin RIA so that the absence of an inhibitory effect was clearly measurable (98±20 pM). Even more different was the threefold increase of the second phase insulin response to GLP-1 (3.18±0.86 nM in fasted Px vs 1.10±0.28 nM in nonfasted Px, $P < 0.035$) to 65% of the fasted control rats (5.06±0.89 nM, P = NS). This value exceeds the predicted fractional β-cell mass in Px by twofold, so that the GLP-1 response was now twice normal.

Pancreas insulin content. In the control rats, insulin content and pancreas weight were unaffected by the fast (Table I, Fig. 3). In nonfasted Px rats, insulin content was 25±2% of the controls. This value is subnormal when viewed in terms of the predicted fractional β-cell mass (35–40% of normal). With fasting, insulin content increased threefold in Px (9.6±0.6 nmol vs 3.6±0.3 nmol, $P < 0.0001$) to 68±4% of the control rats. This value is twofold higher than the predicted fractional β-cell mass of these rats and closely parallels the GLP-1 insulin secretion result.

Islet proinsulin biosynthesis. We investigated the mechanism for the increase in pancreas insulin content in the fasted Px rats by assessing proinsulin biosynthesis in isolated islets using two methods: proinsulin mRNA level and methionine radiolabel incorporation. Fig. 4 shows a representative proinsulin mRNA gel plus combined data from five experiments. In the controls, proinsulin mRNA level fell an average of 30% with fasting ($P < 0.048$). The level in nonfasted Px was higher than nonfasted controls ($P < 0.042$). It fell with fasting but remained above the level of the fasted controls ($P < 0.017$). Identical results were obtained for methionine radiolabel incorporation which was carried out at 2.8 and 16.7 mM glucose (Fig. 5). In nonfasted controls, proinsulin biosynthesis was threefold higher at the high versus low glucose concentration. Both values were lowered 30–50% by the fast. In nonfasted Px, proinsulin biosynthesis was greater than that for nonfasted controls, with the increase being most obvious at 16.7 mM glucose. Fasting lowered the proinsulin biosynthesis rate in Px, but again the decrease was incomplete versus the fasted control rats.

Islet glucose utilization. To investigate the reason for the impaired suppression of proinsulin biosynthesis in the Px rats

Table I. General Characteristics of 40-h Fasted 90% Px and Control Rats

Animal group	Weight before fast	Weight after fast	Blood glucose	Pancreas weight	Insulin content
n	*g*	*g*	*mM*	*g*	*nmol/pancreas*
Controls fasted 40 h (6)	320±9	290±9*	5.9±0.4‡	1.42±0.05	14.1±2.7
Controls nonfasted (5)	330±12	345±12	8.8±0.5	1.50±0.06	14.1±1.2
90% Px fasted 40 h (6)	350±7	312±8§	6.3±0.4‖	0.40±0.01	9.6±0.6¶
90% Px nonfasted (7)	340±9	352±9	10.2±0.3	0.41±0.03	3.6±0.3**

Statistical significance was determined by ANOVA. *$P < 0.004$ between fed and fasted control rats; ‡$P < 0.01$ between fed and fasted control rats; §$P < 0.006$ between fed and fasted Px rats; ‖$P < 0.0001$ between fed and fasted Px rats; ¶$P < 0.001$ between fed and fasted Px rats; **$P < 0.0001$ between fed Px and fed control rats.

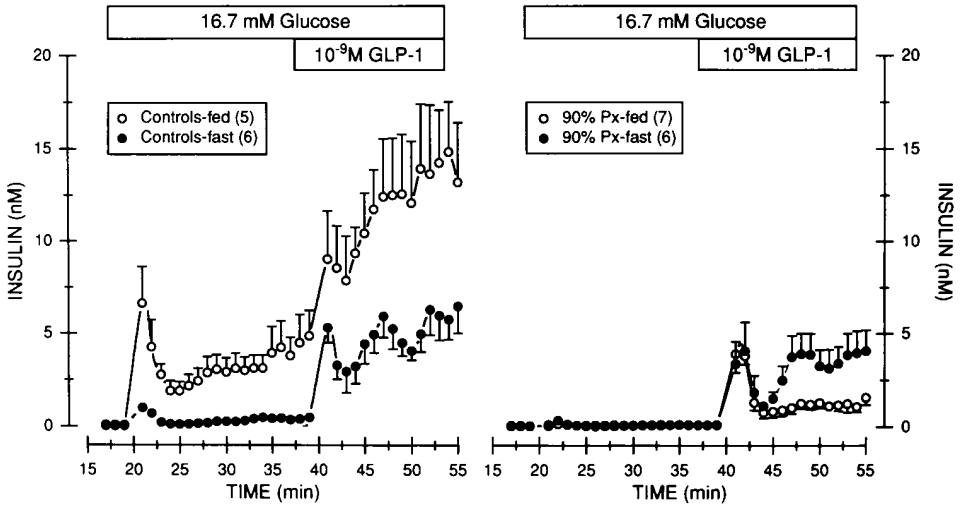

Figure 2. Effect of a 40-h fast on insulin secretion assessed by the in vitro perfused pancreas in rats 4–6 wk after 90% pancreatectomy and age-matched controls. Non-fasted rats were studied in parallel.

with fasting, glucose utilization was measured at 2.8, 8.3, and 27.7 mM glucose in islets from fasted and nonfasted control and Px rats (Table II). The results closely mirrored those for biosynthesis. Fasting the control rats lowered islet glucose utilization 25–35% throughout the glucose range. In nonfasted Px, islet glucose usage was increased versus the nonfasted control rats, with the largest increase occurring at the low glucose level (190±14% of nonfasted control at 2.8 mM glucose and 162±11% at 27.7 mM glucose). Fasting reduced islet glucose utilization in Px rats, with the proportionate reduction being at least equal to the controls. However, because of the higher baseline values, the level remained above that of the fasted controls and was now identical to the nonfasted control islets.

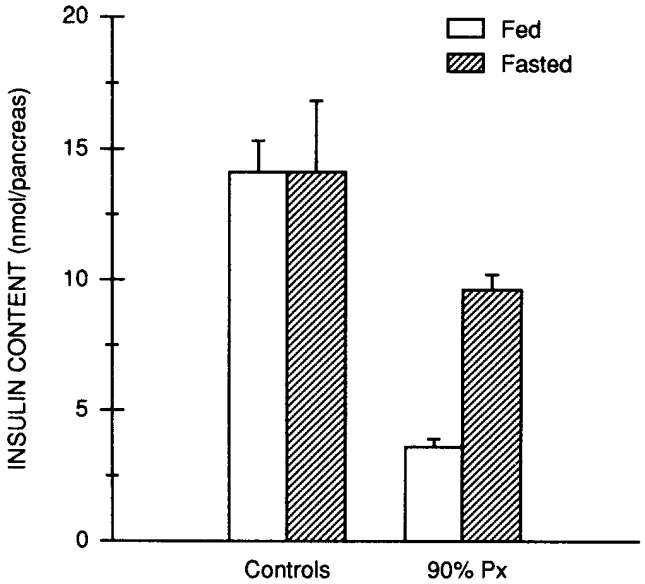

Figure 3. Pancreas insulin content after a 40-h fast in rats 4–6 wk after 90% pancreatectomy ($n = 6$) and age-matched controls ($n = 6$). Nonfasted rats were studied in parallel (Px = 7, controls = 5). Pancreases were collected immediately after the pancreas perfusions shown in Fig. 2.

Discussion

Food restriction is a cornerstone of therapy for NIDDM (29). Known beneficial effects are an increased insulin sensitivity because of conversion from a high fat to a high carbohydrate diet and reduced obesity. Insulin secretion also improves (30), although the mechanism is unclear. It was recently reported that a 4-d fast enhanced insulin secretion in NIDDM (16), which contrasts with the reduced insulin secretory capacity that occurs normally (31). The current study investigated the mechanism of the same finding in 90% Px diabetic rats.

Fasting downregulates glucose-induced and glucose-potentiated insulin secretion by inhibiting the activity of glucokinase in β-cells (32, 33). Glucokinase is the rate-limiting step for glucose metabolism in β-cells (34), which explains the decrease in islet glucose usage noted in the fasted versus nonfasted controls. Insulin secretion and proinsulin biosynthesis also are lowered since the β-cell glucose utilization rate is an important determinant of these functions (34). In Px, the fall in islet glucose usage was incomplete which caused a relative increase in proinsulin biosynthesis so that insulin stores climbed to twice normal (adjusted for the fractional β-cell mass). Our results suggest that the increased insulin content was the cause of the augmented insulin response to high glucose/GLP-1. The key evidence for this interpretation was our finding that the increased insulin secretion occurred in the presence of a lowered islet glucose utilization (albeit incomplete). As such, the normal link between β-cell glucose metabolism and glucose-potentiated insulin responses is missing in Px so that another factor must have assumed control of the β-cell secretory function. We have proposed that depletion of the β-cell insulin stores causes the lowered glucose-potentiated insulin responses in Px. The current study seemingly confirms that idea through the following findings: the paradoxical increase in insulin secretion was paralleled by an aberrant increase in the islet insulin content, the fold increase for each of these was identical (threefold), and the attained responses were identical (twice normal). However, it should be emphasized that our conclusion is based on correlative data, and we cannot exclude some other β-cell factor being the mechanism. Note that glucose-induced insulin secretion was not increased in Px which clearly indicates an alternate pathogenesis for this defect. Thus, this

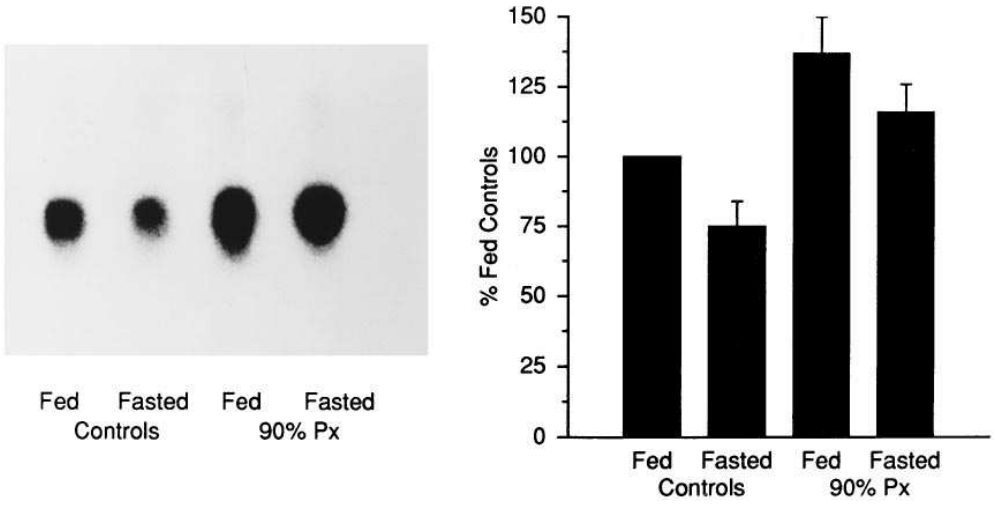

Figure 4. Proinsulin mRNA levels in isolated islets of fasted and nonfasted 90% Px and sham-operated control rats. Proinsulin Northern blots were carried out using a [^{32}P]CTP radiolabeled riboprobe for rat proinsulin I. Results from each gel were quantified by densitometry and expressed in relative terms by assigning the nonfasted control result a value of 100%. The left panel shows a representative gel in which the total extracted RNA from 200 islets was loaded in each lane. The right panel is mean±SEM data from five separate experiments.

study in combination with our previous study with diazoxide (7) provides compelling evidence that some aspect of a hyperstimulated insulin secretion (i.e., the overworked β-cell hypothesis) is the mechanism of the impaired glucose-potentiated insulin responses in 90% Px rats. Also, the parallels with NIDDM as regards defective glucose potentiation (5) and increased insulin responses with diazoxide (10) and fasting (16) suggest similar pathogenic events.

This study was initially undertaken to determine if GLP-1 showed the same secretory dysfunction in Px that we had noted for arginine. Two defects in GLP-1–induced insulin secretion were noted, a lowered glucose set point and impaired glucose-potentiation, perfectly agreeing with the arginine results (6, 7). Finding the lowered glucose set point for insulin output to arginine (6), and now GLP-1, has been critical for the development of the overworked β-cell hypothesis by explaining how the modest hyperglycemia in Px rats could hyperstimulate insulin secretion enough to impair the insulin secretory capacity. The answer came with the recognition that in the presence of the β-cell hypersensitivity for glucose, this degree of hyperglycemia caused Px β-cells to secrete insulin at 90% of capacity versus the normal 10–20% (6). Indicative of this idea are the normal fasting and postmeal plasma insulin values in Px despite the markedly reduced β-cell mass (20).

Our conclusion that glucose potentiation of the GLP-1 insulin response was impaired in Px is based on the understanding that a 90% Px is followed by substantial regeneration of the endocrine and exocrine tissue (20), and assumes a β-cell mass 4–6 wk after surgery that is 35–40% of normal. An alternate interpretation of our results is that the regenerated β-cells are nonfunctional, and the high glucose/GLP-1 insulin response (10% of normal) is normal for a 90% pancreatectomy. Circumstantial evidence which might be viewed as consistent with this idea is the variable β-cell morphology that is found in Px rats (20), plus the recent discovery that the β-cell regeneration in these rats occurs through two distinct pathways (35). However, several findings make this idea untenable. The pancreas insulin content in the Px rats was 25% of normal under basal conditions (7 and current study), and nearly 70% of normal after fasting (seven times the β-cell mass of the original remnant). Also, we have shown reversal of the defect in glucose potentiation for arginine with insulin (36) and diazoxide

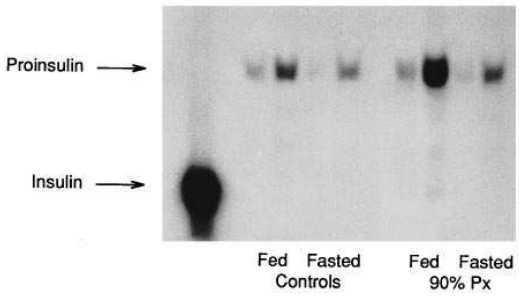

Figure 5. Proinsulin biosynthesis by [^{35}S]methionine incorporation in isolated islets of fasted and nonfasted 90% Px and sham-operated control rats. Islets (100) were radiolabeled at 2.8 or 16.7 mM glucose for 30 min, followed by lysis, immunoprecipitation with antiinsulin serum, alkaline-urea PAGE, and fluorography. A pair of bands is shown for each animal group: the 2.8 mM glucose sample is on the left, and 16.7 mM glucose on the right. The intense band on the far left is ^{125}I-labeled porcine insulin diluted in loading buffer which shows that the labeled bands migrate as a higher molecular weight form (*Proinsulin*).

Table II. Islet Glucose Utilization in 40-h Fasted 90% Px and Control Rats

	Islet glucose utilization		
	Glucose concentration		
Animal group	2.8 mM	8.3 mM	27.7 mM
n	*pmol glucose/90 min per islet*		
Controls fasted 40 h (5)	18±1	53±5	118±7
Controls nonfasted (5)	24±1*	79±3*	159±8*
90% Px fasted 40 h (5)	26±2‡	76±6§	150±12‖
90% Px nonfasted (5)	46±3¶**	114±9¶**	257±17¶**

Statistical significance was determined by ANOVA. *$P < 0.005$ between fed and fasted control rats; ‡$P < 0.01$ between fasted Px and fasted control rats; §$P < 0.016$ between fasted control rats; ‖$P < 0.049$ between fasted Px and fasted control rats; ¶$P < 0.001$ between fed and fasted Px rats; **$P < 0.001$ between fed Px and fed control rats.

(7), and for GLP-1 with fasting in the current study, which excludes nonfunctional β-cells.

The surprising finding which led to our investigating proinsulin biosynthesis and islet glucose utilization was the huge increase in insulin content that occurred in the Px rats with fasting. It rose from 30 to 40% below normal to twice normal (stratified for the β-cell mass), showing a clear dysregulation rather than simply recovery of the insulin stores to normal. Studies of metabolizable versus nonmetabolizable sugars have established the important regulatory role for β-cell glucose metabolism in proinsulin biosynthesis (37). As such, the failure to fully inhibit glucose utilization (and proinsulin biosynthesis) was the underlying cause. What is the mechanism for the incomplete down-regulation of the β-cell glucose utilization in Px? Glucokinase is the dominant regulator of β-cell glucose usage (34). Hexokinase, the low K_m isoform, also is present in β-cells, but it normally has virtually no regulatory role because of end product inhibition by glucose-6-phosphate (38). We measured glucokinase and hexokinase activities in extracts of Px and control islets (39): the major finding was a 250% increase in hexokinase V_{max} in Px as opposed to a minimal increase in glucokinase V_{max}. With fasting (using the same protocol of this study), glucokinase activity fell normally in the Px islets which excludes an aberrant effect of fasting on glucokinase being the mechanism of the increased insulin secretion in this study. Unlike glucokinase, hexokinase activity is not normally down-regulated by fasting, and islet hexokinase activity was unaffected by the fast in either Px or the controls. We speculate from these results that the increased hexokinase activity in Px assumes partial control over β-cell glucose utilization, accounting in part for the increased β-cell sensitivity to glucose which we have observed in these rats (6). The result would be a failure of fasting to fully inhibit glucose utilization; the result noted in this study. We further speculate that this sequence of events is not unique for Px but occurs generally with chronic hyperglycemia. Supporting this concept are recent in vitro transfection studies which overexpressed hexokinase in native or transformed β-cells (40, 41): glucose utilization and glucose-induced insulin secretion both were up-regulated, so that some of the hexokinase activity clearly escaped end product inhibition. Also, increased islet hexokinase activity has been observed in several other diabetic rodent models besides Px (42–44), which is consistent with our suggestion of the global nature of this finding. Of course, this hypothesis is speculative and requires confirmation, including identifying which of the cell types in the Px islets contain an increased hexokinase activity.

A difficult issue was how to design the proinsulin Northern blot and biosynthesis protocols for this study. Northern blot studies usually attempt to equalize samples by loading the same amount of total RNA per lane. However, a 72-h fast in normal rats was found to lower the ratio of islet RNA/DNA by 50% (15). We thus performed gels two ways: loading equivalent amounts of RNA per islet group, also loading the total extracted RNA from the same number of islets for each group. No difference in the methods was noted which may reflect the shorter fasting time of our protocol (40 h). A similar question existed for the [^{35}S]methionine experiments. Results with this technique are usually stratified against total islet protein synthesis as determined from TCA precipitability, again in an attempt to adjust for the amount of tissue in the sample. However, in normal rats fasted for 3 d, this measure increased 30% at a time when proinsulin biosynthesis fell 30% (14). The result would be exaggerated if adjusted for TCA precipitation. To circumvent this problem, we designed our protocol to start with the same number of islets per sample and view the results in absolute terms.

In summary, our results suggest that chronic hyperglycemia impairs both glucose-induced and glucose-potentiated insulin secretion. The pathogenesis of these defects differs. The current results in combination with a previous study with diazoxide (7) indicate that the impaired glucose-potentiation results from a hyperstimulation of insulin secretion. Important in the genesis of this defect is a β-cell hypersensitivity to glucose, with a newly proposed mechanism being an increased activity of hexokinase in β-cells. We speculate that the β-cell insulin stores fall below some critical level, thereby reducing glucose-potentiated insulin responses. Several kinds of evidence support that similar events occur in NIDDM including very similar effects of diazoxide and fasting in Px rats and persons with NIDDM.

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