JCI The Journal of Clinical Investigation

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J Clin Invest. 1988;81(5):1407-1414. https://doi.org/10.1172/JCI113470.

Research Article

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Minimal Chronic Hyperglycemia Is a Critical Determinant of Impaired Insulin Secretion after an Incomplete Pancreatectomy

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Abstract

We now describe experiments that allow one to determine the consequences of B cell reduction alone vs. those that result from superimposed mild hyperglycemia. Male CD rats underwent a 60% pancreatectomy (Px); controls were sham operated. 1 wk later, either 10% sucrose (SUC) was substituted as fluid supply or tap water was continued (WAT).

Plasma glucose and insulin values in Px-WAT remained equal to the sham groups; in Px-SUC the values were euglycemic for 25 d, but then nonfasting plasma glucose rose 15 mg/dl. After 6 wk, B cell mass in Px-WAT was reduced by 45% and non-B cell mass by 57%. In contrast, in Px-SUC both masses were comparable to the sham groups. The insulin response to 27.7 mM glucose was measured using the in vitro perfused pancreas. The responses were reduced in Px-WAT but in proportion to their reduced B cell mass; in contrast, it was 75% less than expected in Px-SUC. Also, the response to arginine given at 16.7 mM glucose was reduced only in Px-SUC.

These results show that a lowering of B cell mass that does not result in hyperglycemia has no adverse effect on the remaining B cells. On the other hand, if even mild hyperglycemia develops, B cell function becomes impaired and results in inappropriately reduced insulin stores and insulin responses to marked stimuli.

Introduction

Considerable evidence is now available showing that a reduction in B cell mass of sufficient magnitude to cause hyperglycemia is inevitably followed by secretory abnormalities in the remaining B cells (1). We have proposed that chronic exposure to the elevated glucose level is what causes their development (recently referred to as the "glucose toxicity" hypothesis), and we have demonstrated similar defects in normal rats made markedly hyperglycemic for 48 h with in vivo glucose infusions (2). On the other hand, the effects of reduced B cell mass alone are not known.

It has been hypothesized that chronic hyperglycemia is responsible for the secretory abnormalities in non-insulin-dependent diabetes mellitus (3, 4), but if this is correct, then barely detectable increases in plasma glucose must be sufficient to cause B cell dysfunction since the early insulin response to an intravenous glucose challenge is already lost in patients with impaired glucose tolerance (5, 6). However, an attempt to demonstrate such an effect experimentally, by making normal rats only mildly hyperglycemic for 48 h with in vivo glucose infusions, was not successful (2). One explanation could be that it may take longer for secretory defects to develop with such a small degree of hyperglycemia.

The aim of this study was to determine if B cell secretory function is altered in any way after a reduction in B cell mass of insufficient magnitude to cause hyperglycemia, and then to assess whether superimposing very mild hyperglycemia has any further effect.

Methods

60% pancreatectomy (Px).[1] 5-wk-old male CD rats weighing 90–100 g (Charles River Breeding Laboratories, Inc., Wilmington, MA) were anesthetized with 100 mg/kg i.p. amobarbital sodium and ether if needed. A midline abdominal incision was made and the splenic lobe of the pancreas mobilized by partially breaking mesenteric connections to the stomach, small bowel, and retroperitoneum. Pancreatic tissue was removed according to the method of Foglia (7) (as modified by Bonner-Weir et al. [8]) by gentle abrasion with cotton applicators, being careful to leave major blood vessels intact. The excised portion was bordered by the spleen and stomach extending to but not including the small flap of pancreas attached to the pylorus; it constituted 57±3% of total pancreatic weight (n = 3). Control rats (shams) underwent laparotomy and mobilization of the pancreas with gentle rubbing of it between the fingers. Postoperatively, all rats received standard laboratory chow and tap water ad lib.

1 wk after surgery, 10% sucrose (SUC) was substituted as the drinking solution in half the rats for either 4 or 6 wk; the others continued to receive tap water (WAT). Bottles were filled every other day after recording the volume ingested. Body weight and nonfasting plasma glucose and insulin values were measured every 7–10 d in blood obtained by tail snipping. Insulin secretion was studied at the end of the 4- or 6-wk period with the in vitro isolated perfused pancreas using one of the protocols shown in Figs. 2–5. In another series of rats, B and non-B cell masses were measured at the end of 6 wk.

In vitro isolated perfused pancreas. This technique has been described previously (9). Animals were anesthetized with 100 mg/kg i.p. amobarbital sodium. The perfusate was a modified KRB buffer which contained 4% dextran (D-4751; Sigma Chemical Co., St. Louis, MO), 2 mM calcium, 1.2 mM magnesium, and 0.2% BSA fraction V (Sigma Chemical Co.). It was bubbled for 20 min with 95% O_2 and 5% CO_2, and then glucose was added and the pH adjusted to 7.4. It was placed in a reservoir maintained at 38°C by water bath; 10 mM arginine was added to perfusate in a second reservoir. Glucose concentrations greater than the baseline were attained with a sidearm syringe driven by syringe pump (Sage Instruments Div., Orion Research Inc., Cambridge, MA) which added 0.2 ml to the usual flow rate of 3 ml/min.

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Received for publication 24 August 1987 and in revised form 30 November 1987.

J. Clin. Invest.

0021-9738/88/05/1407/08 $2.00
Volume 81, May 1988, 1407–1414

1. *Abbreviations used in this paper:* Px, 60% pancreatectomy; SUC, 10% sucrose water; WAT, tap water.

Before delivery, the perfusate passed through an artificial lung to ensure adequate oxygenation (10). After cannulation of the aorta and portal vein, the body cavity was covered with gauze moistened with saline and placed under a heat lamp with the temperature being constantly monitored and maintained at 36–39°C. All perfusions were preceded by a 20-min period during which no samples were taken; thereafter, they were collected according to the protocols at the top of the respective figures for 30 s in chilled tubes that contained 4 mg EDTA, and were kept on ice pending storage at −20°C.

Pancreatic extraction for insulin content. After study with the perfused pancreas, pancreases were removed, cleared of lymph nodes, blotted, weighed, and stored at −20°C in acid ethanol. Later, on a single day, all were individually homogenized using an Ultra Turrax SDT (Tekmar Co., Cincinnati, OH), diluted to a volume of 8 ml, and refrozen pending assay.

Quantitative morphometrics. At the end of 7 wk, pancreases were removed and cleared of fat and lymph nodes; shams were divided into two portions while Px were left whole. Each portion was weighed, placed in Bouin's fixative overnight, and embedded in paraffin. Sections (5–7 μm) were stained by immunoperoxidase using a cocktail of antibodies against non–B islet endocrine cells (non-B cells) that consisted of rabbit anti–synthetic somatostatin (our D-10), rabbit anti–bovine pancreatic polypeptide (gift of Dr. R. Chance, Eli Lilly, Indianapolis, IN), and rabbit anti–porcine glucagon (gift of Dr. M. Appel, University of Massachusetts Medical School, Worchester, MA). Control incubations that used excess antigen added to immune sera, nonimmune sera as primary antibody, and omission of the primary antibody, all resulted in the absence of hormone-specific staining. The relative volumes of the islet B and non–B cells were quantified by the point counting method of Weibel (11): at a magnification of 350 starting at a random point in one corner of the slide, acinar and islet cells were scored in every other field using a 50-point grid, with a minimum of 5,000 points in 108 fields being counted per tissue block. Intercepts over blood vessels, fat, ducts, lymph nodes, and interlobular spaces were subtracted to give the total pancreatic counts. Islet B and non–B cell masses were then calculated for each animal by multiplying the relative volumes times the pancreatic weight.

Analytical methods. Plasma glucose was measured with a glucose analyzer II (Beckman Instruments, Inc., Brea, CA). Insulin concentrations were determined using an RIA that employed charcoal separation (12) and rat insulin standards (gift of Dr. R. Chance, Eli Lilly, Indianapolis, IN).

Data presentation and statistical methods. The insulin concentrations of all samples collected during the perfused pancreas studies are depicted on the figures as single points with brackets that represent the mean and standard error (mean±SEM) for each group. The incremental insulin responses caused by 27.7 mM glucose (taken from Figs. 2 and 3) are listed in Table III. They represent the total amount of insulin released above the secretory rate established at the end of the previous perfusate (2.8 mM glucose), and they were calculated for each animal by subtracting the insulin concentration of the last sample at 2.8 mM glucose from the mean of those taken during 27.7 mM and multiplying by duration (15 min) and flow rate. The mean±SEM for each group was then determined. The incremental response per milligram of B cells was calculated in the 6-wk groups by dividing these values by the B cell masses listed in Table II.

The incremental responses caused by arginine at 2.8 and 16.7 mM glucose (Figs. 4 and 5) are listed in Table IV. The values at 2.8 mM were calculated by subtracting the mean of the three samples taken at 2.8 mM glucose alone from the mean insulin concentration of those collected during the arginine infusion and multiplying by duration (5 min) and flow rate. A similar method was used to calculate the values at 16.7 mM glucose, except that the last sample taken before adding arginine was the only one used to establish the baseline. The incremental responses to arginine at 16.7 mM glucose/mg of B cells were calculated in the 6-wk groups by dividing each result by the B cell mass.

Statistical significance was determined using a one-way analysis of variance (13).

Results

General characteristics of the animal groups. Body weights and nonfasting plasma glucose and insulin values are listed in Table I. The sham-WAT rats tended to be slightly heavier than those of the other groups during much of the test period, but by the sixth week a difference could no longer be demonstrated. Plasma glucose and insulin values in Px-WAT remained equal to those of the sham-WAT group as they also did in the shams given sucrose, although there seemed to be a tendency for plasma insulin to increase in some. In contrast, Px-SUC rats were euglycemic during the first 25 d (samples were also obtained at 10 and 18 d but not included in Table I), but then developed mild hyperglycemia that was not associated with any change in plasma insulin.

The volume of fluid ingested per day averaged over weekly intervals is shown in Fig. 1. Sham and Px rats took in 40–50 ml

Table I. General Characteristics of Animal Groups

	Sham		Px	
	Tap water	10% sucrose	Tap water	10% sucrose
Body weight (*g*)				
25 d	343±6 (7)	316±9 (8)*	326±8 (7)	308±11 (8)
4 wk	369±7 (7)	346±12 (8)	346±7 (7)*	345±12 (8)
6 wk	408±10 (12)	421±12 (14)	427±9 (12)	391±12 (12)
Plasma glucose (*mg/dl*)				
25 d	136±7 (7)	134±4 (8)	146±6 (7)	138±4 (8)
4 wk	139±3 (7)	140±7 (8)	141±3 (7)	154±4 (8)*
6 wk	149±4 (9)	148±5 (10)	150±3 (8)	166±6 (10)*
Plasma insulin (*ng/ml*)				
25 d	2.48±0.25 (7)	3.14±0.49 (8)	2.30±0.36 (7)	2.00±0.23 (8)
4 wk	2.03±0.20 (7)	3.37±0.70 (8)	2.13±0.26 (7)	2.15±0.11 (8)
6 wk	2.03±0.43 (9)	2.81±0.90 (10)	1.63±0.28 (9)	1.89±0.35 (11)

Results are expressed as mean±SEM. The values in parentheses are the number of samples measured. Statistical significance was determined using a one-way analysis of variance; all groups were compared with the sham rats given tap water. *$P < 0.05$.

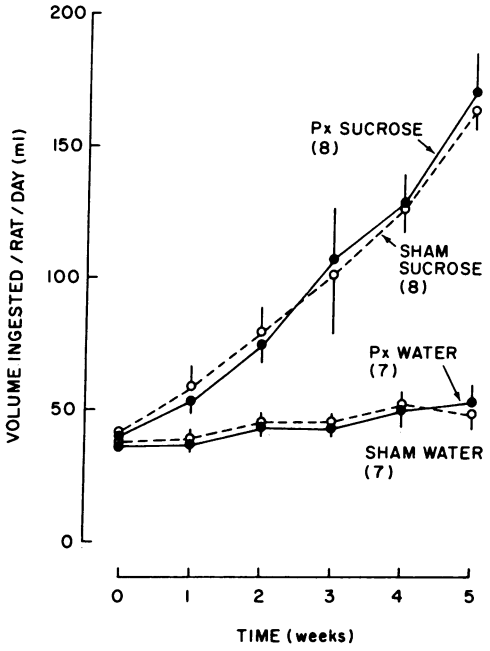

Figure 1. Volume of fluid ingested per day averaged over weekly intervals.

of tap water which did not appreciably change over the test period even though body weight nearly tripled. In contrast, both sucrose groups consumed equal volumes that progressively increased to ~ 150 ml/d.

Islet morphometrics and pancreatic insulin content. B cell mass in Px-WAT averaged 55% of that in shams and non–B cell mass was 43% (Table II). Pancreatic weight and insulin content were similarly reduced which resulted in identical amounts of stored insulin per milligram of B cells in the two tap water groups (12.8±1.1 μg/mg B cells in Px-WAT vs. 13.4±0.8 in sham-WAT).

Sucrose did not significantly alter any of these parameters in shams. In contrast, B and non–B cell masses in Px markedly increased, reaching values close to those of both sham groups. On the other hand, pancreatic content was unchanged so that the amount of insulin stored per milligram of B cells was less than the other groups (7.1±0.5 μg/mg B cells).

Insulin responses to varying glucose concentrations assessed with the in vitro perfused pancreas. The perfusion protocol is shown at the top of Figs. 2 and 3. The baseline perfusate contained 7.8 mM glucose which was decreased to 2.8 mM for 10 min and increased to 27.7 mM for 15 min. Results obtained after 4 wk are shown in Fig. 2 while those after 6 wk are in Fig. 3.

As expected, in shams given tap water for 4 wk, insulin release was fully suppressed during 2.8 mM glucose and was followed by a marked biphasic response to 27.7 mM (Fig. 2). The results in the sham-SUC group were very similar. In contrast, the insulin levels attained during the high glucose perfusate in Px-WAT were considerably less resulting in a 50% reduction in the incremental response (410±53 ng in Px-WAT vs. 741±114 in sham-WAT, $P < 0.03$). Identical results were found in Px-SUC.

When studied after 6 wk, insulin secretion in the two sham groups was again stimulated equally by 27.7 mM glucose (Table III), and the response was again reduced by 50% in Px-WAT. However, this time, an even further decrease was found in Px-SUC (280±56 ng in Px-SUC vs. 538±69 in Px-WAT, $P < 0.02$) so that the incremental response per milligram of B cells was considerably less than that found in the other groups (31±6 ng/mg B cells Px-SUC, 101±13 Px-WAT, 123±17 sham-WAT, 123±11 sham-SUC).

Glucose potentiation of arginine-induced insulin secretion assessed with the perfused pancreas. The perfusion protocol is shown at the top of Figs. 4 and 5. 10 mM arginine was added for 5 min to the baseline perfusate that contained 2.8 mM glucose. After reequilibration at 2.8 mM glucose, the glucose concentration was increased to 16.7 mM and arginine was added again. Results were obtained after 4 (Fig. 4) and 6 (Fig. 5) wk.

As expected, in the shams studied after 4 wk of tap water, the insulin response to arginine at the high glucose background far exceeded that found at a low glucose background (Fig. 4). When given sucrose, arginine-induced release at 2.8 mM glucose was unchanged while that at 16.7 mM more than doubled (Table IV). In both Px groups, results were similar to the sham-WAT rats.

At the end of the 6-wk test period, the two sham groups now had very similar results (Fig. 5). In both Px groups, arginine-induced insulin secretion at 2.8 mM glucose was similar to that of the shams (Table IV); in contrast, the response at the high glucose background was reduced by 50% in Px-WAT and even a little more in Px-SUC, although the incremental values were not statistically different (384±72 ng in Px-WAT vs. 247±49 in Px-SUC, P = NS). When expressed per milligram of B cells, the insulin response caused by arginine at 16.7 mM

Table II. Islet Morphometric Results and Pancreatic Insulin Content

	Sham		Px	
	Tap water	10% sucrose	Tap water	10% sucrose
B cell mass (*mg*)	9.65±0.73 (6)	10.8±0.60 (6)	5.32±0.44 (6)*	8.99±1.66 (6)
Non–B cell mass (*mg*)	3.72±0.23 (6)	3.90±0.05 (6)	1.67±0.18 (6)*	3.19±0.64 (6)
Pancreas weight (*g*)	1.39±0.07 (6)	1.28±0.08 (6)	0.70±0.03 (6)*	0.69±0.03 (6)*
Insulin content (*μg*)	129±7.8 (9)	127±10 (10)	67.9±5.7 (8)*	64.1±4.1 (10)*

Results are expressed as mean±SEM. The values in parentheses are the number of samples measured. B and non–B cell masses were measured using point-counting morphometrics. Insulin content was measured in other pancreases obtained after the perfused pancreas studies. Statistical significance was determined using a one-way analysis of variance; all groups were compared with the sham rats given tap water. *$P < 0.05$.

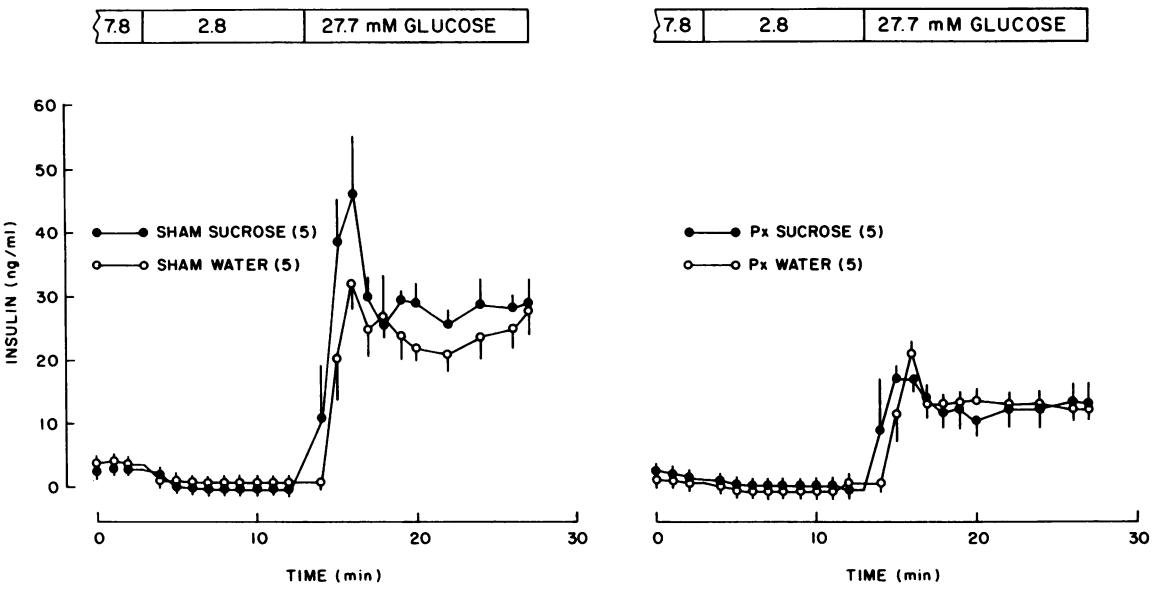

Figure 2. Effects of an acute reduction and then increase in perfusate glucose concentration on insulin secretion. Rats underwent a Px at 5 wk of age or were sham-operated, and then were given tap water or 10% sucrose ad lib. for 4 wk.

glucose in Px-WAT was equal to the response in the sham groups (72±14 ng/mg B cells Px-WAT, 81±20 sham-WAT, 60±8 sham-SUC), but it was reduced in Px-SUC (27±5 ng/mg B cells, $P < 0.0007$ vs. Px-WAT).

Discussion

The results of this study do not show any long-term effects of the 60% pancreatectomy itself that alters the function of the remaining B cells: pancreatic insulin content in Px-WAT was reduced in proportion with the B cell loss as were the insulin responses to 27.7 mM glucose and to arginine when given at the high glucose background. This contrasts with a 90% pancreatectomy where glucose-induced insulin secretion is virtually absent in the face of a B cell mass that (because of regeneration) averages 40% of that in controls (8). Unlike a 60% pancreatectomy, the 90% model develops hyperglycemia with the nonfasting plasma glucose concentration increasing 40 mg/dl, and we have proposed that chronic exposure to this increase is what causes the B cell secretory abnormalities (1, 8).

The present results strongly support this concept, for the addition of sucrose to the water supply caused mild hyperglycemia in Px, and this was associated with a decrease in the insulin response to 27.7 mM glucose, even though pancreatic

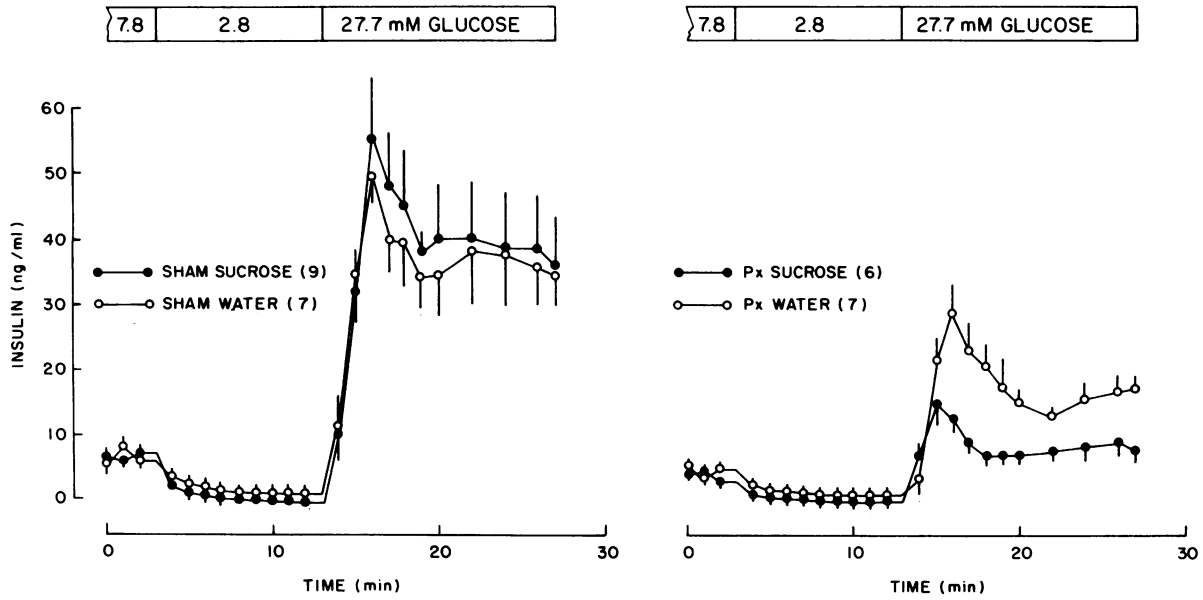

Figure 3. Effects of an acute reduction and then increase in perfusate glucose concentration on insulin secretion. Rats underwent a Px at 5 wk of age or were sham-operated, and then they were given tap water or 10% sucrose ad lib. for 6 wk.

Table III. Insulin Responses to 27.7 mM Glucose Assessed with the In Vitro Perfused Pancreas

	Insulin release		
	2.8 mM glucose	Incremental response 27.7 mM glucose	Incremental response 27.7 mM glucose/ mg B cells
	ng/min	*ng/15 min*	*ng/15 min*
4 wk			
Sham tap water (5)	0.54±0.27	741±114	
Sham 10% sucrose (5)	0.30±0.03	913±76	
Px tap water (5)	0.40±0.13	410±53*	
Px 10% sucrose (5)	0.36±0.09	395±74*	
6 wk			
Sham tap water (7)	0.60±0.15	1,190±162	123±17
Sham 10% sucrose (9)	0.42±0.10	1,330±221	123±11
Px tap water (7)	0.54±0.29	538±69*	101±13
Px 10% sucrose (6)	0.52±0.30	280±56*	31±6*

Insulin release is expressed as mean±SEM. The value at 2.8 mM glucose is the amount released during the last minute of that perfusate. The incremental response to 27.7 mM is the amount released above that baseline during the 15-min infusion period; see text for details. Statistical significance was determined using a one-way analysis of variance; all results were compared with the sham rats given tap water. * $P < 0.05$.

content was unchanged and B cell mass nearly doubled. Also, the ability of glucose to potentiate arginine-induced insulin secretion was markedly reduced when B cell mass was taken into account. One might wonder if a dietary change and not hyperglycemia caused the secretory defects. Although we did not measure how much chow was consumed by the different groups, others have shown using similar systems in normal rats that it is reduced by a third while total calorie intake is 20% higher (14, 15), which results in an overall decrease in protein and fat intake while carbohydrate intake is increased. It has been clearly demonstrated that a low protein-hypocaloric diet tends to depress insulin secretion (16, 17), but when carbohydrate is added to make up the missing calories, secretory responses are unchanged (18). High sucrose diets have also been shown by some investigators to decrease glucose tolerance in normal rats (19–21), but this remains controversial (22–24). More germane to our results, a recent study failed to find any decrease in glucose-induced insulin secretion assessed with the perfused pancreas when normal rats were given a high sucrose diet for a month (25). The most compelling evidence against diet being the cause of the secretory defects in Px-SUC comes from the sham-SUC group; insulin responses remained fully intact despite identical intake of sucrose.

Therefore, we suggest that chronic exposure of the remaining B cells to the higher than normal plasma glucose concentration is what caused the secretory defects. A large body of literature already supports the notion that chronic hyperglycemia impairs B cell function (1, 2, 26); this study is noteworthy because it shows that a very small change in glucose homeostasis also has this effect (nonfasting plasma glucose level was

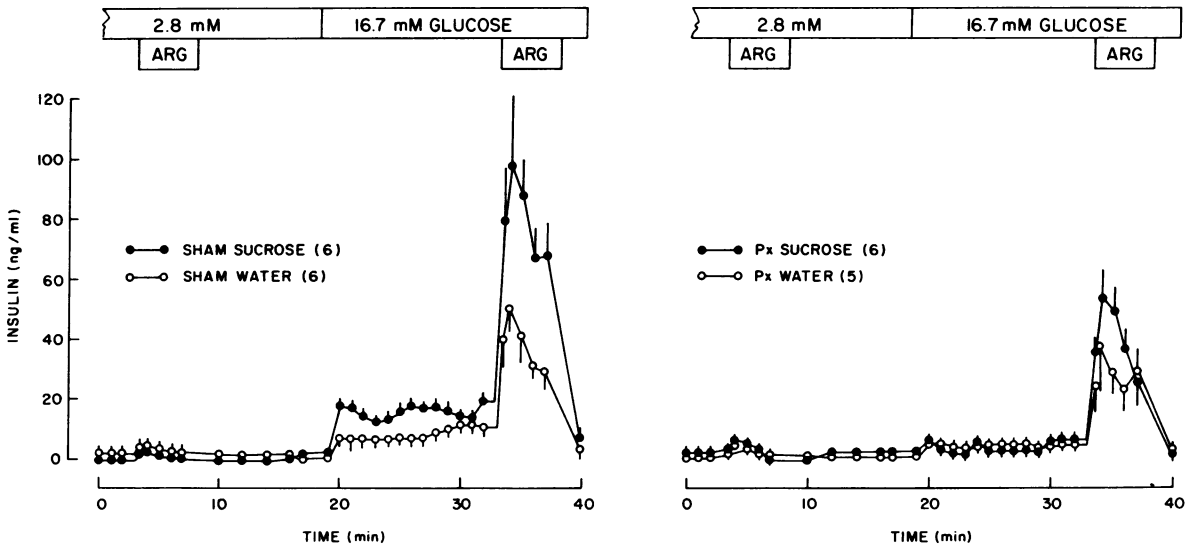

Figure 4. Effects of glucose and 10 mM arginine on insulin secretion. Rats underwent a Px at 5 wk of age or were sham-operated, and then they were given tap water or 10% sucrose ad lib. for 4 wk.

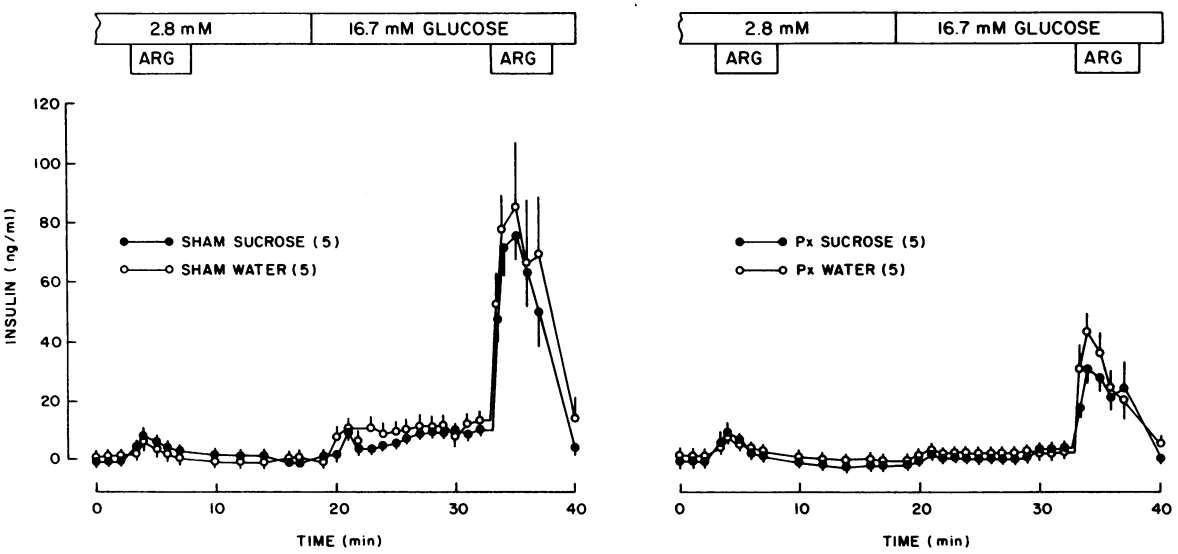

Figure 5. Effects of glucose and 10 mM arginine on insulin secretion. Rats underwent a Px at 5 wk of age or were sham-operated, and then they were given tap water or 10% sucrose ad lib. for 6 wk.

raised only 15 mg/dl). We have previously observed in rats given streptozotocin as neonates that glucose-induced insulin secretion was lost at a time when their glucose level was only slightly elevated (27). However, an attempt to establish a link between the two, using 48-h in vivo glucose infusions in normal rats, was not successful; rats whose plasma glucose was raised 18 mg/dl had no loss of glucose-induced insulin secretion while it was markedly blunted in those who were made more hyperglycemic (2). One explanation could be that it requires a longer period for functional abnormalities to evolve when hyperglycemia is very mild. Possibly consistent with this suggestion is the fact that the insulin responses to 27.7 mM glucose in the Px groups were not different at the end of the 4th wk, a time when hyperglycemia was already well established (plasma glucose levels obtained at the beginning of the perfusion surgery showed the expected 15 mg/dl increase), but marked differences were found 2 wk later.

It has been hypothesized that sustained hyperglycemia has another effect on the islet, namely to accelerate islet growth by increasing the number of cells undergoing mitosis (1). This is based on the fact that glucose is a well-known stimulus for in vitro B cell replication (28), and also because considerable B and non-B cell regeneration has been observed after a 90% pancreatectomy (8) that was associated with a marked increase

Table IV. Insulin Responses to 10 mM Arginine Given at 2.8 and 16.7 mM Glucose Assessed with the In Vitro Perfused Pancreas

	Insulin release		
	Incremental response: 10 mM arginine + 2.8 mM glucose	Incremental response: 10 mM arginine + 16.7 mM glucose	Incremental response: 10 mM arginine + 16.7 mM glucose/ mg B cells
	ng/5 min	*ng/5 min*	*ng/5 min*
4 wk			
Sham tap water (6)	19.2±5.0	355±57	
Sham 10% sucrose (6)	11.9±3.7	740±143*	
Px tap water (5)	19.7±12.1	307±88	
Px 10% sucrose (6)	20.7±3.1	406±77	
6 wk			
Sham tap water (5)	34.7±14.1	786±196	81±20
Sham 10% sucrose (5)	65.4±12.0	649±85	60±8
Px tap water (5)	53.0±11.2	384±72	72±14
Px 10% sucrose (5)	50.4±18.5	247±49*	27±6*

Insulin release is expressed as mean±SEM. The incremental responses are the total amount of insulin released above the baseline established at the end of the 2.8 or 16.7 mM glucose perfusates; see text for the method of calculation. Statistical significance was determined using a one-way analysis of variance; all results were compared with the sham rats given tap water. * $P < 0.05$.

in B cell mitotic frequency (non–B cell mitotic frequency cannot be measured with the same technique due to the smaller populations of cells) (29). Even more direct evidence has recently been obtained, for we have found a 50% increase in B cell mass when normal rats are made markedly hyperglycemic for 96 h with in vivo glucose infusions (30). The present results further support this concept, showing B and non–B cell masses in Px-SUC that were considerably increased over Px-WAT. On the other hand, it is likely that other factors also influence islet-cell growth after a partial pancreatectomy, for we have observed a 20% increase in islet mass 3 wk after a 40% pancreatectomy, despite there being no change in glucose homeostasis (Lee, H. C., J. L. Leahy, S. Bonner-Weir, and G. C. Weir, manuscript submitted for publication). Consistent with this possibility, B cell mass in Px-WAT was 55% of that in shams instead of the expected 40%.

In contrast to the marked B cell regeneration in Px-SUC, insulin content did not increase, which resulted in less stored insulin per milligram of B cells. Although the mechanism for this is unknown, it may reflect an inhibitory effect of hyperglycemia on insulin synthesis (31), although there is some question as to whether such an effect always occurs (32).

In summary, we have described experiments that allow study of the consequences of a reduction in B cell mass alone, and those that result from superimposed, very mild hyperglycemia. When islet mass is reduced to a level that does not cause hyperglycemia, some regeneration occurs and the function of the remaining B cells remains fully intact. Therefore, insulin content is reduced in proportion to the B cell loss as is the amount of insulin released to a marked stimulus. In contrast, if even very mild hyperglycemia develops, islet growth increases with further regeneration of both B and non–B cells. However, B cell function eventually becomes impaired as characterized by secretory defects and reduced insulin stores. Then, the absolute amount of insulin released to a marked stimulus depends upon the balance between how much B cell mass has grown vs. the severity of the B cell defects. For instance, the ability of 16.7 mM glucose to potentiate arginine-induced insulin secretion in the 6-wk Px groups was not different when viewed in absolute terms, but it was inappropriately low in Px-SUC when B cell mass was taken into account. It has been suggested that the ability of a high glucose concentration to potentiate arginine-induced insulin secretion directly depends upon B cell mass and that its measurement can be used clinically to detect subtle degrees of B cell damage (33, 34). Our results are in agreement with this, but only in the absence of hyperglycemia.

These results provide further evidence that mild chronic hyperglycemia resulting from a reduced B cell mass is a critical determinant of impaired B cell function. This concept may be germane to all forms of diabetes. The most analogous situation is insulin-dependent diabetes mellitus in which it has been found that glucose-induced insulin secretion is impaired even in the very early stages when hyperglycemia is not obvious (35, 36). At this point in the evolution of insulin-dependent diabetes mellitus, B cell mass is presumed to be reduced and the plasma glucose levels are considerably below those required for the diagnosis of diabetes. Although unproven, one must suspect that the glucose levels in these individuals are increased enough to initiate and maintain abnormal B cell function. The pathophysiology is more obvious in non–insulin-dependent diabetes mellitus, where a growing body of evidence supports the concept that chronic hyperglycemia, even if very mild, is responsible for the observed secretory defects (1, 3, 4).

Acknowledgments

The authors wish to thank Henrietta Cooper and Deanna Deery for expert technical assistance.

The work was supported by grants AM-35449 and AM-38543 from the National Institutes of Health and 185296 from the Juvenile Diabetes Foundation. Dr. Leahy is the recipient of a Research and Development Award from the American Diabetes Association.

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