Original Articles

Diazoxide Causes Recovery of β-Cell Glucose Responsiveness in 90% Pancreatectomized Diabetic Rats

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Chronic hyperglycemia causes near-total disappearance of glucose-induced insulin secretion. The etiology has been suggested to be a nonsustainable stimulation of insulin release that causes β-cells to become unresponsive to glucose through an undefined mechanism. We used an inhibitor of insulin secretion, diazoxide, to test this hypothesis in 90% pancreatectomized (Px) rats. Px rats were given 5 days of diazoxide (30 mg/kg orally twice a day) or tap water starting on postoperative day 8, 15, or 22. In vitro pancreas perfusions were conducted 36 h posttreatment (2, 3, or 4 weeks after surgery) using a protocol of 15 min of 16.7 mM glucose followed by 15 min of 16.7 mM glucose plus 10 mM arginine. In 2-week Px rats, insulin responses to 16.7 mM glucose and to glucose/arginine were both appropriate for the reduced β-cell mass, i.e., no defect in β-cell glucose responsiveness had yet occurred. Diazoxide had no affect on insulin release at this time. Between 2 and 3 weeks after pancreatectomy, insulin output to 16.7 mM glucose fell 75%, and that to glucose/arginine fell 50%. Diazoxide given at this time partially blocked the fall in glucose-induced insulin secretion and totally prevented that with arginine. The increased insulin secretion caused by diazoxide was accompanied by *1*) lower nonfasting plasma glucose values, *2*) improved glucose tolerance after oral glucose load, and *3*) a 50% increase in pancreatic insulin content. Our results support the concept that excessive insulin secretion is a major cause of the hyperglycemia-induced loss of β-cell glucose responsiveness. A leading candidate for the mechanism of this effect is depleted pancreatic insulin stores. Overstimulation of insulin secretion provides a new target for pharmacological therapy aimed at reducing glucose intolerance in non-insulin-dependent diabetes mellitus. *Diabetes* 43:173–79, 1994

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Px, pancreatectomized; OGTT, oral glucose tolerance test; RIA, radioimmunoassay; NIDDM, non-insulin-dependent diabetes mellitus; .

Normally, the plasma glucose concentration controls insulin secretion through its actions to directly stimulate insulin release and also to modulate insulin output from other secretagogues (1). Chronic hyperglycemia disrupts this relationship such that β-cells become blind to changes in glycemia (2,3). The cellular mechanisms responsible for this effect are incompletely understood (3). Experimental animals with mild degrees of hyperglycemia (plasma glucose values raised <1 mM) have the full-blown secretory defect (4–6). As such, any hypothesis must explain how small increases in glycemia cause β-cell dysfunction.

We recently observed another insulin secretion defect in rats made diabetic by streptozocin administration during the neonatal period. The dose-response curve for glucose-induced insulin secretion was shifted to the left so that half-maximal insulin secretion occurred at 8.3 vs. 13.2 mM glucose in control rats (7). In addition, Sako and Grill (8) reported prevention of the defective glucose-induced insulin secretion in rats made hyperglycemic with glucose infusions when diazoxide, an inhibitor of insulin secretion, was coinfused. They concluded that excessive stimulation of insulin secretion was one mechanism for hyperglycemia-induced β-cell dysfunction.

Based on these results, we hypothesized that chronic hyperglycemia causes a cascade of functional changes in the β-cell. First, the sensitivity for glucose increases such that mild to moderate levels of hyperglycemia cause β-cells to function as if the glycemia were worse than it actually is. This overactivity leads to the next step in the process, the intracellular events that cause glucose-induced insulin secretion to become impaired. We recently tested the first part of this hypothesis, that a β-cell hypersensitivity for glucose precedes the loss of glucose-induced insulin secretion in diabetic rats (9). The diabetic model was rats with a 90% pancreatectomy.

Plasma glucose values in these rats rise 1–2 mM above normal within a week of the surgery (10). Also, the remaining β-cells are known to undergo the typical functional changes seen with chronic hyperglycemia (10,11), although the earliest time point studied until then was 4 weeks after surgery (12). The aforementioned study (9) used rats one week after a 90% pancreatectomy when glucose-stimulated insulin secretion was still intact. Dose-response curves for glucose-induced insulin secretion (half-maximal insulin output at 5.7 vs. 16.5 mM glucose in shams) and glucose potentiation of arginine-induced insulin secretion (half-maximal insulin output at 3.5 vs. 14.8 mM glucose in shams) had left shifts in the pancreatectomized (Px) rats. Extrapolating these in vitro data to the in vivo state, Px β-cells at their usual level of glycemia secreted at 90% of their secretory capacity versus just 10% in normal rats.

This study tests the second part of the hypothesis, that this overactivity of secretory function contributes to the later impairment in glucose-induced insulin secretion. The experimental design consisted of studying the effects of diazoxide treatment on insulin secretory responses in 90% Px rats. Diazoxide is an inhibitor of insulin secretion with a long history of use in humans as a treatment for hypoglycemia (13). Its major action is to open the ATP-sensitive K^+ channels and prevent β-cell depolarization (14,15), although inhibitory effects on mitochondria also have been reported (16). Diazoxide was chosen over the multitude of other inhibitors of insulin secretion because of the report by Sako and Grill (8) showing maintenance of normal glucose-induced insulin secretion in rats made hyperglycemic with glucose infusions when diazoxide was coinfused. However, these authors obtained no recovery of basal glucose-induced insulin secretion in diabetic rats with preexisting β-cell glucose unresponsiveness when given the same diazoxide protocol (48 h intravenous diazoxide) (17). We therefore decided to use a longer treatment period, which required oral administration of the drug. The half-life of oral diazoxide in humans is 28 h, because of protein binding (18). Unfortunately, comparable pharmacological data for rats were not found. As such, the protocol was arbitrary: twice a day dosing of diazoxide or water for 5 days followed by a 36-h waiting period before study with the perfused pancreas to ensure total resolution of the inhibitory effect of diazoxide on insulin secretion. Treatment was administered during one of three periods; some rats were given diazoxide before the β-cell glucose unresponsiveness, others after. Therefore, in addition to studying prevention and/or reversibility of the secretory dysfunction by diazoxide, a second aspect to the study was that insulin responses to glucose and a nonglucose secretagogue (arginine) were systematically mapped out in water-treated 90% Px rats up to the development of the full-blown loss of β-cell glucose responsiveness. As such, the results provide insight into the evolution of the secretory behavior of β-cells exposed to persistently elevated glucose concentrations, a topic that has received relatively little study.

RESEARCH DESIGN AND METHODS

Partial pancreatectomy rat model. Ninety percent pancreatectomies were performed on 5-week-old (100-g) male Sprague-Dawley rats (Taconic, Germantown, NY) using the method of Bonner-Weir et al. (10). During intraperitoneal pentobarbital sodium anesthesia (100 mg/kg), 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 libitum.

Diazoxide treatment. Diazoxide oral suspension (Proglycem, Baker Cummins, Miami, FL) (30 mg/kg) was administered twice a day by gavage tube on postoperative days 8–12 (5 days). Other Px rats received tap water. Nonfasting plasma glucose values were measured in blood obtained by tail snipping at 0900 on days 8 (pretreatment) and 13 (posttreatment). In vitro pancreas perfusions were performed on day 14. Additional groups of rats were treated on days 15–19 (perfusion day 21) and 22–26 (pancreas perfusion day 28). Groups are designated by the time that perfusion studies were conducted (2, 3, or 4 weeks after pancreatectomy).

Oral glucose tolerance test (OGTT). Ninety percent Px rats were treated as above with diazoxide or water on days 22–25 (4 days). Age-matched normal rats were treated in parallel with water. The morning of day 26, after an overnight fast, 3.5 g/kg glucose (1.0 g/ml solution) was given by gavage tube. Blood for plasma glucose was obtained by tail snipping at 0, 30, 60, and 120 min. Pancreases were excised and homogenized in cold acid ethanol. After overnight incubation at 4°C, they were stored at −20°C pending radioimmunoassay (RIA) for insulin content.

In situ perfused pancreas. This technique has been described elsewhere (19). The perfusate was a Krebs-Ringer bicarbonate buffer, pH 7.4, plus 4% dextran T_{70} (Sigma, St. Louis, MO), 2 mM Ca^{2+}, 1.2 mM Mg^{2+}, and 0.2% bovine serum albumin fraction V (Sigma). Flow rate was 3 ml/min. After cannulation of the aorta and portal vein, the body cavity was covered with gauze soaked in saline and maintained at 36–39°C by a heat lamp. The protocol began with a 20-min equilibration. Thereafter, 1-min samples were collected in tubes containing 8 mg EDTA and kept on ice pending storage at −20°C. The protocol was 5.5 mM glucose during the equilibration followed by 15 min of 16.7 mM glucose and 15 min of 10 mM arginine/16.7 mM glucose.

Analytical methods. Plasma glucose was measured with a Beckman Glucose Analyzer II (Beckman, Brea, CA). Insulin concentrations were determined by RIA using charcoal separation (20) and rat insulin standards (Lilly, Indianapolis, IN).

Data presentation and statistical methods. Data are expressed as means ± SE. Values for insulin output in Table 2 are the mean insulin concentration of all samples collected during the designated perfusate condition. No

TABLE 1
Body weight and nonfasting plasma glucose values in diazoxide-treated Px rats

	Weight (g)			Plasma glucose (mM)		
	2 weeks	3 weeks	4 weeks	2 weeks	3 weeks	4 weeks
Water-treated Px rats	197 ± 12	241 ± 13	285 ± 17	9.6 ± 0.7	12.7 ± 2.4	10.1 ± 0.8
n	4	5	3	4	5	3
Diazoxide-treated Px rats	200 ± 16	226 ± 7	280 ± 9	11.0 ± 0.7	9.3 ± 0.7	8.2 ± 0.6
n	5	4	5	5	4	5

Data are means ± SE; *n*, number of animals in each group. Nonfasting plasma glucose values and body weights were measured in 90% Px rats after a 5-day treatment of diazoxide (30 mg/kg orally twice a day) or water. The treatment period was postoperative days 8–12 (data obtained day 13), 15–19 (data obtained day 20), and 22–26 (data obtained day 27).

appreciable differences were noted in the secretory results obtained at 3 and 4 weeks after pancreatectomy. As such, the results have been combined into a single group (3–4 weeks). Statistical significance was determined with the two-tailed, unpaired Student's *t* test.

RESULTS

General characteristics of 90% Px rats. The pancreatic surgery was performed in 100 g rats. Body weight rose thereafter at 40–50 g per week with no differences in the water-treated versus diazoxide-treated rats (Table 1). Nonfasting plasma glucose values averaged 9.6–12.7 mM in water-treated Px rats. Diazoxide had no affect on this hyperglycemia (glucose values in normal rats averaged 8 mM) when it was given up to 2 weeks after surgery. Surprisingly, thereafter, nonfasting plasma glucose values at the end of the treatment period were lowered by diazoxide (8.7 ± 0.4 vs. 10.4 ± 0.5 mM glucose in 3- to 4-week diazoxide-treated and water-treated Px rats, respectively, $P < 0.024$).

OGTT. Px rats treated with diazoxide or water on postoperative days 22–25 underwent an OGTT on the morning of day 26 (Fig. 1). Normal rats treated with water were studied in parallel. In the normal rats, plasma glucose peaked 30 min after the glucose load at 10.5 ± 0.6 mM, then gradually fell to 7.2 ± 0.8 mM by 120 min. Both Px groups had raised fasting plasma glucose values (5.6 ± 0.1 in the normal rats vs. 6.9 ± 0.3 mM in the water-treated Px rats, $P < 0.018$, and 6.8 ± 0.4 mM in the diazoxide-treated Px rats, $P < 0.023$). Water-treated Px rats were grossly glucose intolerant; plasma glucose at 30 min was 17.6 ± 1.3 mM and remained elevated at 120 min (13.4 ± 1.8 mM, $P < 0.019$ vs. the normal rats). In the diazoxide-treated Px rats, plasma glucose was elevated at 30 min (15.9 ± 1.1 mM, $P < 0.005$ vs. normal rats) but was back to normal at 120 min (6.8 ± 0.4 mM).

Insulin secretion assessed by the in situ perfused pancreas. The perfusion protocol of 15 min of 16.7 mM glucose followed by 15 min of 16.7 mM glucose/10 mM arginine was performed 2, 3, and 4 weeks after a 90% pancreatectomy. Insulin secretory responses in the control Px group (water-treated) underwent notable changes during this period. Two weeks after surgery, a clear, biphasic insulin response to the high glucose was observed with a further 6.2 ± 1.1-fold rise in insulin output when arginine was added (Fig. 2). By 3 weeks, insulin release to 16.7 mM glucose had fallen 75% (360 ± 85 pM at 2 weeks, $n = 4$, vs. 89 ± 26 pM at 3 weeks, $n = 5$, $P < 0.01$), and insulin release to arginine had fallen nearly 50% (2,049 ± 555 pM at 2 weeks vs. 1,184 ± 287 pM at 3 weeks, NS). Because the percentage fall in the response to 16.7 mM glucose was larger than the fall with arginine, an increase in the fold insulin response to arginine versus that to high glucose alone was observed (17.9 ± 4.3, $P < 0.05$ vs. 2-week water-treated Px rats). Diazoxide had no effect on insulin release when given up to 2 weeks after surgery; insulin output to glucose and to arginine were identical in the water-treated and diazoxide-treated groups (Table 2). In contrast, in the 3- to 4-week groups, diazoxide partially prevented the fall in insulin release to 16.7 mM glucose and totally prevented the fall to arginine (Fig. 2). Viewed together, the 3- to 4-week results showed a near-identical 120% increase in the insulin responses to 16.7 mM glucose (213 ± 38 vs. 99 ± 22 pM in diazoxide-treated and water-treated Px rats, respectively, $P < 0.024$) and to 16.7 mM glucose/10 mM arginine (2,594 ± 217 vs. 1,172 ± 236 pM in diaz-

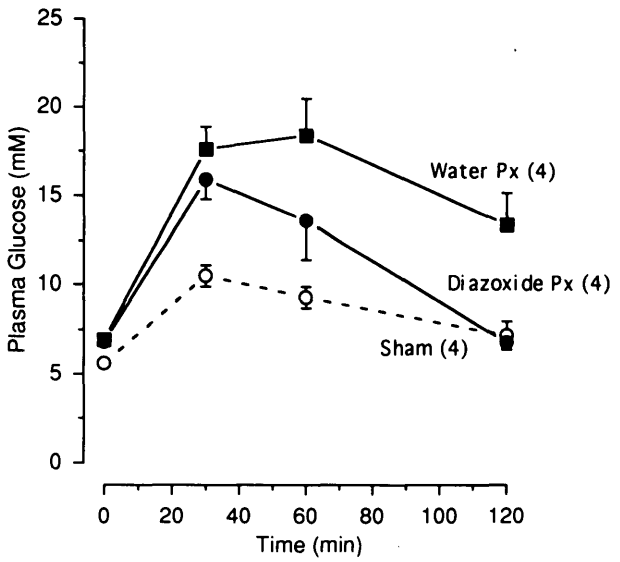

FIG. 1. Plasma glucose values during an OGTT. Px rats were treated with 30 mg/kg diazoxide (●—●) or water (■—■) twice a day by gavage tube on postoperative days 22–25. Age-matched normal rats were treated in parallel with water (○—○). The morning of day 26, after an overnight fast, 3.5 g/kg glucose was given by gavage tube. Blood for plasma glucose was obtained by tail snipping at 0, 30, 60, and 120 min.

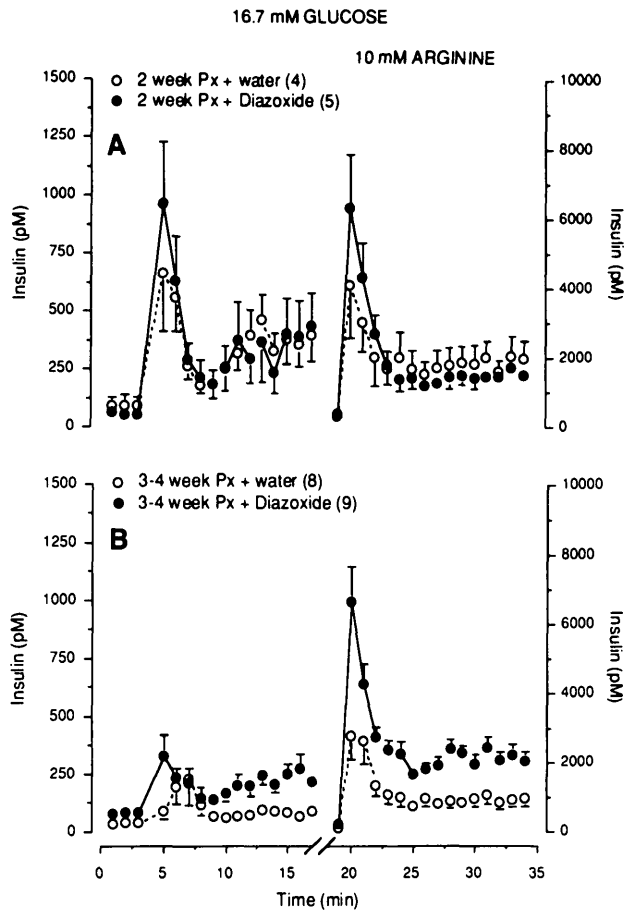

FIG. 2. Insulin secretion assessed with the in vitro perfused pancreas. Rats underwent a 90% pancreatectomy. Diazoxide (30 mg/kg) (●—●) or water (○—○) was administered twice a day by gavage tube on postoperative days 8–12 followed by pancreas perfusion on day 14 (*A*). Additional groups of rats were treated with diazoxide or water on postoperative days 15–19, pancreas perfusion day 21; or postoperative days 22–26, pancreas perfusion day 28 (results are combined in *B*). Note the separate *y* axes for the insulin responses to 16.7 mM glucose and to 16.7 mM glucose plus 10 mM arginine.

oxide-treated and water-treated Px rats, respectively, $P < 0.001$). Because the proportionate increases in these responses were identical, no change occurred in the fold rise of insulin output to arginine over that to the high glucose alone (15.1 ± 3.1 vs. 14.2 ± 3.2 pM in diazoxide-treated and water-treated Px rats, respectively).

Pancreas insulin content. Pancreas insulin content was measured in the rats that underwent the oral glucose challenge. As expected, the remnant pancreas of water-treated Px rats contained a small fraction (25%) of the insulin content of a normal pancreas (2.80 ± 0.36 vs. 11.30 ± 1.41 nmol Px and normal rats, respectively, $P < 0.001$). Diazoxide increased insulin content in the 90% Px rats by 50% (4.26 ± 0.46 nmol, $P < 0.05$ vs. water-treated Px rats) (Fig. 3).

DISCUSSION

The plasma glucose concentration controls insulin secretion through a direct effect and by modulating insulin responses to other secretagogues (1). Considerable experimental evidence has shown that chronic hyperglycemia makes β-cells glucose unresponsive (2,3). This toxic effect of hyperglycemia was clearly seen in our study; insulin output to 16.7 mM glucose fell precipitously in the water-treated Px rats between 2 and 3 weeks after the surgery. This study in combination with another study (9) has furthered our understanding of this functional defect by mapping patterns of insulin release in Px rats at weekly intervals after the surgery. One week post-Px was characterized by left-shifted curves for glucose-induced insulin secretion and glucose potentiation of arginine-induced insulin output (9). This increase in glucose sensitivity when combined with the rat's hyperglycemia caused the remaining β-cells to secrete insulin at 90% of their capacity versus the normal 10–20%. Sometime later, a near-total reduction in glucose-induced insulin secretion occurred, although our results on that point are somewhat contradictory. We previously observed a large fall in the insulin response to 16.7 mM glucose between 1 and 2 weeks after surgery, whereas the insulin re-

TABLE 2
Insulin secretion in diazoxide-treated Px rats

Time postsurgery	*n*	Insulin secretion (pM) 16.7 mM glucose	Insulin secretion (pM) 16.7 mM glucose plus 10 mM arginine	Fold response arginine/glucose
2 weeks				
Water-treated Px rats	4	360 ± 85	2049 ± 555	6.2 ± 1.1
Diazoxide-treated Px rats	5	386 ± 121	2055 ± 305	7.8 ± 2.1
3–4 weeks				
Water-treated Px rats	8	99 ± 22*	1172 ± 236†	14.2 ± 3.2
Diazoxide-treated Px rats	9	213 ± 38‡	2594 ± 217§	15.1 ± 3.1

Data are means ± SE; *n*, number of animals in each group. Mean insulin responses to 15 min of 16.7 mM glucose and 15 min of 16.7 mM glucose plus 10 mM arginine measured in 90% Px rats using the in vitro perfused pancreas. Rats were treated with diazoxide (30 mg/kg orally twice a day) or water on postoperative days 8–12 (perfusion day 14), 15–19 (perfusion day 21), or 22–26 (perfusion day 28). The 3- and 4-week data were combined into a single group.
*$P < 0.002$ vs. 2-week water-treated Px rats.
†$P < 0.042$ vs. 2-week diazoxide-treated Px rats.
‡$P < 0.024$ vs. 3- to 4-week water-treated Px rats.
§$P < 0.001$ vs. 3- to 4-week water-treated Px rats.

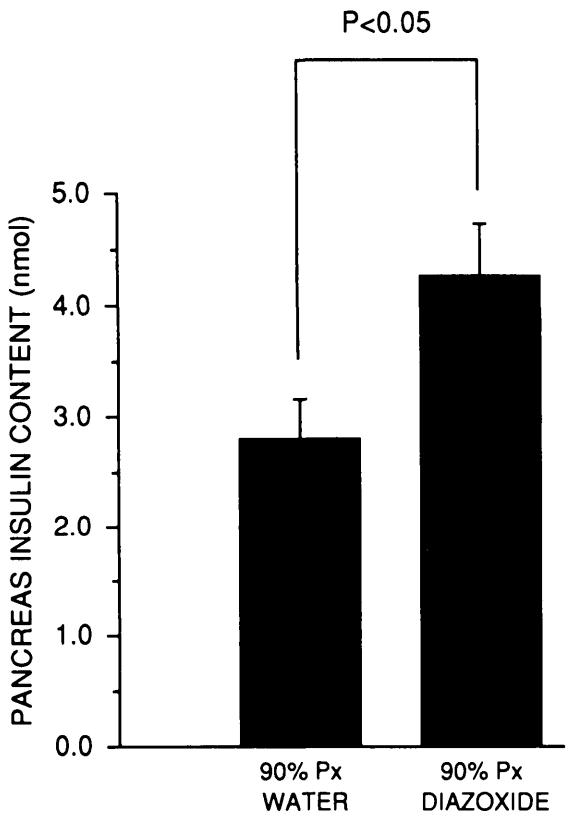

FIG. 3. Insulin content in the pancreatic remnant of 90% Px rats. Rats were treated with 30 mg/kg diazoxide or water twice a day by gavage tube on postoperative days 22–25. On day 26, after an OGTT, pancreases were excised and homogenized in cold acid ethanol. Insulin content was measured by RIA.

sponse to 16.7 mM glucose/10 mM arginine did not change (9). In our present study, instead, we found insulin responses in 2-week Px rats that were virtually identical to those noted previously at 1 week (9). Regardless, by 3 weeks, the hyperglycemia-associated defect in glucose-induced insulin output was fully established. Unexpectedly, the insulin response to 16.7 mM glucose/10 mM arginine also underwent a substantial reduction, although the large SE prevented statistical significance. Similar results have been reported in diabetic mice grafted with an insufficient number of islets to restore euglycemia; after 4 weeks of hyperglycemia, the grafted islets had impaired insulin responses to both glucose and arginine (21). These results clash with the dogma that chronic hyperglycemia impairs insulin secretion to glucose without affecting insulin secretion to nonglucose secretagogues (2,3). Instead, reductions in glucose and non-glucose-mediated insulin secretion were both observed. The second is presumed to represent a defect in glucose potentiation rather than reduced effectiveness of arginine. This idea clarifies a previous observation in Px rats, i.e., why insulin responses to 16.7 mM glucose and to 16.7 mM glucose/10 mM arginine both increased after insulin therapy (12).

Two subtle aspects to these results deserve mention. First, the fall in the insulin response to 16.7 mM glucose (75%) exceeded that to arginine (50%). As such, the ratio of insulin output to arginine/high glucose increased. The ratio in 2-week Px rats (6.1 ± 1.1) was like that of sham rats (4.7 ± 0.3) (J.L.L., unpublished observations), i.e., it was normal. Therefore, 3-week Px rats were characterized by the first appearance of impaired glucose-induced insulin secretion and the first observation of an abnormal arginine:high glucose ratio. The ratio, therefore, appears to be a marker for hyperglycemia-induced β-cell glucose unresponsiveness. Second, despite the marked decline in in vitro insulin secretion between 2 and 3 weeks after surgery, morning plasma glucose values did not change. Rats eat at night; whether postprandial levels of glycemia rose is unknown.

The question addressed in this study is whether the heightened secretory function in the Px rats played any role in the subsequent impairment in β-cell glucose responsiveness. Our results showed a protective effect of diazoxide on secretory output. This effect was observed only after the decline in insulin secretion at 3 weeks; 2-week Px rats were totally unaffected by diazoxide. The increases in insulin output to 16.7 mM glucose and to 16.7 mM glucose/arginine in the 3- to 4-week diazoxide-treated Px rats show that excessive insulin release is causing part of the reduced β-cell secretory capacity in these rats. Potential mechanisms for this effect include hyperglycemia-induced depletion or alteration of key β-cell substrates, cofactors, or regulatory enzymes. A candidate from our results is depleted insulin stores because the protective effect of diazoxide on insulin secretion was paralleled by a 50% increase in pancreatic insulin content. Additional support for this idea has come from Björklund and Grill (22) who used diazoxide to prevent the β-cell insensitivity to glucose induced by culturing or perifusing islets at a high glucose concentration. The protective effect of diazoxide was again paralleled by a higher islet insulin content. Moreover, high glucose perifusions were performed at 22°C versus the normal 37°C that blocks insulin secretion, thereby conserving insulin content without disrupting earlier steps in β-cell glucose metabolism. Under these conditions, the protective effect of diazoxide on glucose-induced insulin secretion was attenuated, supporting the need for a reduced insulin content to see the full protective effect of diazoxide on insulin output. However, an argument against this effect causing the results in this study is that the β-cells of the water-treated Px rats were not obviously degranulated. Insulin content of the remnant pancreas had risen from 10% of normal at the time of surgery to 25% of normal by the time of the experiment (4 weeks after surgery). The key question is what is the proportionate β-cell mass of the pancreatic remnant at the same time? The remnant β-cell mass undergoes compensatory growth after the surgery, rising from the initial 10% of normal to 42% of normal 8 weeks after the surgery (10). Unfortunately, intervening time periods have not been measured so that the proportionate β-cell mass at 4 weeks after surgery is not known. Even if overt degranulation is not present, depletion of the readily releasable pool of insulin could theoretically cause the reduced insulin responses (23).

One interpretation of the results in this study is that the near-total disappearance of glucose-induced insulin se-

cretion in the 3-week Px rats represents a combination of two independent defects. One defect is the well-known specific impairment in glucose-induced insulin secretion (the defect sometimes referred to as glucotoxicity). This defect is represented in the results as the portion of the insulin response to 16.7 mM glucose that was not prevented by diazoxide. Its hallmark is a raised ratio of insulin output to arginine/high glucose. Being unresponsive to diazoxide (shown by the lack of change in the ratio of insulin output to arginine/glucose in the diazoxide-treated Px rats) means that it is unrelated to excessive insulin secretion. Instead, the mechanism is presumed to be a direct inhibitory effect of the high glucose level on some critical step in glucose-induced insulin secretion. The second secretory defect is more global, reducing insulin output to glucose and nonglucose secretagogues alike. This defect was reversed by diazoxide therapy, making it dependent on excessive insulin secretion. In the Px rats, each defect contributed ~50% to the overall decline in insulin secretion at 16.7 mM glucose.

A second interpretation of the results is that the reduction in glucose-induced insulin secretion represents a single defect that was fully reversed by diazoxide. However, part of this effect was lost during the posttreatment period. The protocol was originally designed with the 36-h waiting period to allow full dissipation of the inhibitory effect of diazoxide on insulin secretion. The strategy was successful, as shown by the lack of any lowering of insulin responses in the 2-week diazoxide-treated versus water-treated Px rats. As such, what was observed in this study were the cumulative effects of 5 days of suppressed insulin secretion on β-cell secretory capacity rather than any direct effect of diazoxide. However, in retrospect, a shorter waiting period was likely possible based on the results of Gomis et al (24). They treated rats 3 times a day for 3 days with 0.33 g/kg of diazoxide. Isolated islets from these rats had normal insulin responses to glucose 10 h after the last diazoxide dose. As such, it is possible that the β-cells in the diazoxide-treated Px rats were re-exposed to as much as 24 h of hyperglycemia in the absence of a significant presence of diazoxide. We therefore cannot eliminate waning of the protective effect of diazoxide during this period, although our findings of the intact arginine response and the marked increase in insulin content at the end of the waiting period lower the concern over this possibility. Additional experiments are needed to distinguish which of these two possibilities is correct.

In summary, our results suggest a different mechanism than commonly appreciated for the loss of glucose-induced insulin secretion in diabetic rats. Until now, it has generally been accepted that the defect represents a direct inhibitory effect of chronic hyperglycemia on some critical step in β-cell glucose metabolism or some second messenger. Instead, our results indicate that a substantial portion (if not all) of the reduction in glucose-induced insulin secretion is caused by excessive insulin secretion. A second manifestation of this defect was a fall in the insulin response to 10 mM arginine plus 16.7 mM glucose. This defect may be the rat counterpart to the impairment in glucose potentiation that has been described in human non-insulin-dependent diabetes mellitus (NIDDM) (1,25). This idea is supported by data from 1976 showing substantial increases in insulin responses to glucagon and tolbutamide in NIDDM after 10 days of diazoxide treatment (26,27). To date, little has been learned in humans about the pathogenesis of this secretory defect or its reversibility. This study has suggested that depleted insulin stores may underlie it. In addition, full reversal was found with diazoxide. As such, an exciting prospect is that diazoxide or some related compound may provide a new kind of pharmacological therapy for NIDDM. Consistent with this idea was the observation of reduced nonfasting glucose values and improved glucose tolerance in the diazoxide-treated Px rats.

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REFERENCES

1. Porte D Jr: β-cells in type II diabetes mellitus. *Diabetes* 40:166–80, 1991
2. Leahy JL: Natural history of β-cell dysfunction in NIDDM. *Diabetes Care* 13:992-1010, 1990
3. Leahy JL, Bonner-Weir S, Weir GC: β-cell dysfunction induced by chronic hyperglycemia: current ideas on the mechanism of the impaired glucose-induced insulin secretion. *Diabetes Care* 15:442–55, 1992
4. Trent DF, Fletcher DJ, May JM, Bonner-Weir S, Weir GC: Abnormal islet and adipocyte function in young β-cell deficient rats with near-normoglycemia. *Diabetes* 33:170–75, 1984
5. Leahy JL, Bonner-Weir S, Weir GC: Minimal chronic hyperglycemia is a critical determinant of impaired insulin secretion after an incomplete pancreatectomy. *J Clin Invest* 81:1407–14, 1988
6. Leahy JL, Cooper HE, Weir GC: Impaired insulin secretion associated with near normoglycemia: study in normal rats with 96-h in vivo glucose infusions. *Diabetes* 36:459–64, 1987
7. Chen C, Thorens B, Bonner-Weir S, Weir GC, Leahy JL: Recovery of glucose-induced insulin secretion in a rat model of NIDDM is not accompanied by return of the β-cell GLUT2 glucose transporter. *Diabetes* 41:1320–27, 1992
8. Sako Y, Grill VE: Coupling of β-cell desensitization by hyperglycemia to excessive stimulation and circulating insulin in glucose-infused rats. *Diabetes* 39:1580–83, 1990
9. Leahy JL, Bumbalo LM, Chen C: β-cell hypersensitivity for glucose precedes loss of glucose-induced insulin secretion in 90% pancreatectomized rats. *Diabetologia*. In press
10. Bonner-Weir S, Trent DF, Weir GC: Partial pancreatectomy in the rat and subsequent defect in glucose-induced insulin release. *J Clin Invest* 71:1544–53, 1983
11. Leahy JL, Bonner-Weir S, Weir GC: Abnormal glucose regulation of insulin secretion in models of reduced β-cell mass. *Diabetes* 33:667–73, 1984
12. Leahy JL, Weir GC: β-cell dysfunction in hyperglycemic rat models: recovery of glucose-induced insulin secretion with lowering of the ambient glucose level. *Diabetologia* 34:640–47, 1991
13. Black J: Diazoxide and the treatment of hypoglycemia: an historical review. *Ann NY Acad Sci* 150:194–203, 1968
14. Henquin JC, Meissner HP: Opposite effects of tolbutamide and diazoxide on $86Rb^+$ fluxes and membrane potential in pancreatic β-cells. *Biochem Pharmacol* 31:1407–15, 1982
15. Trube G, Rorsman P, Shosaku O: Opposite effects of tolbutamide and diazoxide on the ATP-dependent K^+ channel in mouse pancreatic β-cells. *Pfluegers Arch Eur J Physiol* 407:493–99, 1986
16. MacDonald MJ: High content of mitochondrial glycerol-3-phosphate dehydrogenase in pancreatic islets and its inhibition by diazoxide. *J Biol Chem* 256:8287–90, 1981
17. Sako Y, Grill VE: Diazoxide infusion at excess but not at basal hyperglycemia enhances β-cell sensitivity to glucose in vitro in neonatally streptozotocin-diabetic rats. *Metabolism* 41:738–43, 1992
18. Anonymous: Diazoxide: a review of its pharmacologic properties and therapeutic use in hypertensive crises. *Drugs* 2:78–137, 1971

19. Weir GC, Knowlton SD, Martin DB: Glucagon secretion from the perfused rat pancreas: studies with glucose and catecholamines. *J Clin Invest* 54:1403–12, 1974
20. Albano JDM, Ekins RP, Maritz G, Turner RC: A sensitive, precise radioimmunoassay of serum insulin relying on charcoal separation of bound and free hormone moieties. *Acta Endocrinol* 70:487–509, 1972
21. Korsgren O, Jansson L, Andersson A: Effects of hyperglycemia on function of isolated mouse pancreatic islets transplanted under kidney capsule. *Diabetes* 38:510–15, 1989
22. Björklund A, Grill V: β-cell insensitivity in vitro: reversal by diazoxide entails more than one event in stimulus-secretion coupling. *Endocrinology* 132:1319–28, 1993
23. Gold G, Landahl HG, Gishizky ML, Grodsky GM: Heterogeneity and compartmental properties of insulin storage and secretion in rat islets. *J Clin Invest* 69:554–63, 1982
24. Gomis R, Plaza C, Malaisse WJ: Diazoxide-induced long-term hyperglycemia. I. Preservation of β-cell insulin-releasing capacity. *Diabetes Res* 9:183–86, 1988
25. Ward WK, Bolgiano DC, McKnight B, Halter JB, Porte D Jr: Diminished β-cell secretory capacity in patients with non-insulin-dependent diabetes mellitus. *J Clin Invest* 74:1318–28, 1984
26. Greenwood RH, Mahler RF, Hales CN: Improvement in insulin secretion in diabetes after diazoxide. *Lancet* 1:444–47, 1976
27. Greenwood RH, Mahler RF, Hales CN: Therapeutic modifications of the kinetics of insulin secretion. In *Diabetes Research Today: Meeting of the Minkowski Prize-Winners*. Renold A, Creutzfeldt W, Pfeiffer EF, Eds. Stuttgart, Schattauer-Verlag, 1976, p. 175–90