Compensatory Growth of Pancreatic β-Cells in Adult Rats After Short-Term Glucose Infusion

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The extent to which adult pancreatic β-cells can respond in vivo to a sustained glucose stimulus by increasing their mass through either hyperplasia or hypertrophy has remained unanswered. Therefore, we studied the in vivo effect of short-term (96-h) hyperglycemia on the growth of β-cells by infusing adult rats with 35 or 50% glucose or 0.45% saline. After 96 h of glucose infusion, the β-cell mass, quantified by point-counting morphometrics of immunoperoxidase-stained paraffin sections, showed a 50% increase (9.57 ± 0.87 mg, *n* = 5, 50% glucose infused; 9.50 ± 1.23, *n* = 7, 35% glucose infused; 6.15 ± 0.55, *n* = 6, 0.45% saline infused). This growth was selective for β-cells; the non–β-cell mass was unchanged. The mitotic index, measured by accumulated mitotic frequency after a 4-h colchicine treatment, increased fivefold in glucose-infused animals compared to saline-infused animals. This enhanced replication of β-cells provides evidence for increase in cell number or hyperplasia. In addition, hypertrophy of the β-cells was also quantified. Mean cell volume, determined from the mean cell cross-sectional area measured planimetrically from low-magnification electron micrographs, increased to 150% of control values after 96 h of 50% glucose infusion. Seven days after the 96-h infusion, in reversal experiments, the β-cell mass had not returned to saline-infused levels. In addition, the non–β-cell mass of glucose-infused animals had increased. The mitotic index of the β-cells of glucose-infused rats was, however, significantly lower than that of the saline controls, but the mean cell volume of the β-cells remained elevated. Thus, with a short-term in vivo stimulus, adult β-cells have a far greater capacity to respond with compensatory growth by hyperplasia and hypertrophy than has been appreciated before. Even 7 days after discontinuation of the stimulus, β-cell mass remains elevated. *Diabetes* 38:49–53, 1989

Glucose	1 mM = 18 mg/dl
Insulin	1 pM = 0.139 μU/ml

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Received for publication 25 April 1988 and accepted in revised form 6 July 1988.

Compensatory growth has been defined as the capacity of an organ to grow to meet its physiological responsibilities (1,2). Such responses to functional demands vary from organ to organ (see ref. 1 for review). The liver is well known for its regenerative ability after resection. After unilateral nephrectomy, the kidney undergoes compensatory hypertrophy. Various endocrine organs—thyroid, parathyroid, and adrenal—are capable of compensatory growth by increase in cell number (hyperplasia) and/or increase in cell volume (hypertrophy) (1).

The ability of the endocrine pancreas, particularly the insulin-producing β-cells, to respond to a functional demand is unclear. Although it is accepted that islets continue to grow until adulthood (3), adult islets, or more specifically β-cells, are thought to have limited growth capacity (4). However, Woerner (5) in 1938 reported that islet tissue of guinea pigs increased after continuous glucose infusions. Numerous other studies have confirmed this finding, some suggesting hyperplasia, others hypertrophy (4,6–9). However, the extent of the growth of the islets in the face of continued glucose challenge has been unanswered. We undertook this study to quantify the changes in the β-cell mass and explore the contributions of hyperplasia and hypertrophy in adult rats infused with glucose for 96 h. In addition, we looked at the same parameters 7 days after stopping the infusion.

MATERIALS AND METHODS

Male CD rats weighing 225–275 g (Charles River Breeding Laboratories, Wilmington, MA) were infused for 96 h with either 35% glucose (wt/vol), 50% glucose (wt/vol), or the

diluent 0.45% NaCl via an indwelling jugular catheter at 2 ml/h (10). In later experiments, termed *reversal experiments*, after a 96-h infusion, the animals were disconnected from the pumps and allowed food and water ad libitum for 7 days.

Four hours before death, rats received colchicine (2.5 mg/kg body wt; Sigma, St. Louis, MO) intravenously either by tail vein or jugular catheter for the accumulated mitotic index to be determined (see below). Colchicine was not used in experiments for electron microscopy. Animals infused with 35% glucose were not included in the reversal or electron-microscopy series.

Blood for plasma glucose measurements was obtained by tail snipping at 24, 48, 72, and 96 h and 7 days after infusion in the reversal experiments. Plasma was assayed with a Beckman Glucose Analyzer II (Brea, CA). In some experiments, blood for plasma insulin measurements was obtained from the jugular catheter (10). The plasma was separated by centrifugation and stored at −20°C before assaying. Plasma insulin was measured by radioimmunoassay with rat insulin (Lilly, Indianapolis, IN) for standards and charcoal separation (11).

Immunoperoxidase staining. Freshly excised pancreases were cleared of fat and lymph nodes, divided into halves and blotted, and each portion was weighed and separately cassetted. Each pancreas was oriented in the same direction to minimize variations due to the uneven distribution of islet tissue. After fixation in Bouin's solution, pancreas was embedded in paraffin. Sections (5–7 μm) were stained by the peroxidase–antiperoxidase method of Sternberger (12), with an overnight incubation for primary antibodies. As previously reported , we stain the mantle of non–β-cells to clearly delineate the borders of the islets (13). This staining does not hinder the counting of mitotic figures in the β-cells (see below). To do this, we used as primary antibody a mixture of rabbit anti-porcine glucagon (gift of Dr. M. Appel, University of Massachusetts Medical School, Worcester, MA), rabbit anti-bovine pancreatic polypeptide (gift of Dr. R. Chance, Lilly), and rabbit anti-synthetic somatostatin-14 (our laboratory's D-10).

Quantification of β- and non–β-cell mass. One immunoperoxidase-stained section from each of the two blocks for each animal was counted with the morphometric point-counting technique of Weibel (14). Starting at one corner of a section, at least 108 nonoverlapping fields, systematically chosen with the markings of the stage micrometer, were scored with a 50-point grid at a final magnification of ×350; at least 10,800 points/animal were used. The relative or volume density (%) of either the β- or non–β-cells was the number of intercepts over that specific tissue as a proportion of the total counts over pancreatic tissue. To get an absolute mass (mg), the relative density for each pancreatic portion (block) was multiplied by the weight of that portion; the values for the two portions per animal were summed to get the value for each animal. Sample sizes were chosen to given estimated standard errors of ≤10%.

Mitotic-index determinations. Mitotic indices were determined by the stathmokinetic protocol of Swenne (15,16) as previously described (17). With the same sections described above, at least 1000 nuclei of β-cells were scored at ×400 magnification on coded slides. The mitotic index (%) was measured by the accumulated frequency of arrested mitotic figures over the 4-h colchicine treatment and was expressed as a percentage of the total nuclei scored. Sections were scanned for islets, and all islets >5 cells in cross section were scored until the required number of nuclei had been counted.

Measurement of mean β-cell volume. Animals in separate series of experiments but comparable in all respects were used in these studies. Without receiving colchicine, the animals were killed, and splenic pancreatic tissue was rapidly excised and minced in 2.5% glutaraldehyde and 0.1 M phosphate buffer at pH 7.4. After several hours of fixation at room temperature, islets were hand dissected and processed for routine electron microscopy. One to three islets of similar size (≥200 μm) from each animal were sectioned and viewed on a Philips 301 electron microscope. To minimize the variations of orientations of the cells to the plane of section, centrally located β-cells that had a visible capillary face and a nuclear cross section were photographed on micrographs at ×3800 final magnification. The cross-sectional area of β-cells and their nuclei were measured with the electronic planimeter MOP-2 (C. Zeiss, Thornwood, NY). Estimates of cellular (not cytoplasmic) and nuclear volumes were calculated with the formula for the volume of a sphere, even though the β-cell is a truncated pyramid.

Data presentation and statistical methods. Data is presented as means ± SE with the sample number in parentheses. Statistical significance was determined with the unpaired two-tailed Student's *t* test.

RESULTS

Plasma glucose and insulin values. The plasma glucose values at 0, 24, 48, 72, and 96 h of the animals used for light-microscopic morphometrics are in Fig. 1. These values are similar to those previously reported by us (18) and those in the other series of experiments presented in this study. However, in the small sample used in this aspect of the study, the values at 96 h are not different for the 35% glucose–infused (7.84 ± 0.4 mM, n = 7) and 50% glucose–infused

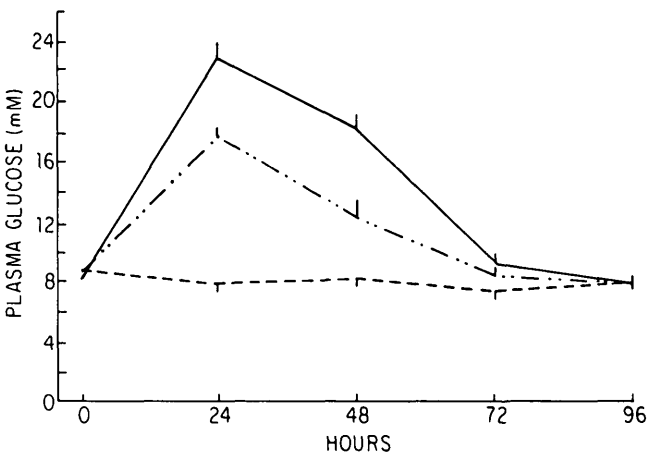

FIG. 1. Plasma glucose values during 96-h infusion of 0.45% NaCl (*dashed line*), 50% glucose (*solid line*), and 35% glucose (*dashed and dotted line*) of animals used for quantification of β- and non–β-cell mass and mitotic index. Values are comparable for values found in other series.

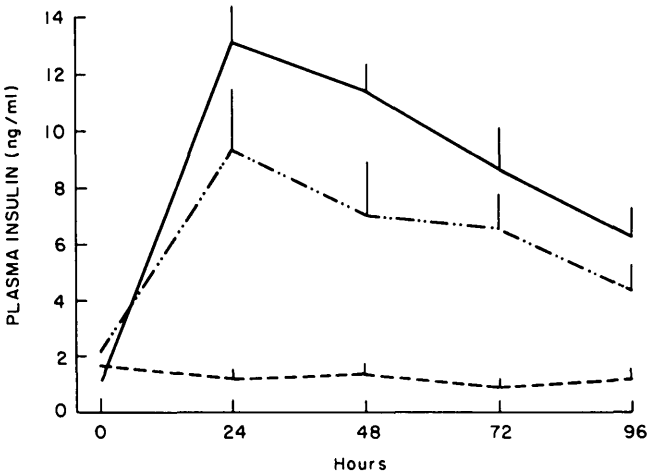

FIG. 2. Plasma insulin values during 96-h infusion of 0.45% NaCl (*dashed line*), 50% glucose (*solid line*), and 35% glucose (*dashed and dotted line*) in same animals in Fig. 1. Insulin values were not measured in other series.

(7.8 ± 8 mM, n = 5) animals or the saline-infused controls (7.7 ± 0.2 mM, n = 6).

Figure 2 presents the plasma insulin values for the same animals. As previously reported, the plasma insulin values of both glucose-infused groups remain elevated through the 96-h infusion, even at the end when the animals are normoglycemic (18).

β-Cell and non–β-cell mass after 96-h infusion. After 96 h of infusion with either 35 or 50% glucose, the β-cell mass had increased markedly compared to the saline-infused controls (Table 1). In fact, there is a 50% increase in the β-cell mass in both of the glucose-infused groups. The relative volume values (data not shown) differ even more dramatically, but some of the difference is attributable to the significantly lower weight of the pancreas after glucose infusion. This finding provides further evidence of the greater reliability of expressing morphometric data in absolute rather than relative values. The increase in β-cell mass was selective; there was no significant change in the non–β-cell mass (Table 1).

β- and non–β-cell mass 7 days after stopping infusion. Seven days after stopping the infusion, the β-cell mass in both the 50% glucose– and saline-infused animals had not changed significantly from the end of the 96-h infusion (Table 1). Animals infused with 35% glucose were not studied at this time point. Interestingly, the non–β-cell mass of the 50% glucose–infused animals was also significantly increased compared to that of the saline-infused animals 7 days after stopping the infusion (Table 1).

Mitotic index of β-cells at end of 96-h infusion and 7 days after infusion. At the end of the 96 h of infusion, both the 35 and 50% glucose–infused animals had significantly increased mitotic index compared with the saline-infused controls (Table 2). Even though the plasma glucose levels of all three groups were not different 4 h before death, the mitotic index of the 50% glucose–infused group was fivefold greater and that of the 35% glucose–infused group was threefold greater than that of saline-infused controls.

Seven days after stopping the infusion, the saline-infused animals had a mitotic index similar to that of the controls at the end of the infusion (Table 2). Surprisingly, the mitotic index of 50% glucose–infused animals was not only lower than that of similarly infused animals at the end of the infusion, but the mitotic index of these reversed animals was significantly lower than that of the saline-infused animals 7 days after stopping the infusion.

Changes in individual β-cells. After 96 h of 50% glucose infusion, the β-cells showed significant increases in mean cross-sectional area (measured) and the mean cell volume (calculated) compared to those of the saline-infused controls (Table 3). Both parameters were ~150% of the control value, indicating considerable hypertrophy of the β-cells.

In the later experiments, after a 7-day reversal period, mean cross-sectional β-cell area of the glucose-infused animals was not significantly increased compared to saline-infused animals. However, the mean β-cell volume remained significantly elevated in the glucose-infused animals. The validity of comparing the values found after 96 h of infusion to those after 7-day reversal is unclear because the experiments were performed several months apart.

We have no indication that the shape of the β-cell changed during or after the glucose infusion; however, the ultrastructural appearances did differ. After 96 h of saline infusion the β-cells had a normal well-granulated appearance, but after 96 h of glucose infusion the β-cells were usually quite degranulated (data not shown). Within a given islet, not all β-cells were degranulated to the same extent, but it is not yet known whether there is any pattern to the location of these cells with varying granulation. After the 7-day reversal the β-cells are regranulated and are visually indistinguishable from those of saline-infused animals (data not shown). At 96 h the nuclei of the glucose-infused animals were striking in their greater cross-sectional area (32.6 ± 0.8 μm², n = 162 nuclei, glucose infused vs. 23.0 ± 0.6 μm², n = 139 nuclei, saline infused), their prominent nucleoli, and their being more eu-chromatic.

TABLE 1
Quantification of β-cell and non–β-cell mass after 96 h of infusion and 7 days of reversal

Group	n	Body weight at death (g)	Pancreatic weight (g)	β-Cell mass (mg)	Non–β-cell mass (mg)
Saline infused for 96 h	6	252 ± 8	0.92 ± 0.05	6.15 ± 0.55	2.23 ± 0.16
50% glucose infused for 96 h	5	236 ± 9	0.67 ± 0.02*	9.57 ± 0.87*	2.75 ± 0.40
35% glucose infused for 96 h	7	235 ± 13	0.66 ± 0.04*	9.50 ± 1.23†	2.90 ± 0.27
Saline infused for 7 days	5	296 ± 9	1.12 ± 0.06	5.23 ± 0.44	2.39 ± 0.29
50% glucose infused for 7 days	5	275 ± 7	0.96 ± 0.04	8.33 ± 0.57*	3.57 ± 0.31*

*$2P \leq .01$, †$2P \leq .05$ compared with appropriate saline-infused group.

TABLE 2
Mitotic index of β-cells after 96 h of infusion and 7 days later

	Saline	*n*	35% Glucose	*n*	50% Glucose	*n*
96 h	0.64 ± 0.12	6	2.06 ± 0.16*	7	3.11 ± 0.26*	5
7 days later	0.73 ± 0.06	5	ND		0.41 ± 0.10†	5

Mitotic index measured by accumulated mitotic figures after 4 h of colchicine treatment; number of mitotic figures/100 cells counted = %/4 h. ND, not determined.
*$2P \leq .05$ compared with saline control at 96 h.
†$2P \leq .05$ compared with saline control 7 days later.

DISCUSSION

In this study, we have shown that with a short-term (96-h) continuous glucose stimulus the pancreatic β-cell mass of the adult rat markedly increases by both hyperplasia and hypertrophy. Furthermore, even 7 days after the glucose infusion is stopped, the β-cell mass has not returned to normal. It is unclear whether the β-cell mass at these two time points is the same; statistics can only tell us that they are not different. Nonetheless, this study provides clear evidence of rapid compensatory growth of adult pancreatic β-cells. Even though the β-cell mitotic index and mean cell volume were quantified, the actual contributions of each to the increased β-cell mass cannot clearly be attributed. The data show that the β-cell hypertrophy could account for much of the increased β-cell mass. Nonetheless, enhancement of the mitotic index at 96 h is not trivial and certainly is a significant component. The actual increase in cell number remains undetermined. Cell death as a major counterbalancing factor to the increased mitosis is unlikely because there is no evidence of dead or dying cells within the islets. Presumably the mitotic index at previous time points in the infusion period is less than at 96 h. In other studies of in vivo stimulated β-cell growth, there was a 24- to 36-h lag with a burst of β-cell replication at 3 days after the removal of an insulinoma (19) or injections of insulin antibodies (20).

In liver regeneration after partial resection, hypertrophy is seen within 4 h, whereas increased mitotic activity is not seen until 24 h (21,22). It remains to be seen if β-cell hypertrophy is also an immediate response to a stimulus, preceding enhanced mitotic activity. However, it is of interest that mean cell volume had not returned to normal 7 days later, even though the plasma glucose and insulin levels and the function of the β-cells had returned to normal (23). It remains to be determined whether the β-cell mass would revert to normal after a longer period of observation. In humans, obese nondiabetic and diabetic individuals have greater β-cell mass than their lean nondiabetic or diabetic counterparts (24), and this could be a glucose-driven change.

The protocol for these glucose infusions has previously been shown to induce a functional blindness to glucose, leading to defects in insulin secretion, e.g., the loss of glucose-stimulated insulin release and altered glucose influence on arginine-stimulated insulin release (10,18). Thus, this increased mass of β-cells functioned similarly to the reduced β-cell mass found in partially pancreatectomized (13) and adult neonatal streptozocin-treated rats (25). Seven days after stopping the glucose infusions, the defects are reversed (23). The mechanisms responsible for these functional alterations are unknown. β-Cell replication and insulin release has been shown to be dissociable in cultured islet cells, although both seemed dependent on glucose utilization (26).

Although glucose is known to be a stimulus in culture for β-cell replication (27), the factors influencing the growth of the non–β-cells (i.e., glucagon-producing A, somatostatin-producing D, and pancreatic polypeptide-producing PP cells) are unknown. After 96 h of infusion, the mass of the non–β-cells had not changed significantly, and yet, 7 days after the infusion a significant increase of approximately the same magnitude as the β-cell growth was seen. It is unclear whether there is a delayed effect of the glucose infusion itself or some stimuli (e.g., hormonal, neural, and/or metabolic) occurring only after the cessation of the infusion. The analysis of which cells increase needs further study.

At 96 h the mitotic index of the β-cells was fivefold increased. The cells of the saline-infused animals increased ~0.6% over a 4-h period, which, if extrapolated to 24 h, corresponds to the 3%/day found in Swenne's (28) studies for adult islets. Swenne also showed that the cell cycle did not change with age or stimulus. With the assumption that the rate is relatively stable for 24 h, then the glucose-infused animals would have an 18% proliferative rate at 96 h, even though the animals were normoglycemic. A similar elevated β-cell replication at a time of normoglycemia was reported by Logothetopoulos and Bell (20) in a study in which anti-insulin antibodies were injected into mice. With multiple injections for up to 3 wk, the animals were hyperglycemic and had enhanced β-cell replication. However, if the injections

TABLE 3
Changes in cross-sectional area and volume of β-cell after 96 h of infusion and 7 days later

	β-Cells (*n*)	Islets (*n*)	Animals (*n*)	β-Cell cross-sectional area (μm^2)	β-Cell volume (μm^3)
After 96-h infusion					
Saline	83	6	3	111 ± 3	869 ± 26
50% glucose	128	9	4	151 ± 4*	1383 ± 36*
7 days later					
Saline	56	4	3	129 ± 4	1098 ± 36
50% glucose	77	7	3	139 ± 4	1218 ± 35*

Sample size is number of cells counted in number of islets from number of animals. Mean values are based on cell counts. If values were pooled to have islets as representative sample, or even to have animals as sample, values are slightly different but differences are still significant at $2P \leq .025$.
*$2P \leq .005$ compared with saline control for same period.

were stopped after 2 days, the animals became normoglycemic on the third and fourth days but still had enhanced β-cell replications. In both of these cases, the β-cells may have been programmed for replication during the earlier hyperglycemia. Our knowledge of how cells are triggered to enter the proliferative cycle is minimal. The reduced mitotic index 7 days after stopping the glucose infusion suggests a feedback of some sort, perhaps mediated by a mild lowered plasma glucose seen immediately after glucose infusions are stopped.

There has been little previous quantitative evidence of hypertrophy of β-cells when stimulated, although pathologists have widely held this as true. Logothetopoulos and Valiquette (29) found that islets isolated after 72 h of glucose infusion had a 55% increase of islet volume per microgram of DNA. Even though size was not evaluated, they suggested that the β-cell "size likely doubled after 72 hour infusion." In a study of the effect of glyburide on isolated mouse islets, Borg and Anderson (30), with electron-micrographic morphometrics, found that after 7 days of culture in high (27.7 mM) glucose, the control islets had a 160% increase in cell and nuclear volume.

The ultrastructural findings of the β-cells were similar to those of markedly stimulated β-cells (30–32) and were consistent with the large amount of insulin being synthesized and secreted during the glucose infusions. In a recent study defining different patterns of degranulation in peripheral and central β-cells after glucose infusions for up to 24 h, the volume density of insulin granules decreased as the volume density of rough endoplasmic reticulum and Golgi apparatus increased (32). No detectable differences in degranulation between peripheral and central β-cells by 24 h of infusion were found; similarly, we have not distinguished a pattern of the less-degranulated cells after 96-h infusion. Interestingly, they found no change in volume density of the nucleus. However, volume density is a relative value, so the lack of change does not preclude parallel hypertrophy of the cell and nucleus as an initial response.

ACKNOWLEDGMENTS

We thank Chris J. Cahill and Henrietta Cooper for excellent technical assistance and Jim Evans for expert preparation of the manuscript.

Support for this work has come from NIH Grants DK-35449 and DK-38543, BRSG S07-RR-05673 for partial support of the electron-microscopy facility, and NIH Grant DK-36836 for support of the Diabetes Endocrinology Research Center animal research core.

This work was presented in part at the 47th annual meeting of the American Diabetes Association, Indianapolis, Indiana, June 1987.

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