Evolution of Abnormal Insulin Secretory Responses During 48-h In Vivo Hyperglycemia

JOHN L. LEAHY AND GORDON C. WEIR

Recent in vitro studies have shown that insulin release caused by continuous exposure to high glucose concentration markedly falls within a few hours. We wanted to determine if a similar effect occurs in vivo with chronic intravenous infusions in normal rats. Male CD rats (200–250 g) were infused with 50% glucose at 2 ml/h for 6, 14, 24, or 48 h, whereas controls received 0.45% NaCl, and insulin responses were tested with the in vitro isolated perfused pancreas. Plasma glucose averaged 352 ± 20 mg/dl after 4 h and 396 ± 11 mg/dl after 24 h versus 137 ± 5 mg/dl in controls; plasma insulin at the same times was 8.94 ± 1.44 and 12.1 ± 2.62 ng/ml versus 1.69 ± 0.19 ng/ml in controls. The incremental insulin response caused by an increase in perfusate glucose from 2.8 to 16.7 mM was not significantly reduced after 24 h of glucose infusion; in contrast, paradoxical suppression was seen after 48 h. A second protocol examined glucose potentiation by giving 10 mM arginine at 2.8 and 16.7 mM glucose; a hyperresponse to arginine at the lower glucose level was present after just 14 h of infusion. Therefore, these results do not support the hypothesis that β-cells lose their sensitivity to glucose within hours of being exposed to higher than normal glucose concentrations. *Diabetes* 37:217–22, 1988

Insulin secretion is altered in human diabetes, and selective loss of the β-cell response to glucose has been described in both non-insulin-dependent (1–3) and early insulin-dependent (4,5) diabetes mellitus. Although the cause of this defect is not known, we have proposed that prolonged exposure of β-cells to an elevated plasma glucose concentration may be an important pathogenic factor. Indeed, we have shown that glucose-stimulated insulin secretion is lost, whereas the response to arginine remains fully intact in normal rats made markedly hyperglycemic for 48 h with continuous glucose infusions (6). A possible mechanism for this finding has been suggested by the recent observation made with in vitro systems that the increased insulin release that occurs with continued presence of a high glucose concentration tends to wane within a few hours, perhaps indicating that β-cells rapidly grow insensitive to the stimulatory effects of glucose (7–9).

The aim of this study is to determine whether β-cell desensitization also occurs with in vivo hyperglycemia and whether it can explain the altered insulin responses described in normal rats after chronic glucose infusions.

From the Research Division, Joslin Diabetes Center, and the Departments of Medicine, New England Deaconess Hospital and Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts.
Address correspondence and reprint requests to Dr. Jack Leahy, Research Division, Joslin Diabetes Center, One Joslin Place, Boston, MA 02215.
Received for publication 17 February 1987 and accepted in final form 30 June 1987.

MATERIALS AND METHODS

Chronic intravenous infusion. This method has been described previously (6). Indwelling jugular venous catheters were placed into male CD rats weighing 200–250 g (Charles River, Wilmington, MA). The next day, at 1000 h, the infusions were started with either 50% glucose (wt/vol) or 0.45% NaCl at 2 ml/h with a Sage syringe pump (Orion, Cambridge, MA) and an infusion device that consisted of a swivel above the cage and a hollow metal cable attached to the rat by a metal neck ring and Velcro vest (Emdie, Goochland, VA). The animals were unrestrained and had free access to food and water.

In vitro isolated perfused pancreas. This technique has been described previously (10). The animal was anesthetized with amobarbital sodium (100 mg/kg i.p.) and removed from its harness. The perfusate was a modified Krebs-Ringer bicarbonate buffer containing 4% dextran (D-4751, Sigma, St. Louis, MO), 2 mM calcium, 1.2 mM magnesium, and 0.2% bovine serum albumin fraction V (Sigma). It was bubbled for 20 min with 95% O_2/5% CO_2, glucose was added to reach baseline concentration (2.8 mM), and pH was adjusted to 7.4. It was placed in a reservoir maintained at 38°C by water bath; 10 mM arginine was added to the perfusate in a second reservoir. The higher glucose concentration (16.7 mM) was

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/2/217/512823/37-2-217.pdf by guest on 31 March 2023

obtained with a sidearm syringe driven by syringe pump that added only 0.2 ml to the usual flow rate of 3 ml/min. Before delivery, the perfusate passed through an artificial lung (11). After surgery, the body cavity was covered with gauze moistened with saline and placed under a heat lamp with the temperature constantly monitored and maintained at 36–39°C. All perfusions were preceded by a 20-min equilibration period, during which no samples were taken; samples were then collected for 30 s into chilled tubes containing 4 mg EDTA. The tubes were kept on ice until storage at −20°C pending assay.

Pancreatic extraction for hormone content. After the perfused-pancreas studies, 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 with an Ultra Turrax SDT (Tekmar, Cincinnati, OH) and re-stored pending assay.

Protocols. To determine plasma glucose and insulin values at several time points during the infusion, all rats were infused with 0.45% NaCl for 24 h, and then 0.5 ml blood was collected by tail snipping (time 0) in a heparin-coated 6 × 50-mm glass tube kept on ice until centrifugation and storage of the plasma at −20°C pending assay for glucose and insulin measurements. The infusion was then restarted with either 50% glucose or 0.45% NaCl, and the sampling was repeated at 2, 4, 6, and 24 h.

In the first protocol, to evaluate in vitro insulin secretion, rats were infused with 0.45% NaCl for 18 h and then switched to 50% glucose for the next 6 h; the control group received 0.45% NaCl in parallel. The β-cell responsiveness to a glucose challenge was then tested with the in vitro isolated perfused pancreas. The protocol was later extended to include groups infused with 50% glucose for 14, 24, or 48 h and a control group given 0.45% NaCl for 48 h. These infusions lasted a total of 48 h; those groups given 50% glucose for 14 or 24 h initially received 0.45% NaCl and then were switched to 50% glucose with the appropriate time remaining in the infusion period.

In the second protocol, to evaluate in vitro insulin secretion, rats were infused with 50% glucose for 14, 24, or 48 h, whereas controls received 0.45% NaCl for 48 h, and then glucose modulation of arginine-stimulated insulin and glucagon secretion was tested with the perfused pancreas.

Analytical methods. Plasma glucose was measured with a Beckman Glucose Analyzer II (Brea, CA). Insulin concentration was determined with a radioimmunoassay that used charcoal separation (12) and rat insulin standards (Lilly, Indianapolis, IN). Glucagon measurements were made by radioimmunoassay with 04A antiserum and charcoal separation (10).

Data presentation and statistical methods. Insulin concentrations of the individual samples from the perfused-pancreas studies are shown in the figures as means ± SE for each group. Total insulin output was calculated for each animal by multiplying the mean insulin concentration of all samples collected during that perfusate condition by the flow rate and duration; the mean ± SE for each group was then determined. Incremental insulin responses were calculated by subtracting the insulin concentration of the last sample taken before beginning that perfusate from the mean insulin concentration, then repeating the same calculation.

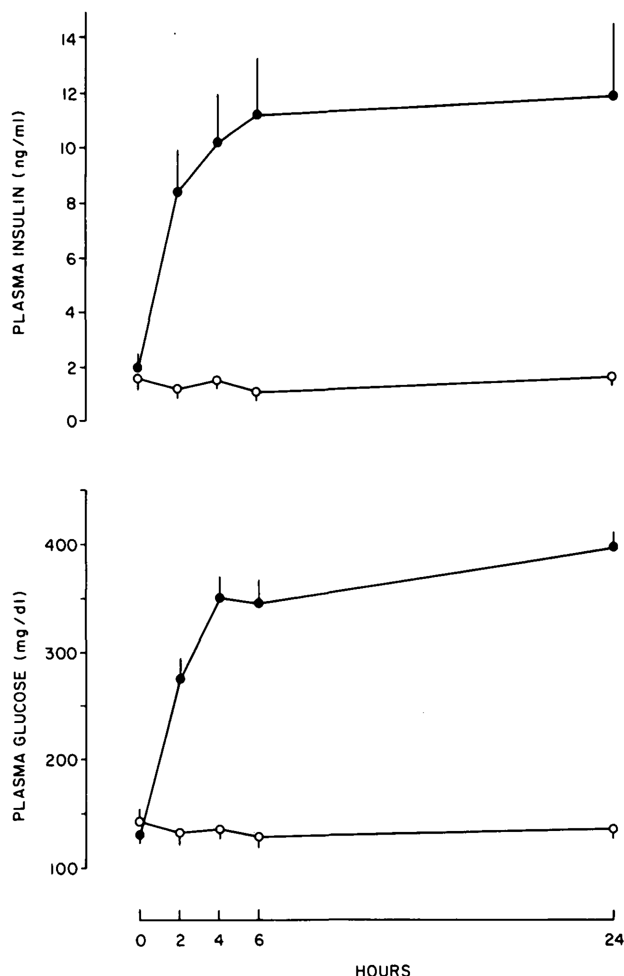

FIG. 1. Plasma glucose and insulin concentrations measured 0, 2, 4, 6, and 24 h after starting infusion of 50% glucose (●, *n* = 7) or 0.45% NaCl (○, *n* = 6). Values are means ± SE from perfused-pancreas studies.

Statistical significance was calculated with a one-way analysis of variance and the Newman-Keuls multiple-range test (13).

RESULTS

Plasma insulin and glucose concentrations. As expected, plasma insulin and glucose values did not vary to any significant degree during the saline infusion (Fig. 1). In the glucose-infused rats, plasma glucose rose to 352 ± 20 mg/dl by 4 h, with a slight upward drift thereafter. The plasma insulin profile closely mirrored that of plasma glucose, showing a rise to a plateau within 4 h that was sustained through the rest of the 24-h test period.

Pancreatic hormone content. Pancreatic insulin content was unaffected by 6 h of 50% glucose; in contrast, it decreased 60% during a 14-h infusion, with an additional 15% drop in the 24- and 48-h groups. Glucagon content, however, was similar in all rats (Table 1).

In vitro insulin response to various perfusate glucose concentrations. The perfusion protocol and results are shown in Figs. 2 and 3. The baseline perfusate contained 16.7 mM glucose, which was decreased to 2.8 mM for 10

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/2/217/512823/37-2-217.pdf by guest on 31 March 2023

TABLE 1
Pancreatic insulin and glucagon content

Treatment	n	Insulin content (μg/pancreas)	Glucagon content (μg/pancreas)
0.45% NaCl			
24 h	5	65 ± 5	
48 h	12	78 ± 5	5.7 ± 0.3
50% Glucose			
6 h	5	71 ± 5	
14 h	10	32 ± 5*	6.1 ± 0.3
24 h	10	21 ± 2*	5.4 ± 0.3
48 h	10	19 ± 2*	5.9 ± 0.3

Values are means ± SE. Statistical significance determined with the Newman-Keuls multiple-range test.
*P < .05 compared with rats infused with 0.45% NaCl for 48 h.

min and returned to 16.7 mM for 15 min. Total insulin output and incremental insulin responses to the reintroduction of 16.7 mM glucose are listed in Table 2.

The rats infused with 0.45% NaCl for 24 h showed rapid suppression of insulin release after the glucose reduction and a marked biphasic response on return of the high glucose (Fig. 2). The results in the group given 50% glucose for 6 h were similar except that insulin release was not as completely inhibited by 2.8 mM glucose (Table 2).

The pattern of response in rats infused with saline for 48 h was qualitatively very similar to that of the 24-h control group, although the amount of insulin released during 16.7 mM glucose was considerably less (incremental response 935 ± 219 ng in 24-h controls vs. 341 ± 91 in 48-h controls, P < .05). After a 14-h glucose infusion, the fall in insulin release after glucose reduction was somewhat sluggish compared with controls, but the response to reintroduction of high glucose was essentially identical in both groups (Table 2). The baseline insulin level in the 24-h glucose-infused group was twice that of controls. After the glucose reduction, there was a marked transient paradoxical rise in insulin output, which then fell to only half the baseline. The return of 16.7 mM glucose caused a discrete insulin spike, closely followed by a gradual increase in insulin concentration such that the incremental insulin response was not statistically different from that in the rats infused with saline for 48 h, although it was reduced 30% when viewed in absolute terms. In the group infused for 48 h, a transient paradoxical insulin response was also seen when the low-glucose perfusate was added, but the insulin concentration leveled out at a value equal to baseline. Return of 16.7 mM glucose then caused insulin release to decrease for much of the 15-min infusion period.

Effect of glucose on in vitro arginine-stimulated insulin release. The protocol and results are shown in Fig. 4. The baseline perfusate contained 2.8 mM glucose, to which 10 mM arginine was added. After reequilibration at 2.8 mM glucose, it was increased to 16.7 mM and arginine was added again. Incremental insulin and glucagon responses to arginine at 2.8 and 16.7 mM glucose are listed in Table 3.

In saline-infused rats, arginine-induced insulin release at 2.8 mM glucose was trivial compared with that at 16.7 mM glucose. In rats infused with 50% glucose for 14 h, a 25-fold increase in the incremental response at 2.8 mM glucose was found (Table 3); there was also a near doubling of that at 16.7 mM glucose, although this difference was not significant (623 ± 126 vs. 1020 ± 184 ng). In the 24-h group, the marked response at 2.8 mM glucose remained intact, whereas that at high glucose returned to a value similar to controls. When infused for 48 h, the response to arginine at 2.8 mM glucose further increased and was significantly greater than that of the 14- and 24-h glucose-infused groups and the control rats. In contrast, that at 16.7 mM glucose did not change.

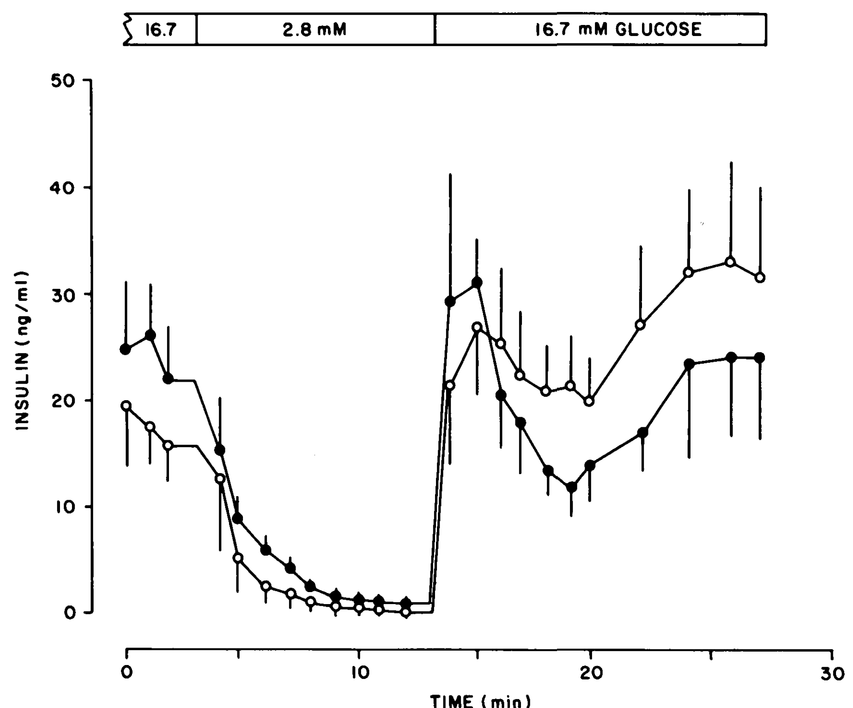

FIG. 2. Effect of acute reduction and then increase in perfusate glucose concentration on in vitro insulin secretion in rats infused wth 50% glucose (●) for 6 h and rats given 0.45% NaCl (○) for 24 h. n = 5 in each group. Values are means ± SE from perfused-pancreas studies.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/2/217/512823/37-2-217.pdf by guest on 31 March 2023

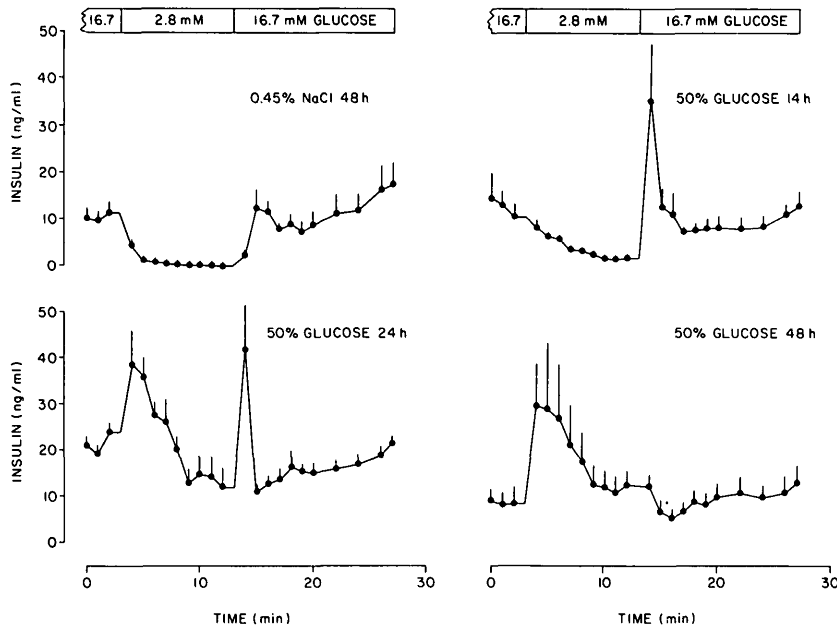

FIG. 3. Effect of acute reduction and then increase in perfusate glucose concentration on in vitro insulin secretion in rats infused with 50% glucose for 14, 24, or 48 h and rats given 0.45% NaCl for 48 h. n = 5 in each group. Values are means ± SE from perfused-pancreas studies.

Effect of glucose on in vitro arginine-stimulated glucagon release. The incremental glucagon responses taken from the arginine portions of the protocol shown in Fig. 4 are listed in Table 3. The values at 2.8 mM glucose fell with increasing length of exposure to the glucose infusion. In contrast, values at 16.7 mM glucose did not change.

DISCUSSION

The results of this study confirm the observation that β-cell secretory responses are severly altered in normal rats made markedly hyperglycemic for 48 h with continuous glucose infusions (6). The methodology used provides an opportunity not previously afforded with other animal models to view β-cell secretory defects at various stages of evolution. In Fig. 3, for example, the paradoxical response to the glucose reduction first appears in the 24-h group, but it is not sustained as long as that in 48-h rats. Also, the hyperresponse to arginine at low glucose (Fig. 4) is present after 14 h but then continues to evolve. If the timing of these results is carefully examined, an interesting dichotomy is seen: the direct effect of glucose on insulin secretion as assessed in Fig. 3 is not yet changed in the 14-h group, but its modulating influence on arginine-induced insulin release is already altered, possibly suggesting different molecular mechanisms for these two defects.

The cause of the β-cell dysfunction in this model is not known, but we have proposed that chronic exposure of β-cells to abnormally high glucose levels may be an important pathogenic factor. One possible mechanism has been suggested by the recent observation made with in vitro techniques that β-cells maintained in the presence of a high glucose concentration tend to rapidly become refractory to the stimulatory effects of glucose on insulin secretion (7–9). These studies used chronic perifusions of isolated islets and long-term perfused pancreas preparations and showed that the increase in insulin release that occurs with continuous delivery of a high-glucose perfusate tapers off within 5–7 h,

TABLE 2
Effects of increasing length of in vivo glucose infusion on insulin response to 16.7 mM glucose measured with perfused rat pancreas

		Insulin release		
Treatment	n	2.8 mM glucose (ng/min)	Total output at 16.7 mM glucose (ng/15 min)	Incremental response at 16.7 mM glucose (ng/15 min)
0.45% NaCl				
24 h	5	0.6 ± 0.3	944 ± 218	935 ± 219
48 h	5	0.4 ± 0.1	347 ± 91	341 ± 91
50% Glucose				
6 h	5	2.6 ± 0.6	658 ± 200	623 ± 196
14 h	5	3.8 ± 1.3	369 ± 89	312 ± 73
24 h	5	26.7 ± 7.1*	624 ± 78*	252 ± 156
48 h	5	29.7 ± 6.1*	296 ± 81	−129 ± 89

Values are means ± SE. Value at 2.8 mM glucose is amount of insulin released during last minute of that perfusate; total output is total of insulin released during 15-min infusion period; incremental response is amount of insulin released above baseline established at end of 2.8 mM glucose perfusate (see MATERIALS AND METHODS for method of calculation). Statistical significance determined with the Newman-Keuls multiple-range test; glucose-infused rats are compared with their respective saline-infused controls.
*$P < .05$ compared with appropriate saline-infused rats.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/2/217/512823/37-2-217.pdf by guest on 31 March 2023

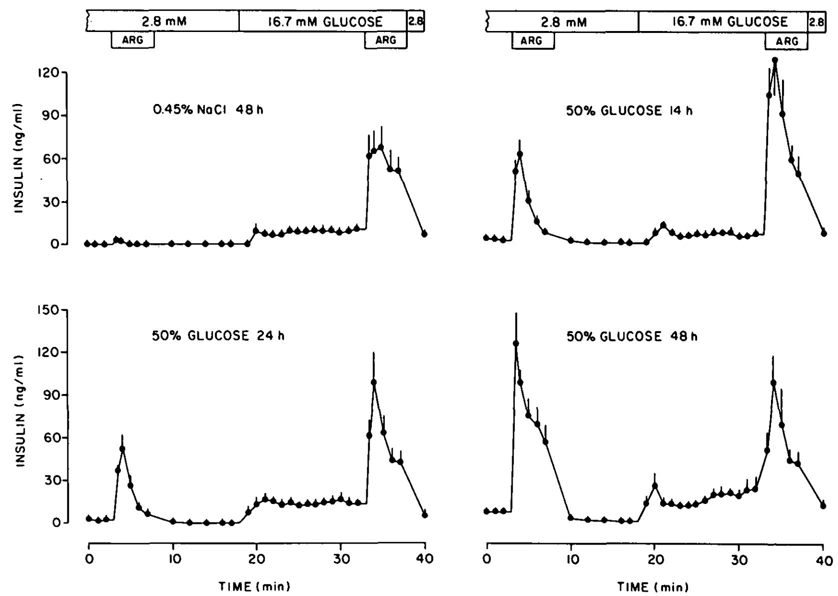

FIG. 4. Effect of background glucose concentration on arginine-induced insulin secretion in rats infused with 50% glucose for 14, 24, or 48 h (n = 5) and rats given 0.45% NaCl for 48 h (n = 7). Values are means ± SE from perfused-pancreas studies.

reaching a new steady state that averages only 20% of the initial peak. Our results, however, do not show a similar effect in vivo, at least within 24 h, because plasma insulin in the glucose-infused rats did not fall in the face of continued hyperglycemia. Moreover, with 96-h infusions, we observed only a 35% decrease despite plasma glucose having returned to near normal (14). Of course, factors other than the level of glycemia could be driving the β-cell, but the fact that the insulin response to a glucose challenge as measured with the perfused pancreas also remained mostly intact after 24 h provides further evidence in support of our conclusion. Finally, if the pattern of insulin release in rats infused for 48 h is carefully examined (Fig. 3), it is clear that a change in glucose sensitivity alone does not adequately explain the results, because β-cell function is aberrant, showing paradoxical responses to both an increase and a decrease in glucose concentration. On the other hand, the glucose challenge is relatively short term, and we do not know if the insulin responses in the 14- and 24-h groups would be sustained comparably to those of the controls had it been longer. Also, these findings do not rule out the possibility that an effect similar to that described in vitro may occur in vivo with less-marked hyperglycemia; in rats infused with 35% glucose for 48 h whose plasma glucose is raised only 37 mg/dl, we have found that the insulin response to an in vitro glucose challenge is blunted (6).

Table 2 shows that the insulin response to the glucose challenge in the rats infused with saline for 48 h was less than half that of the 24-h control group. We have described a similar observation when rats infused with 0.45% NaCl for 48 h were compared with noninfused normal rats (6), and we presumed that this decrease was secondary to stress from wearing the Velcro vest, such as occurs with immobilization (15). In support of this possibility, the insulin response improves when the infusion period is increased to 96 h, suggesting that the rats become tolerant of this effect (14). Therefore, with this model, control rats must be infused for the same period as test rats.

Insulin content fell in the glucose-infused rats, a finding made even more impressive by the likelihood of an increase in β-cell mass (16,17). Our results, however, do not implicate this as being important in the secretory defects, because β-cell function was relatively preserved in the 24-h group, whereas β-cell responses were markedly altered in the

TABLE 3
Effects of increasing length of in vivo glucose infusion on insulin and glucagon responses to 10 mM arginine measured with perfused rat pancreas

Treatment	n	Incremental insulin response (ng/5 min)		Incremental glucagon response (ng/5 min)	
		2.8 mM glucose + 10 mM arginine	16.7 mM glucose + 10 mM arginine	2.8 mM glucose + 10 mM arginine	16.7 mM glucose + 10 mM arginine
0.45% NaCl					
48 h	7	13 ± 7	623 ± 126	14.3 ± 2.4	1.7 ± 0.5
50% Glucose					
14 h	5	357 ± 61*	1020 ± 184	11.4 ± 2.8	1.5 ± 0.9
24 h	5	278 ± 31*	584 ± 90	5.6 ± 2.8*	0.5 ± 0.4
48 h	5	859 ± 38*	415 ± 107	2.0 ± 0.8*	0.8 ± 0.3

Values are means ± SE. Incremental responses are total amount of hormone released above baseline established at end of 2.8 mM or 16.7 mM glucose perfusates (see MATERIALS AND METHODS for method of calculation). Statistical significance determined with the Newman-Kuels multiple-range test.
*$P < .05$ compared with rats infused with 0.45% NaCl for 48 h.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/2/217/512823/37-2-217.pdf by guest on 31 March 2023

48-h group, despite the fact that content was comparably decreased in both. Moreover, the fact that the insulin response to arginine given at 16.7 mM glucose did not fall in the 24- and 48-h glucose-infused rats argues against the level of stored insulin having any significant influence on insulin secretion. Regulation of insulin content remains poorly understood, being a balance of synthesis, release, and intracellular degradation. The level of glycemia in our model also seems to influence the process, for rats infused with 35% glucose for 48 h have normal pancreatic content despite plasma insulin levels equal to those of rats infused with 50% glucose (6). It has been postulated that hyperglycemia has a long-term effect of suppressing insulin synthesis (18), and it may be that this can be seen earlier with the high levels of glycemia attained in this study.

A finding particularly interesting is the paradoxical suppression of insulin secretion noted after the glucose challenge in the rats infused with glucose for 48 h. A similar observation has been made in human diabetes (19–21), which suggests that the pathophysiologic events occurring in both situations may be very similar.

In summary, the pancreatic response to a relatively short 15-min in vitro glucose challenge remains relatively intact in normal rats after 24 h of marked hyperglycemia; in contrast, paradoxical suppression is seen after 48 h. These results do not support the recent suggestion that β-cells become desensitized to glucose when exposed to higher than normal glucose levels for a few hours. Instead, the responses to glucose are clearly intact after 6 and 14 h and were largely preserved after 24 h, even though the pattern of response at this time has become perceptibly altered. The glucose infusion model provides a way to study the effects of chronic hyperglycemia in vivo on normal β-cells, and because glucose concentration and duration of infusion can be varied, it has a flexibility never before available in animal models.

ACKNOWLEDGMENTS

We thank H.E. Cooper for expert technical assistance.

This work was supported by Grants AM-35449 and AM-38543 from the National Institutes of Health and Grant 185296 from the Juvenile Diabetes Foundation. J.L.L. is the recipient of a Research and Development Award from the American Diabetes Association.

REFERENCES

1. Seltzer HS, Allen EW, Herron AL, Brennan MT: Insulin secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. *J Clin Endocrinol Metab* 46:323–35, 1967
2. Palmer JP, Benson JW, Walter RM, Ensinck JW: Arginine-stimulated acute phase of insulin and glucagon secretion in diabetic subjects. *J Clin Invest* 58:565–70, 1976
3. Ward WK, Beard JC, Porte D Jr: Clinical aspects of islet B-cell function in non-insulin-dependent diabetes mellitus. *Diabetes Metab Rev* 2:297–315, 1986
4. Ganda OP, Srikanta S, Brink SJ, Morris MA, Gleason RE, Soeldner JS, Eisenbarth GS: Differential sensitivity to β-cell secretagogues in "early," type I diabetes mellitus. *Diabetes* 33:516–21, 1984
5. Srikanta S, Ganda OP, Rabizadeh A, Soeldner JS, Eisenbarth GS: First degree relatives of patients with type I diabetes mellitus: islet cell antibodies and abnormal insulin secretion. *N Engl J Med* 313:461–64, 1985
6. Leahy JL, Cooper HE, Deal DA, Weir GC: Chronic hyperglycemia is associated with impaired glucose influence on insulin secretion: a study in normal rats using chronic in vivo glucose infusions. *J Clin Invest* 77:908–15, 1986
7. Bolaffi JL, Heldt A, Lewis LD, Grodsky GM: The third phase of in vitro insulin secretion: evidence for glucose insensitivity. *Diabetes* 35:370–73, 1986
8. Curry DL: Insulin content and insulinogenesis by the perfused rat pancreas: effects of long term glucose stimulation. *Endocrinology* 118:170–75, 1986
9. Hoenig M, MacGregor LC, Matschinsky FM: In vitro exhaustion of pancreatic B-cells. *Am J Physiol* 250:E502–11, 1986
10. Weir GC, Knowlton SD, Martin DB: Glucagon secretion from the perfused rat pancreas. *J Clin Invest* 54:1403–12, 1974
11. Hamilton RL, Berry MN, Williams MC, Severinghaus EM: A simple and inexpensive membrane "lung" for small organ perfusion. *J Lipid Res* 15:182–86, 1974
12. Albano JDM, Ekins RP, Maritz G, Turner RC: A sensitive, precise radioimmunoassay of serum insulin relying on charcoal separation of bound and free hormone moities. *Acta Endocrinol* 70:487–509, 1972
13. Snedecor GW, Cochran WG: *Statistical Methods*. Ames, Iowa State Univ. Press, 1967
14. Leahy JL, Cooper HE, Weir GC: Impaired insulin secretion associated with near normoglycemia: a study in normal rats with 96-h in vivo glucose infusions. *Diabetes* 36:459–64, 1987
15. Shah JH, Wongsurawat N, Aran PP, Motto GS, Bowser EN: A method for studying acute insulin secretion and glucose tolerance in unanesthetized and unrestrained rats: the effect of mild stress on carbohydrate metabolism. *Diabetes* 26:1–6, 1977
16. Brodsky GM, Kern HF, Logothetopoulos J: Ultrastructure, mitotic activity, and insulin biosynthesis of pancreatic beta cells stimulated by glucose infusions (Abstract). *Fed Proc* 31:250, 1972
17. Bonner-Weir S, Deery D: Selective growth of adult B-cells is seen during short term in vivo glucose infusions (Abstract). *Diabetes* 36 (Suppl. 1):6A, 1987
18. Orland MJR, Chyn R, Permutt MA: Modulation of proinsulin messenger RNA after partial pancreatectomies in rats: relationship to glucose homeostasis. *J Clin Invest* 75:2047–55, 1985
19. Robertson RP, Brunzell JD, Hazzard WR, Lerner RL, Porte D Jr: Paradoxical hypoinsulinemia: an alpha-adrenergic-mediated response to glucose. *Lancet* 2:787–89, 1972
20. Metz SA, Halter JB, Robertson RP: Paradoxical inhibition of insulin secretion by glucose in human diabetes mellitus. *J Clin Endocrinol Metab* 48:827–35, 1979
21. Hellman B, Hällgren R, Abrahamsson H, Bergsten P, Berne C, Gylfe E, Rorsman P, Wide L: The dual action of glucose on the cytosolic Ca^{2+} activity in pancreatic B cells: demonstration of an inhibitory effect of glucose on insulin release in the mouse and man. *Biomed Biochim Acta* 44:63–70, 1985

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/2/217/512823/37-2-217.pdf by guest on 31 March 2023