Rapid Publications

Coupling of β-Cell Desensitization by Hyperglycemia to Excessive Stimulation and Circulating Insulin in Glucose-Infused Rats

YASUHIRO SAKO AND VALDEMAR E. GRILL

Nondiabetic rats were infused with glucose for 48 h to maintain moderate or marked hyperglycemia (mean blood glucose 13.2 ± 0.7 or 22.8 ± 0.3 mM, respectively). The two levels of hyperglycemia increased plasma insulin levels severalfold but decreased the insulin response to 27 mM glucose by 19 and 95%, respectively, versus saline infusion. Diazoxide (5 mg · kg^{-1} · h^{-1}), when continuously infused during the hyperglycemia protocols, completely inhibited the glucose-induced rise in plasma insulin levels. Diazoxide transformed β-cell insensitivity to stimulation: glucose-induced insulin release was thus increased 318% after moderate hyperglycemia and 707% after marked hyperglycemia. These stimulatory effects of diazoxide were reversed by exogenous insulin infusion (8 or 2 U/24 h) in a dose-dependent manner. It is concluded that excessive β-cell stimulation rather than glucotoxicity underlies hyperglycemia-induced β-cell insensitivity. Effects of hyperinsulinemia can form part of the mechanisms whereby excessive stimulation affects β-cell secretion.
***Diabetes* 39:1580–83, 1990**

Insensitivity of β-cells to glucose is found in animal diabetes of various origin (for review, see ref. 1). Several observations suggest hyperglycemia as a major cause of insensitivity. Thus, chronic hyperglycemia induced by 48-h glucose infusions in nondiabetic rats leads to β-cell insensitivity (2). Furthermore, prolonged glucose stimulation in vitro results in β-cell insensitivity, at least under certain conditions (3). Also, glucopenia in vitro, i.e., perfusion with a glucose-free medium, partially restores the insulin response to glucose in the perfused pancreas of neonatally streptozocin-induced diabetic rats (4).

However, previous studies have not dissociated the effects of chronic hyperglycemia per se (glucotoxicity) from those of continuous stimulation of secretion. We attempted to analyze these factors separately. This was done by studying in vivo and in vitro responses to 48 h of hyperglycemia during which insulin secretion was inhibited by diazoxide or potentiated by tolbutamide. Results from these protocols showed that β-cell insensitivity was correlated to in vivo insulin levels. Therefore, we also tested the influence of exogenous insulin in vivo on β-cell responsiveness to glucose.

RESEARCH DESIGN AND METHODS

Male Sprague-Dawley rats were obtained from ALAB (Stockholm). At the time of experiments, they weighed between 240 and 300 g. They had free access to water and a standard pelleted diet (EWOS-ALAB Brood Stock Feed for Rats and Mice).

The preparation for infusion has been described previously (5). A catheter was inserted under anesthesia into the vena cava by way of an external jugular vein. For double-infusion protocols, another catheter was inserted in the other jugular vein. After the catheter operation, a recovery period followed of 3–5 days. The infusion tubes ran inside a metal spiral that was fixed to a ball bearing. This procedure allowed complete freedom of movement to the rat. All rats were infused for 48 h starting ~1000. Throughout the infusion, rats had free access to water and food.

The pancreas was isolated free from adjacent organs (6) and perfused with a Krebs-Henseleit bicarbonate buffer (7) containing 20 g/L bovine albumin (Sigma, St. Louis, MO) and, when not otherwise indicated, 3.9 mM glucose. Twenty minutes of perfusion with this medium was allowed (not recorded in tables or figures) before the start of the actual protocol. At the end of perfusion, the pancreas was quickly frozen and stored at −20°C. Insulin content was later extracted by acid-ethanol (8).

From the Department of Endocrinology, Karolinska Hospital, Stockholm, Sweden.

Address correspondence and reprint requests to Valdemar E. Grill, Department of Endocrinology, Karolinska Hospital, S-104 01 Stockholm, Sweden.

Received for publication 17 July 1990 and accepted in revised form 4 September 1990.

TABLE 1
Levels of blood glucose and plasma insulin during 48-h infusions

Infusions	Blood glucose (mM)						Plasma insulin (pM)		
	0 h	6 h	24 h	30 h	48 h	Mean/48 h	0 h	24 h	48 h
Saline	5.7 ± 0.1		5.9 ± 0.2		6.1 ± 0.3	5.9 ± 0.1	302 ± 29	345 ± 22	294 ± 29
Moderate hyperglycemia	6.8 ± 0.4	13.7 ± 1.4	15.1 ± 1.1	16.4 ± 0.9	8.7 ± 0.5	13.2 ± 0.7	330 ± 50	2369 ± 115	1257 ± 194
Marked hyperglycemia	6.5 ± 0.3	22.4 ± 0.6	24.7 ± 0.5	25.2 ± 0.3	24.6 ± 1.3	22.8 ± 0.3	323 ± 36	2879 ± 151	3375 ± 165
Diazoxide	6.2 ± 0.2	9.0 ± 0.4	8.5 ± 0.4	8.8 ± 0.8	7.2 ± 0.5	8.3 ± 0.3	273 ± 50	129 ± 14	151 ± 29
Diazoxide + moderate hyperglycemia	5.8 ± 0.3	11.3 ± 0.4	18.9 ± 2.6	16.6 ± 1.6	9.3 ± 0.9	13.7 ± 1.0	294 ± 29	230 ± 36	208 ± 36
Diazoxide + marked hyperglycemia	6.5 ± 0.3	15.9 ± 0.8	25.3 ± 0.8	29.0 ± 1.3	20.5 ± 2.6	21.6 ± 0.9	381 ± 43	223 ± 57	302 ± 65
Diazoxide + moderate hyperglycemia + 8 U/24 h insulin	6.9 ± 0.5	11.7 ± 1.6	14.7 ± 2.0	14.7 ± 1.8	7.3 ± 1.1	12.0 ± 1.0	388 ± 36	20,118 ± 2427	14,267 ± 5636
Diazoxide + moderate hyperglycemia + 2 U/24 h insulin	7.0 ± 0.3	14.2 ± 2.2	14.7 ± 0.8	15.1 ± 1.1	7.2 ± 0.7	12.8 ± 0.9	409 ± 14	1228 ± 165	1300 ± 172
Tolbutamide + moderate hyperglycemia	6.4 ± 0.3	18.8 ± 0.9	16.5 ± 1.5	17.3 ± 1.4	9.2 ± 1.0	15.1 ± 0.4	381 ± 72	2355 ± 359	1580 ± 366

Values are means ± SE. Number of experiments is given in Table 2. Data on significance testing are given in RESULTS.

A solution of diazoxide (Hyperstat, Schering, Bloomfield, NJ) was added to either 0.9% saline or 45% glucose. The pH of these infusates was adjusted to 9.5. Tolbutamide (a gift of Farbwerke Hoechst, Frankfurt, FRG) was dissolved in saline. The pH of tolbutamide-containing infusates was adjusted to 9.5. Insulin (Actrapid human, Novo-Nordisk, Bagsvaerd, Denmark) was added to 0.9% saline or 45% glucose solution. The secretagogues tested were from Sigma.

Plasma samples were assayed undiluted and at 3- to 20-fold dilution. Bound and unbound insulin was separated by charcoal (9). The insulin antibodies used were raised in our laboratory against pork insulin.

Results are expressed as means ± SE. Incremental responses to 27 mM glucose in vitro were calculated as the mean secretion rate above the secretion rate recorded immediately before a switch from the low to the high glucose concentration. Tests of significance were carried out with two-tailed Student's *t* test for paired or unpaired differences.

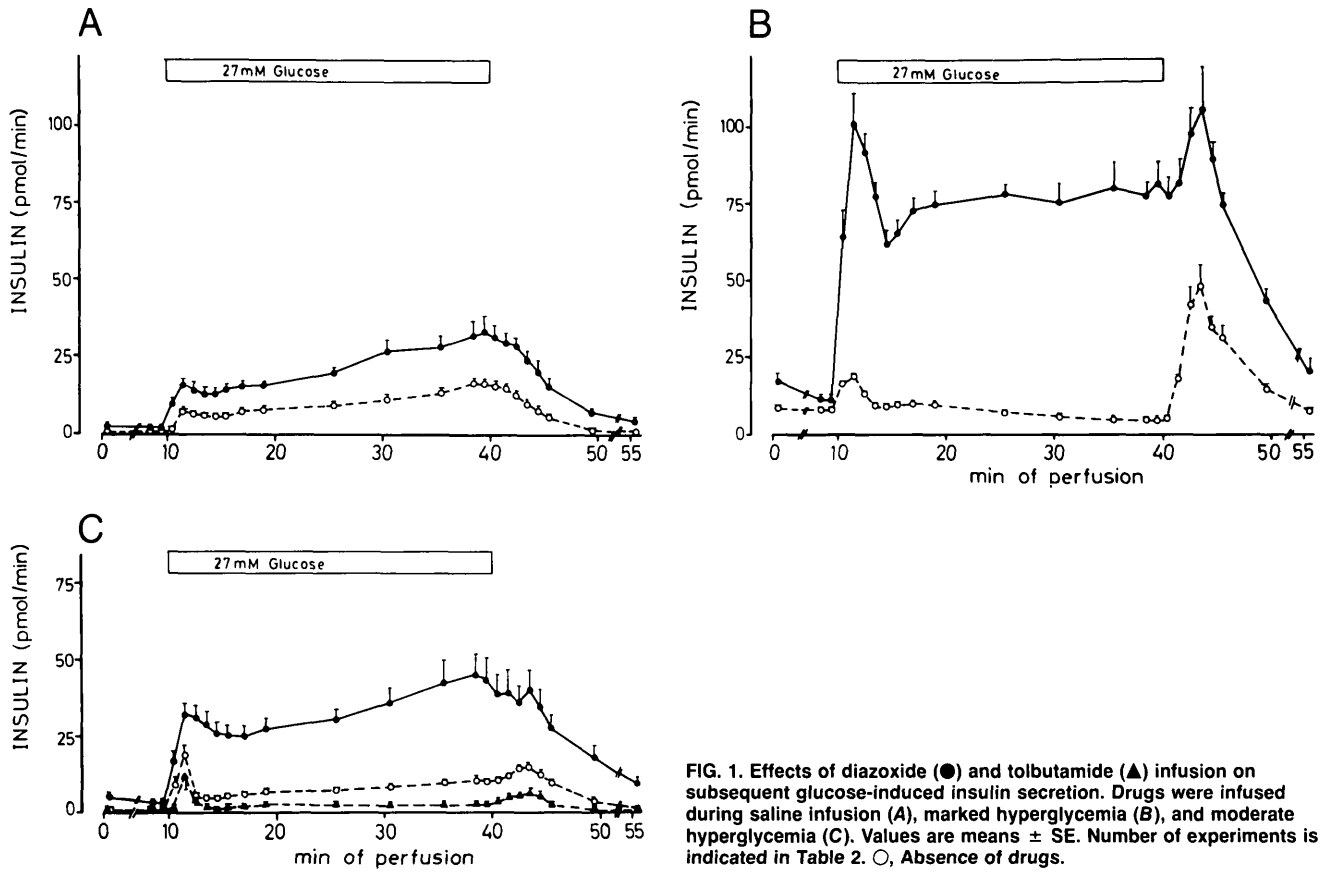

FIG. 1. Effects of diazoxide (●) and tolbutamide (▲) infusion on subsequent glucose-induced insulin secretion. Drugs were infused during saline infusion (*A*), marked hyperglycemia (*B*), and moderate hyperglycemia (*C*). Values are means ± SE. Number of experiments is indicated in Table 2. ○, Absence of drugs.

TABLE 2
Effects of 48-h infusions on insulin secretion from perfused pancreas and pancreatic insulin content

Infusions	*n*	Incremental insulin response (pmol/min)	Pancreatic insulin (nmol/pancreas)
Saline	6	9.50 ± 1.13	24.05 ± 3.37
Moderate hyperglycemia	5	7.65 ± 0.76	9.46 ± 1.58*
Marked hyperglycemia	5	0.49 ± 1.59*	5.23 ± 0.49*
Diazoxide	4	19.62 ± 2.19*	27.85 ± 1.22
Diazoxide + moderate hyperglycemia	5	30.25 ± 4.46*	28.93 ± 2.51
Diazoxide + marked hyperglycemia	4	67.13 ± 1.19*	26.55 ± 3.43
Diazoxide + moderate hyperglycemia + 8 U/24 h insulin	3	3.23 ± 1.29†	16.59 ± 1.72†
Diazoxide + moderate hyperglycemia + 2 U/24 h insulin	4	9.63 ± 2.26†	18.40 ± 2.48†
Tolbutamide + moderate hyperglycemia	4	2.43 ± 0.65‡	10.03 ± 1.23*

Values are means ± SE. *n*, Number of experiments. Incremental insulin was measured in response to 27 mM glucose.
*$P < 0.02$ or less vs. saline.
†$P < 0.05$ or less for effect of insulin.
‡$P < 0.005$ for effect of tolbutamide.

RESULTS

In vivo effects of saline, glucose, and diazoxide. Rats infused with saline for 48 h had levels of blood glucose and plasma insulin that were similar throughout the infusion (Table 1). Rats infused with 2 ml 45% glucose displayed moderately elevated glucose and markedly elevated insulin levels (Table 1). Increases in glucose and insulin levels partly subsided toward the end of the infusion.

In another protocol, we attempted to uphold more pronounced and continuous hyperglycemia. To this end, 45% glucose was infused at a rate between 2.3 and 4 ml/h (mean ± SE 2.69 ± 0.7 ml/h). The rate of infusion was higher during the 2nd than the 1st day (2.87 ± 0.11 vs. 2.51 ± 0.05 ml/h, $P < 0.05$). Under these conditions, blood glucose was markedly and stably elevated as were insulin levels (Table 1). Both of these parameters were significantly more elevated ($P < 0.05$ or less) than during the 2-ml/h protocol.

The effects of 5 mg · kg^{-1} · h^{-1} diazoxide were tested during infusion with saline or glucose. When coperfused with saline, diazoxide moderately but significantly elevated blood glucose (Table 1, $P < 0.001$ vs. control). When diazoxide was infused with glucose, we adjusted the rate of glucose infusion to approach the levels reached during the glucose infusion alone. The glucose levels reached were comparable whether the goal was moderate hyperglycemia or marked hyperglycemia (Table 1). With regard to insulin levels, diazoxide did not significantly diminish basal but completely inhibited glucose-induced increases in plasma insulin (Table 1). Coinfusion with tolbutamide (200 mg · kg^{-1} · 24 h^{-1}) and glucose resulted in glucose and insulin levels that were not significantly different from those obtained with the 2-ml/h infusion of glucose alone (Table 1).

Effects on insulin secretion in vitro. In response to 27 mM glucose, the moderate-hyperglycemia protocol tended to cause inhibition, whereas the marked-hyperglycemia protocol caused profound inhibition (Fig. 1; Table 2). These inhibitory effects of hyperglycemia were completely reversed by diazoxide. Instead, increasing degrees of hyperglycemia dramatically enhanced glucose-induced insulin secretion (Fig. 1; Table 2). On the other hand, tolbutamide inhibited glucose-induced insulin secretion.

Effects of exogenous insulin. The dramatic effects of diazoxide prompted us to investigate a role for circulating insulin on β-cell secretion. A continuous infusion of insulin was added to the glucose plus diazoxide protocol. Increasing the rate of glucose infusion prevented the blood-glucose–lowering effect of exogenous insulin; the levels of blood glucose obtained during the insulin plus glucose plus diazoxide protocol were in fact similar to those achieved by glucose plus diazoxide (Table 1). Insulin infused at 8 U/24 h raised insulin to pharmacological levels in plasma (but possibly within the range of intraislet concentrations). Infusion of 2 U insulin/24 h raised plasma insulin levels three- to fourfold above basal, i.e., to levels similar to or below those achieved when glucose was infused alone (Table 1). Exogenous insulin profoundly inhibited both first- and second-phase insulin secretion (Fig. 2). Inhibition after 8 U/24 h was 89% and after 2 U/24 h was 68% (Table 2).

Pancreatic insulin content. Glucose infusions with ensuing hyperglycemia diminished pancreatic insulin content (content calculated to include insulin released during perfusions; Table 2). Diminishment was proportional to the degree of in

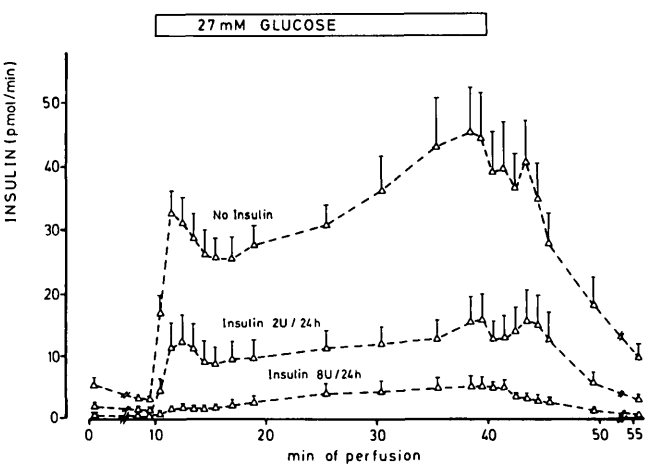

FIG. 2. Effects of insulin infusion on subsequent glucose-induced insulin secretion. Values are means ± SE. Number of experiments is indicated in Table 2.

vivo hyperglycemia. When glucose infusions were combined with diazoxide, insulin content was similar to that after saline infusion and was not measurably affected by the degree of hyperglycemia. The insulin content after insulin (2 or 8 U/24 h plus diazoxide plus glucose protocols) was significantly lower (57 and 63%, $P < 0.05$) than after diazoxide plus glucose.

DISCUSSION

Our results show that diazoxide in vivo transforms the β-cell insensitivity due to chronic hyperglycemia into marked enhancement of glucose-induced insulin secretion. Note that the effects of diazoxide increased with increasing levels of hyperglycemia, whereas the opposite was true for different levels of hyperglycemia alone.

Diazoxide inhibits insulin secretion through interaction with K^+ channels in the cell membrane of β-cells, leading to hyperpolarization (10). To our knowledge, this drug has not been reported to affect glucose metabolism. Our observations thus strongly disfavor the notion of glucotoxicity behind β-cell insensitivity if the latter concept is defined as a negative effect of glucose per se or glucose metabolites on β-cells.

By which mechanisms does diazoxide reverse β-cell insensitivity? The effect does not seem secondary to the accumulation of insulin in β-cells. Pancreatic insulin content was thus similar after infusion of diazoxide during moderate or marked hyperglycemia, yet the enhancement of glucose-induced insulin secretion was much greater after marked hyperglycemia. It seems possible that the drug protects from exhaustion modalities regulating cell membrane potential and K^+ and Ca^{2+} fluxes over the membrane. This notion is consonant with our finding that tolbutamide, which exerts an effect opposite to diazoxide on K^+ channels (10), aggravates β-cell insensitivity to glucose.

Our results with exogenous insulin indicate that some of the effects of diazoxide are secondary to prevention of glucose-induced hyperinsulinemia. We demonstrated that raising plasma insulin to levels comparable to or lower than those achieved during hyperglycemia alone produced marked inhibition of glucose-induced insulin secretion. In contrast to a previous study (11), the interpretation of our results is not complicated by hypoglycemia occuring concomitant with hyperinsulinemia. Instead, moderate hyperglycemia was upheld to a degree similar to that during experiments with diazoxide alone.

The inhibitory effects of exogenous insulin are likely to be direct, because exogenous insulin in vitro inhibits glucose-induced insulin secretion at concentrations comparable to those achieved by our insulin infusions (12,13). The precise mechanisms whereby the insulin molecule inhibits glucose-induced secretion are not known. Previous evidence indicated that exposure to elevated concentrations of insulin in vitro inhibits some or several components of glucose utilization in β-cells (12) and that this effect is initiated by binding of insulin to specific insulin receptors (14). Our results show that pancreatic insulin content is diminished after insulin treatment. This observation indicates that circulating insulin decreases biosynthesis and/or increases degradation of insulin within β-cells.

Our results pertain to situations of hyperglycemia in nondiabetic rats. Further studies are needed to assess the importance of excessive stimulation and circulating insulin for β-cell insensitivity in non-insulin-dependent diabetes.

ACKNOWLEDGMENTS

This study was supported by the Swedish Medical Research Council (Grant 04540), the Swedish Diabetes Association, the Magnus Bergvall Foundation, the Hoechst Fund for Diabetes Research, and the Nordic Insulin Foundation. Y.S. was a recipient of stipends from the Karolinska Institute and the Swedish Diabetes Foundation.

REFERENCES

1. Weir GC, Leahy JL, Bonner-Weir S: Experimental reduction of β-cell mass: implications for the pathogenesis of diabetes. *Diabetes Metab Rev* 2:125–61, 1986
2. 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
3. Bolaffi JL, Heldt A, Lewis LD, Grodsky GM: The third phase of in vivo insulin secretion: evidence for glucose insensitivity. *Diabetes* 35:370–73, 1986
4. Grill V, Westberg M, Östenson C-G: β-Cell insensitivity in a rat model of non-insulin-dependent diabetes: evidence for a rapidly reversible effect of previous hyperglycemia. *J Clin Invest* 80:664–69, 1987
5. Roos KA, Meurling S, Sandberg G: Growth and nitrogen utilization in rats on continuous and intermittent parenteral nutrition with and without fat (Intralipid 20%). *Acta Chir Scand* 147:459–64, 1981
6. Loubatières AL, Mariani MM, Ribes G, de Malbosc H, Chapal J: Étude expérimental d'un nouveau sulfamide hypoglycémiant particulierement actif le HB419 ou glibenclamide. *Diabetologia* 5:1–10, 1969
7. Umbreit WW, Burris RH, Stauffer JF: *Manometric Techniques*. Minneapolis, MN, Burgess, 1957, p. 149–50
8. Grill V, Efendic S: Loss of priming effect of glucose on A- and D-cell secretion in perfused pancreas from alloxan-diabetic rats: role of insulin and alloxan. *Diabetologia* 24:47–51, 1983
9. Herbert V, Lau KS, Gottlieb CW, Bleicher SK: Coated charcoal immunoassay of insulin. *J Clin Endocrinol Metab* 25:1375–84, 1965
10. 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
11. Kruszynska Y, Villa-Komaroff L, Halban P: Islet β-cell dysfunction and the time course of recovery following chronic overinsulinisation of normal rats. *Diabetologia* 31:621–26, 1988
12. Ammon HPT, Verspohl E: Pyridine nucleotides in pancreatic islets during inhibition of insulin release by exogenous insulin. *Endocrinology* 99:1469–76, 1976
13. Draznin B, Goodman M, Leitner JW, Sussman KE: Feedback inhibition of insulin on insulin secretion in isolated pancreatic islets. *Endocrinology* 118:1054–58, 1986
14. Verspohl E, Ammon HPT: Evidence for presence of insulin receptors in rat islets of Langerhans. *J Clin Invest* 65:1230–37, 1980