Regulation of α-Cell Function by the β-Cell in Isolated Human and Rat Islets Deprived of Glucose: the "Switch-off" Hypothesis

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The "switch-off" hypothesis to explain β-cell regulation of α-cell function during hypoglycemia has not been assessed previously in isolated islets, largely because they characteristically do not respond to glucose deprivation by secreting glucagon. We examined this hypothesis using normal human and Wistar rat islets, as well as islets from streptozotocin (STZ)-administered β-cell–deficient Wistar rats. As expected, islets perifused with glucose and 3-isobutryl-1-methylxanthine did not respond to glucose deprivation by increasing glucagon secretion. However, if normal rat islets were first perifused with 16.7 mmol/l glucose to increase endogenous insulin secretion, followed by discontinuation of the glucose perifusate, a glucagon response to glucose deprivation was observed (peak change within 10 min after switch off = 61 ± 15 pg/ml [mean ± SE], $n = 6$, $P < 0.01$). A glucagon response from normal human islets using the same experimental design was also observed. A glucagon response (peak change within 7 min after switch off = 31 ± 1 pg/ml, $n = 3$, $P < 0.01$) was observed from β-cell–depleted, STZ-induced diabetic rats whose islets still secreted small amounts of insulin. However, when these islets were first perifused with both exogenous insulin and 16.7 mmol/l glucose, followed by switching off both the insulin and glucose perifusate, a significantly larger ($P < 0.05$) glucagon response was observed (peak change within 7 min after switch off = 71 ± 11 pg/ml, $n = 4$, $P < 0.01$). This response was not observed if the insulin perifusion was not switched off when the islets were deprived of glucose or when insulin was switched off without glucose deprivation. These data uniquely demonstrate that both normal, isolated islets and islets from STZ-administered rats can respond to glucose deprivation by releasing glucagon if they are first provided with increased endogenous or exogenous insulin. These results fully support the β-cell switch-off hypothesis as a key mechanism for the α-cell response to hypoglycemia. *Diabetes* 53:1488–1495, 2004

Glucagon secretion during hypoglycemia stimulates glycogenolysis, thereby restoring normoglycemia. Loss of the glucagon response to hypoglycemia is an important problem for patients with type 1 diabetes and for patients with diabetes who require insulin-based therapy for glycemic control (1–15). Cryer et al. (15) attributed loss of the glucagon response in diabetes to an absence of an essential signal from the β-cell. This signal is a sudden drop in the level of insulin flowing from the β-cell to the α-cell via the portal circulation of the islet. This hypothesis posits that the switch-off signal is not possible in diabetic patients with greatly diminished or absent β-cells, so that the α-cell cannot respond to low glucose concentrations. However, regulation of the glucagon response to hypoglycemia is multifactorial, as reviewed by Taborsky et al. (1). Signals to the pancreatic α-cell to release glucagon when it is exposed to low glucose concentrations are also provided by the central nervous system and circulating catecholamines. Isolated pancreatic islets typically do not have a glucagon response when exposed to buffers or media containing low glucose concentrations, although they do secrete glucagon when stimulated by amino acids. This lack of glucagon responsiveness to low glucose concentrations is used by some as an argument that inputs to the α-cell coming from the central nervous system and circulating catecholamines during hypoglycemia are more important than the low glucose signal itself.

To examine the issue of absent glucagon responses from isolated islets when they are exposed to low glucose concentrations and to assess the insulin "switch-off" hypothesis in vitro in the context of glucagon responses to glucose deprivation, we designed experiments using perifused isolated rat and human islets to: *1*) examine glucagon responses in normal human and rat islets that have and have not been exposed to an antecedent period of high glucose levels to increase endogenous insulin release, followed by cessation of glucose provision and insulin secretion to provide an insulin switch-off signal; and *2*) determine whether provision of exogenous insulin by perifusion to isolated islets from streptozotocin (STZ)-induced diabetic animals, followed by a discontinuation of the insulin perifusion to provide a switch-off signal, enables α-cells in β-cell–depleted islets to secrete glucagon in response to zero, normal, and high glucose concentrations.

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STZ, streptozotocin.

RESEARCH DESIGN AND METHODS

Isolated islets. Both normal male Wistar rats and rats made diabetic by injections of 80 mg/kg STZ were used (16). Two weeks were allowed to elapse after the STZ injection. On the day of pancreas removal, pancreata were cannulated for infusion of collagenase and then removed from the animal for processing, according to the method of Lacy and Kostianovsky (17). After isolation, the islets were hand picked and split into groups of 50 islets each, which were then placed into eight separate perifusion chambers for simultaneous study. The islets were placed in the chambers on top of mesh filters small enough to prevent islets from escaping into the effluent. Human islets were obtained from the Human Islet Distribution Core of the Puget Sound Blood Center in Seattle, Washington. When human islets were used, 100 islets were placed in each chamber. Perifusion was performed using Kreb's Ringer buffer, 3-isobutryl-1-methylxanthine, regular insulin (Humulin, 300 μU/ml), and various concentrations of glucose (0, 3.0, 5.6, and 16.7 mmol/l). A solution containing 19 amino acids (alanine, 1.64 mmol/l; argininine, 0.70 mmol/l; asparagine, 0.15 mmol/l; citrulline, 0.35 mmol/l; glutamic acid, 0.45 mmol/l; glutamine, 1.88 mmol/l; glycine, 1.12 mmol/l; histadine, 0.29 mmol/l; isoleucine, 0.35 mmol/l; leucine, 0.61 mmol/l; lysine, 1.39 mmol/l; methionine, 0.18 mmol/l; ornithine, 0.26 mmol/l; phenylalanine, 0.31 mmol/l; proline, 1.31 mmol/l; serine, 2.13 mmol/l; threonine, 1.01 mmol/l; tryptophan, 0.28 mmol/l; and valine, 0.75 mmol/l) was used as an alternate stimulator for glucagon secretion. Insulin was measured by the method of Morgan and Lazarow (18), and glucagon was measured by the method of Harris et al. (19).

Statistical analysis. Results for each perifusion experiment were calculated as the mean ± SE of all eight lanes and considered as $n = 1$. Comparisons were by paired Student's t test. P values <0.05 were considered significant.

RESULTS

Isolated islets from normal Wistar rats. Isolated islets were perifused with 5.6 mmol/l glucose for 60 min, and then the perifusate was switched to 0 mmol/l glucose for 30 min. In three separate experiments, no glucagon response was observed (Fig. 1*A*). In six other experiments, islets were first perifused with 3 mmol/l glucose for 60 min and then with 16.7 mmol/l glucose for 30 min. This was followed by a switch to perifusion with buffer containing no glucose. In these experiments, the expected rise in endogenous insulin secretion (0 min = 13 ± 3 and 30 min = 54 ± 8 μU/ml; $P < 0.02$) and fall in glucagon secretion (0 min = 54 ± 10 and 8 min = 38 ± 7 pg/ml; $P < 0.02$) was observed during the perifusion with 16.7 mmol/l glucose (Fig. 1*B*). When the perifusion was switched to a zero glucose concentration, there was a fall in insulin levels (30 min = 54 ± 8 and 60 min = 15 ± 3 μU/ml; $P < 0.01$) and a prompt rise in glucagon levels (peak change within 10 min after switch off = 61 ± 15 pg/ml [mean ± SE], $n = 6$, $P < 0.01$).

Human isolated islets. Human islets were perifused with 3 mmol/l glucose for 60 min, and then the perifusion was switched to 16.7 mmol/l glucose to characterize β-cell function. Upon exposure to the high glucose concentration, first-phase (0 min = 8 ± 1 and 3 min = 30 ± 9 μU/ml; $n = 2$) and second-phase insulin secretion was observed (Fig. 2*A*). Exposure of human islets to 5.6 mmol/l glucose for 60 min, followed by perifusion with 0 mmol/l glucose failed to elicit glucagon secretion (Fig. 2*B*). In another experiment, human islets were first perifused with 3 mmol/l glucose for 60 min, followed by perifusion with 16.7 mmol/l glucose for 30 min and then by a switch to 0 mmol/l glucose for 30 min. During the infusion with 16.7 mmol/l glucose, insulin secretion increased (0 min = 14 and 30 min = 58 μU/ml) and glucagon secretion decreased (0 min = 151 and 30 min = 132 pg/ml) (Fig. 2*C*). Upon switching to 0 mmol/l glucose, insulin levels fell and a glucagon response was observed (30 min = 132 and 35 min = 168 pg/ml).

β-Cell–depleted isolated islets obtained from STZ-administered Wistar rats. In contrast to normal islets, isolated islets from rats made diabetic with STZ released a small amount of insulin during perifusion with 16.7 mmol/l glucose and had a glucagon response when the perifusate was switched from 16.7 to 0 mmol/l glucose (peak change within 7 min after switch off = 31 ±1 pg/ml, $n = 3$, $P < 0.001$) (Fig. 3). However, provision of exogenous insulin via perifusion during the last 15 min of the 16.7-mmol/l glucose infusion, followed by discontinuation of the exogenous insulin at the same time as the glucose perifusion was switched to 0 mmol/l glucose, elicited a significantly ($P < 0.05$) larger glucagon response (peak change within 7 min after switch off = 71 ± 11 pg/ml, $n = 4$, $P < 0.01$) (Fig. 4). This glucagon response did not occur when insulin was not switched off (Fig. 5) or when insulin was switched off without glucose deprivation (Figs. 6 and 7). However, a glucagon response to a solution of amino acids was observed, indicating functional α-cells (45 min = 38 ± 4 and 48 min = 237 ± 89 pg/ml; $n = 2$) (Fig. 7).

DISCUSSION

Isolated pancreatic islets, unlike islets in intact pancreata, characteristically do not release glucagon when they are exposed to very low concentrations of glucose. This has variously been attributed to lack of innervation or damage to α-cells during isolation procedures. Our studies were designed to examine the lack of α-cell responsiveness to low glucose levels in the context of regulation of the α-cell by insulin. We posited that the lack of periportal blood flow and delivery of insulin to downstream α-cells renders impossible the provision of an insulin switch-off signal during exposure of the islet to low glucose concentrations. As expected, we observed that the exposure of normal rat and human islets to a zero glucose concentration did not release glucagon. However, if we first perifused the islets with a high glucose concentration to stimulate endogenous insulin secretion and then followed this with discontinuation of the glucose infusion to provide an endogenous insulin switch-off signal, we observed a glucagon response. We noted that the glucagon response occurred before insulin levels in the effluent decreased. We speculate that this is an artifact caused by the perifusate washing away insulin that had collected in the capsules during the time taken to switch perifusate solutions at the 30-min time point. We also examined isolated islets obtained from STZ-induced diabetic Wistar rats. They secreted a small amount of insulin when they were first exposed to a high glucose concentration and released a small amount of glucagon when deprived of glucose. However, when exogenous insulin and high glucose were provided by perifusion before exposing the islets to a zero glucose concentration, switching off the glucose and insulin perifusate elicited a significantly greater glucagon response. Thus, in both normal and β-cell–deficient isolated islets, provision of an insulin switch-off signal utilizing either endogenous or exogenous insulin signaled the α-cells to respond to hypoglycemic conditions. In control experiments, during which the insulin perifusion was not switched off, no glucagon response to zero glucose concentration was observed. In all conditions where we observed a glucagon response, we also noted that gluca-

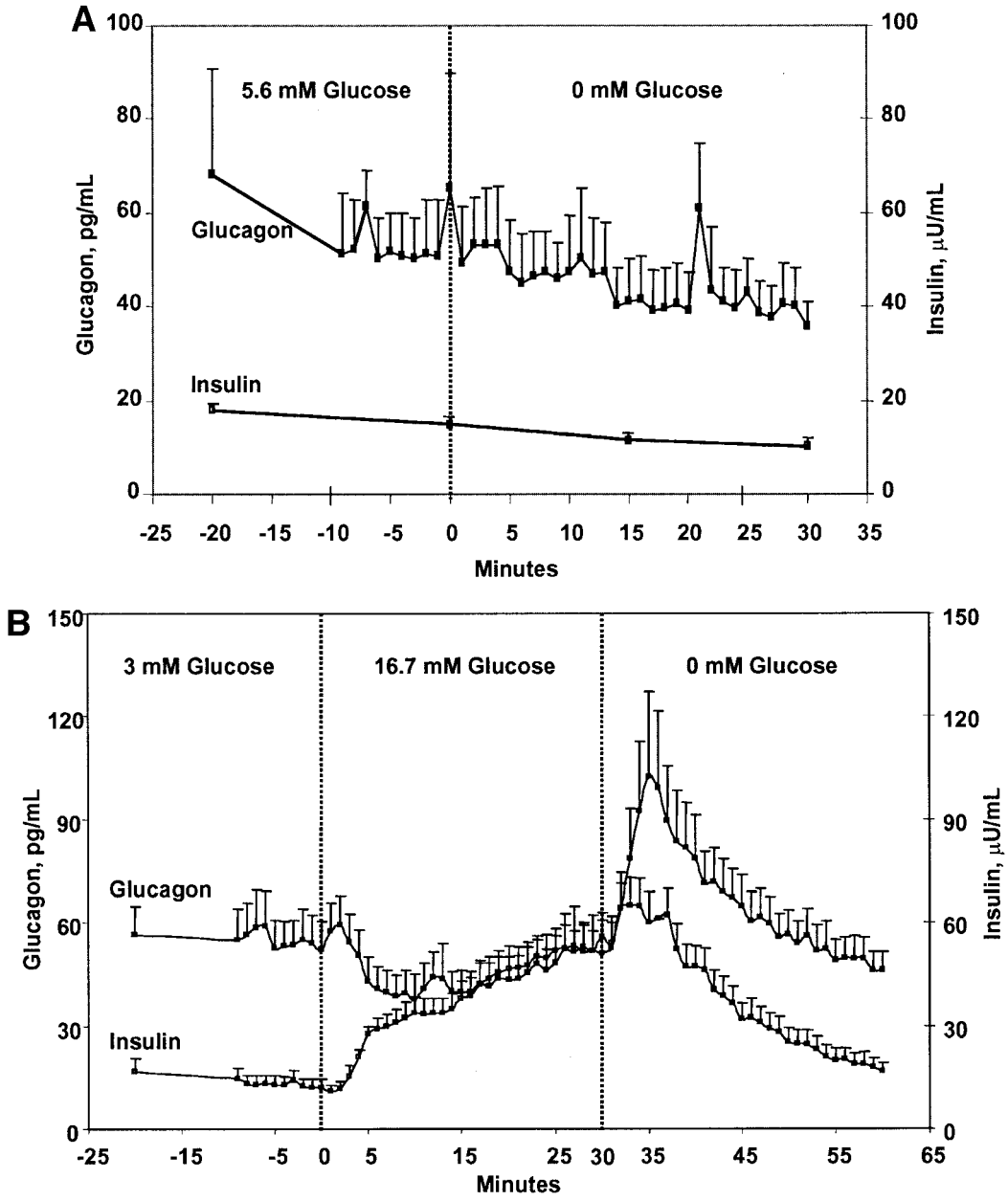

FIG. 1. ***A*****: Isolated control rat islets were perifused using buffer containing 5.6 mmol/l glucose and then switched to buffer containing 0 mmol/l glucose. No glucagon response was observed. Results are expressed as mean ± SE of three replicate perifusion experiments.** ***B*****: Control rat islets were first perifused with buffer containing 3 mmol/l glucose and then 16.7 mmol/l glucose to stimulate insulin secretion, followed by 0 mmol/l glucose to switch off insulin secretion. A glucagon response to zero glucose was observed ($P < 0.01$). Results are expressed as mean ± SE of six replicate perifusions.**

gon levels rose no higher than "basal levels." The question these results raise is whether the α-cell perceived that a new basal level had been set by prior exposure to a high glucose concentration and that the amount of glucagon released during zero glucose was the appropriate response. This obviously could not be the case in an in vivo situation wherein defense mechanisms intervene to stimulate glucagon release until normal glucose levels are reached. However, the isolated islet operates in an artificial situation in which it may have a programmed response unrelated to its basal level of secretion. That hypoglycemia was required for a glucagon response was shown in other experiments wherein physiologic or supraphysiologic levels of glucose were included in the perifusate. In these instances, the insulin switch-off signal was not sufficient to elicit glucagon secretion. Consequently, both low glucose concentrations and switching off α-cell exposure to insulin are required for the glucagon response. These conclusions are supported by the findings reported in the accompanying work by Zhou et al. (16), which describes in vivo experiments using STZ-administered rats receiving regional insulin infusions via the pancreaticoduodenal artery.

Evidence for the regulation of α-cell function by the secretory product of the β-cell has been previously provided by several research groups. Weir et al. (20) demonstrated that pancreata from rats made diabetic with STZ had enhanced glucagon secretion and that exogenous insulin could suppress this enhancement. Stagner and Samols (21) re-

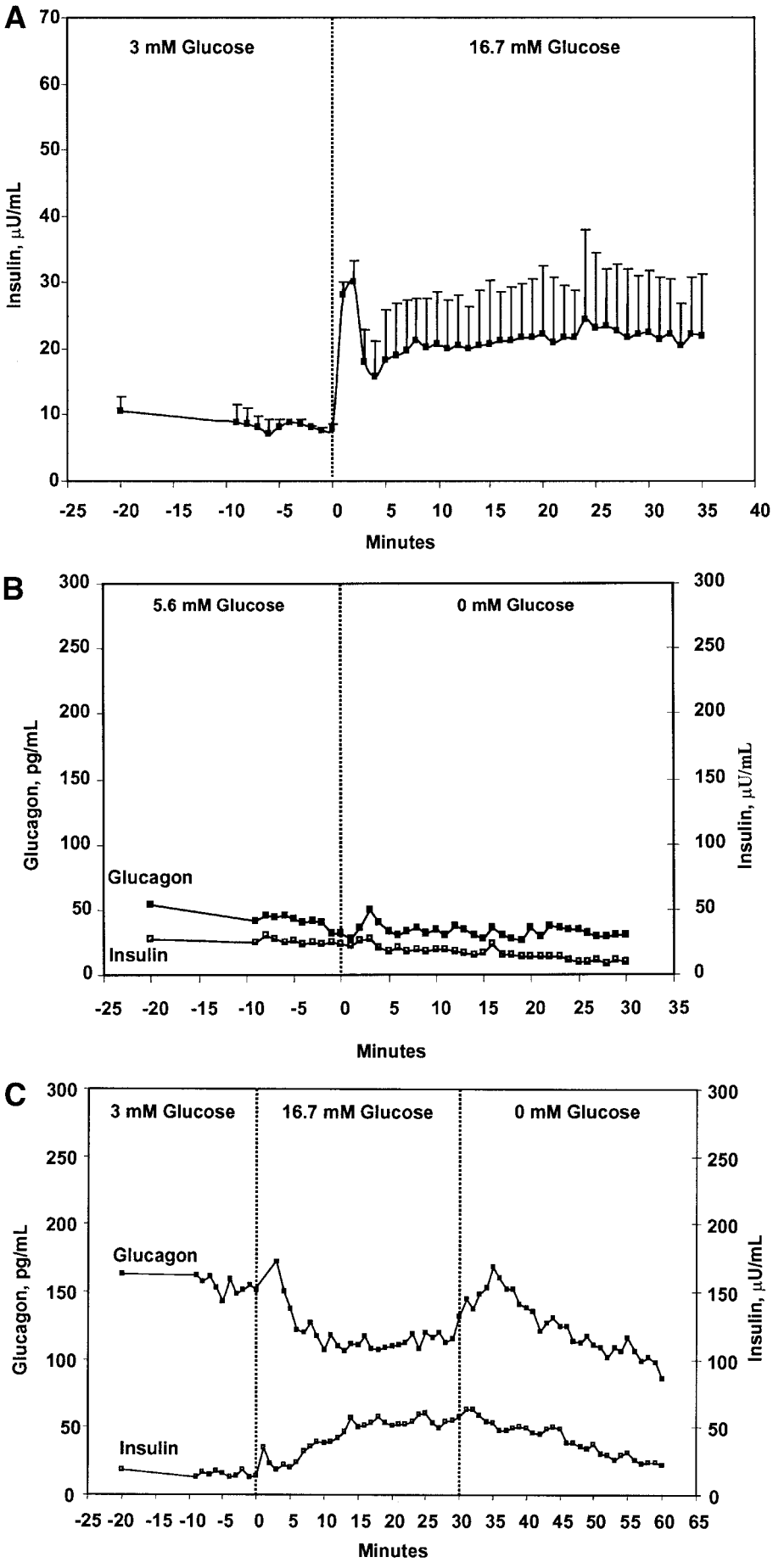

FIG. 2. *A*: **Human islets were perifused with buffer containing 3 mmol/l glucose followed immediately by 16.7 mmol/l glucose. Normal first- and second-phase responses to glucose were observed. Results are expressed as mean ± SD of two replicate perifusions.** *B*: **Human islets were perifused using buffer containing 5.6 mmol/l glucose and then switched to buffer containing 0 mmol/l glucose in a single experiment. No glucagon response to 0 mmol/l glucose was observed.** *C*: **Human islets were first perifused with buffer containing 3 mmol/l glucose and then 16.7 mmol/l glucose to stimulate insulin secretion, followed by 0 mmol/l glucose to switch off insulin secretion in a single experiment. A glucagon response to 0 mmol/l glucose was observed.**

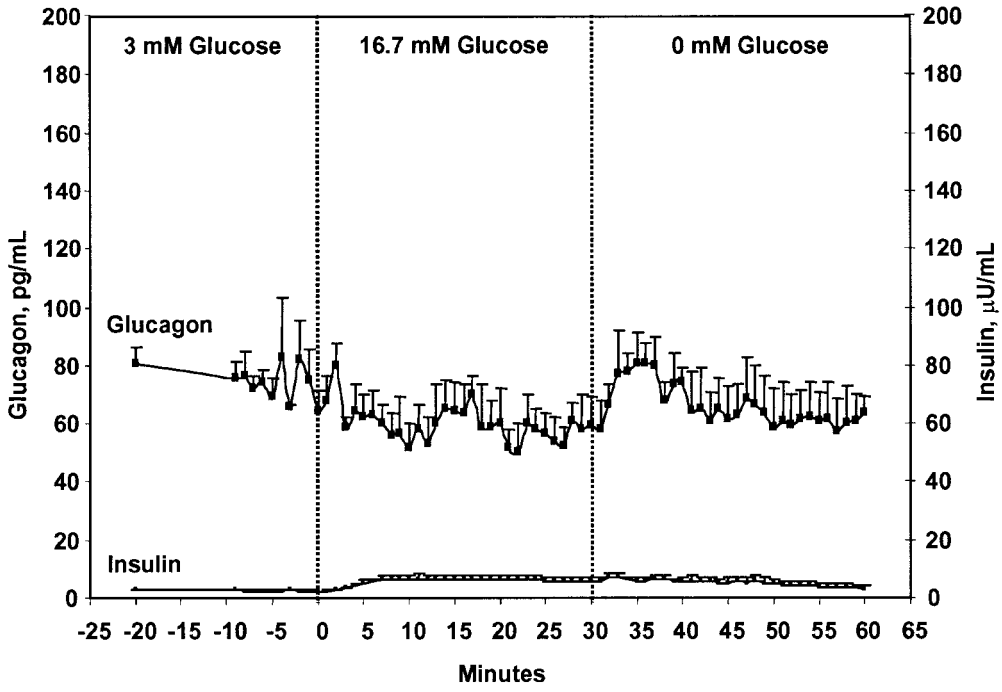

FIG. 3. Islets from STZ-administered rats were perifused with buffer containing 3 mmol/l glucose, 16.7 mmol/l glucose, and then switched to 0 mmol/l glucose. No glucagon response to 0 mmol/l glucose was observed. Results are expressed as mean ± SE of three replicate perifusions.

ported that retrograde perfusion of a constant glucose concentration increased mean glucagon secretion from dog pancreas and explained this phenomenon by suggesting that it was due to the prevention of insulin in the islet portal circulation from reaching the α-cell downstream. Maruyama et al. (22) perfused anti-insulin serum in rat pancreas and observed a significant rise in glucagon secretion, whereas nonimmune guinea pig serum had no effect. They concluded that insulin maintains an ongoing restraint on α-cell secretion and that loss of this inhibition by insulin may account for the hyperglucagonemia observed in insulin-deficient states. These initial observations were followed by a series of in vivo and in vitro experiments (23–30) that reinforced the hypothesis of β-cell regulation of α-cell function. More recently, McCrimmon et al. (31) have reported reversal of the hypoglycemia-specific defect in glucagon secretion in the diabetic BB rat through the use of a combination of a noninsulin glucose-lowering agent (5-aminoimidazole-4-carboxamide

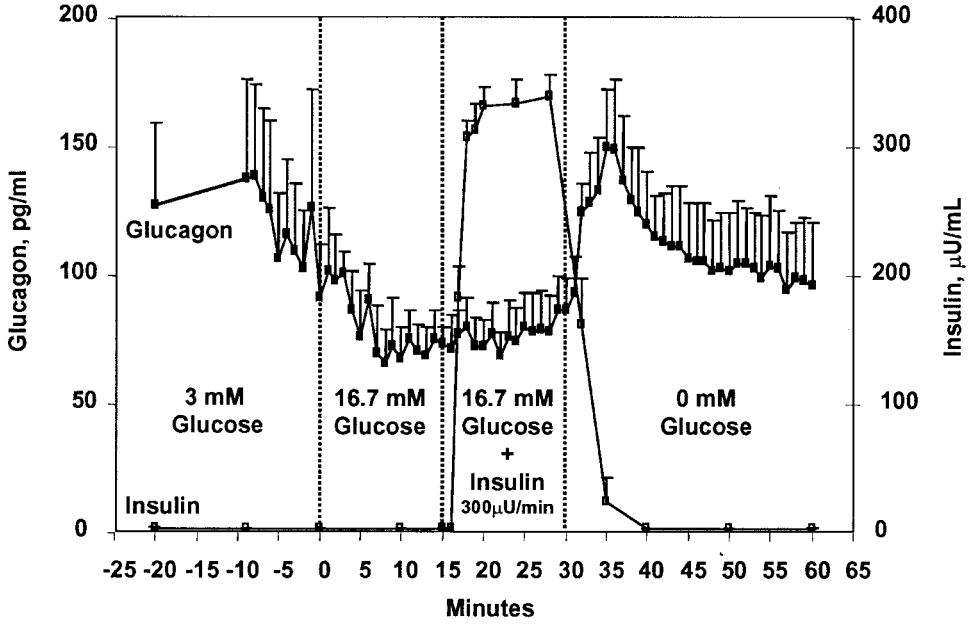

FIG. 4. Islets from STZ-administered rats were perifused with buffer containing 3 mmol/l glucose, 16.7 mmol/l glucose, and then switched to 0 mmol/l glucose. Exogenous insulin (300 μU/ml) was infused for the last 15 min of the 16.7-mmol/l glucose period and then promptly switched off at the beginning of the 0-mmol/l glucose period. A glucagon response to 0 mmol/l glucose was observed ($P < 0.02$). Results are expressed as mean ± SE of four replicate perifusions.

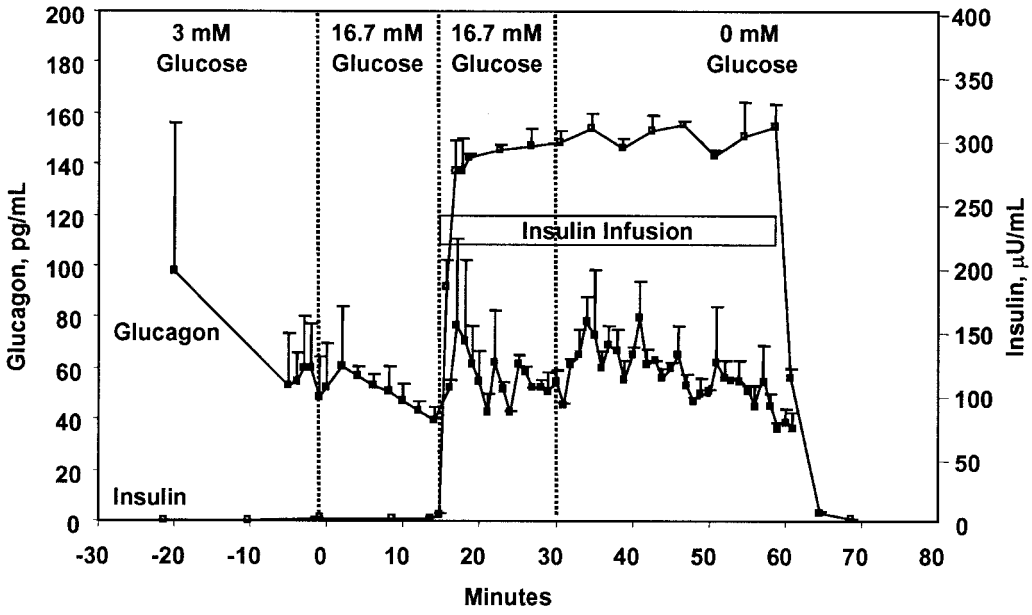

FIG. 5. Islets from STZ-administered rats were perifused with buffer containing 3 mmol/l glucose, 16.7 mmol/l glucose, and then switched to 0 mmol/l glucose. Exogenous insulin (300 μU/ml) was infused for the last 15 min of the 16.7-mmol/l glucose period and then continued throughout the 0-mmol/l glucose period. Results are expressed as mean ± SD of two replicate perifusions.

[AICAR]) and phlorizin. This combination induced moderate and equivalent hypoglycemia in both diabetic and nondiabetic animals in the absence of marked hyperinsulinemia. Glucagon responses were improved during the hypoglycemia caused by these drugs, and the improvement was attenuated by infusion of exogenous insulin. The authors concluded that α-cell glucagon secretion and response to hypoglycemia are not defective in this diabetic model if intraislet hyperinsulinemia is prevented. Banarer et al. (15) reported a decreased glucagon response to hypoglycemia during tolbutamide infusion in normal subjects, suggesting that enhanced β-cell secretion of insulin dampens the glucagon response to hypoglycemia. These latter two reports provided strong evidence supporting the switch-off hypothesis. Our studies, which fully support the switch-off hypothesis, more directly assessed this issue by providing insulin to and then removing it from islets from β-cell–depleted diabetic animals.

The role of the central nervous system as a regulator of glucagon secretion is a critically important one. The degree to which the central nervous system is required for normal glucagon responses in vivo during hypoglycemia has recently been reviewed by Taborsky et al. (1). The authors considered three different mechanisms for regulation of the α-cell response to hypoglycemia: direct

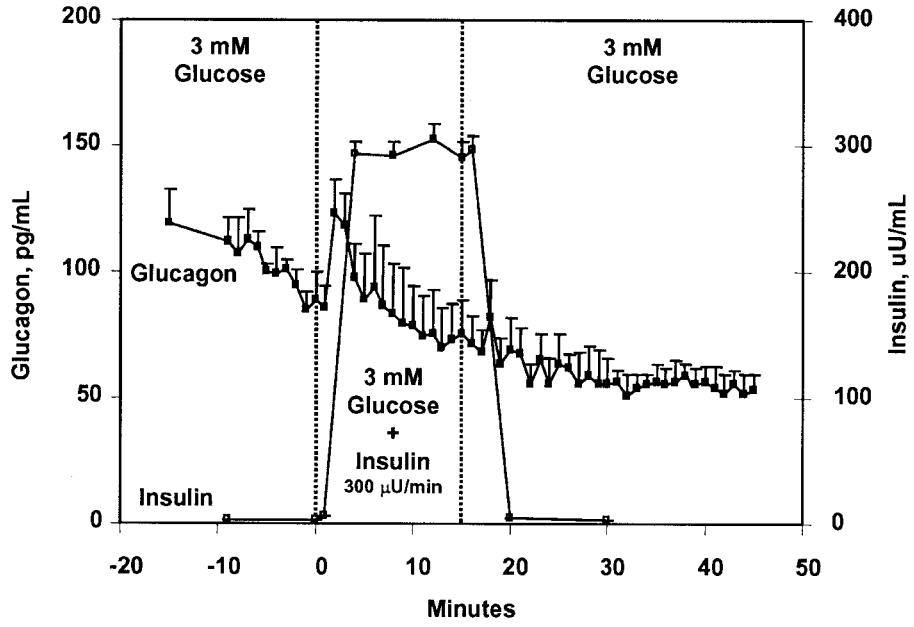

FIG. 6. Islets isolated from STZ-administered rats were perifused with buffer containing 3 mmol/l glucose, while 300 μU/ml exogenous insulin was infused and then switched off after 15 min, and then 3 mmol/l glucose was continued. No glucagon response was observed. Results are expressed as mean ± SD of three duplicate perifusions.

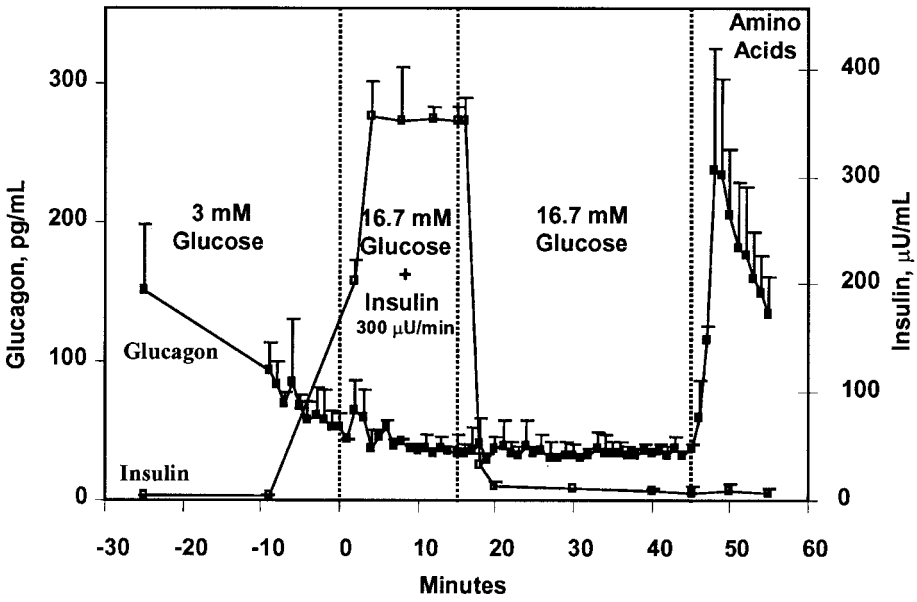

FIG. 7. Islets isolated from STZ-administered rats were perifused with buffer containing 16.7 mmol/l glucose, while 300 μU/ml exogenous insulin was infused and then switched off after 15 min. A mixture of amino acids was infused for 10 min. No glucagon response was observed after insulin was switched off, although a response to amino acids was present. Results are expressed as mean ± SE of two replicate perifusions.

stimulation of glucagon secretion by low glucose concentrations, local effects of endogenous insulin secretion on neighboring α-cells, and circulating epinephrine as well as autonomic inputs to the α-cells via sympathetic and parasympathetic nerves. They reviewed studies (32–37) that have examined the relative importance of the autonomic nervous system in the regulation of glucagon secretion during hypoglycemia through the use of surgical techniques and pharmacologic agents. One of the strongest arguments that the central nervous system may play the dominant role in regulating α-cell responses to hypoglycemia has been the failure to observe glucagon secretion from isolated islets when they are exposed to very low glucose concentrations. The results described in this work show for the first time that isolated islets can secrete glucagon during glucose deprivation. This result clearly demonstrates that the central nervous system and circulating epinephrine are not required for the glucagon response to hypoglycemia. This finding is consistent with the observation made by Diem et al. (38) that human recipients of ectopically placed and denervated pancreas transplants have intact glucagon responses to hypoglycemia, even during infusion of intravenous propranolol, which blocks a possible glucagon-stimulatory contribution of circulating epinephrine.

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REFERENCES

1. Taborsky GJ Jr, Ahren B, Havel PJ: Autonomic mediation of glucagon secretion during hypoglycemia: implications for impaired α-cell responses in type 1 diabetes. *Diabetes* 47:995–1005, 1998
2. Gerich JE, Langlois M, Noacco C, Karam JH, Forsham PH: Lack of glucagon response to hypoglycemia in diabetes: evidence for an intrinsic pancreatic alpha cell defect. *Science* 182:171–173, 1973
3. Fukuda M, Tanaka A, Tahara Y, Ikegami H, Yamamoto Y, Kumahara Y, Shima K: Correlation between minimal secretory capacity of pancreatic β-cells and stability of diabetic control. *Diabetes* 37:81–88, 1988
4. Diamond MP, Hallarman L, Starick-Zych K, Jones TW, Connolly-Howard M, Tamborlane WV, Sherwin RS: Suppression of counterregulatory hormone response to hypoglycemia by insulin per se. *J Clin Endocrinol Metab* 72:1388–1390, 1991
5. Liu D, Moberg E, Kollind M, Lins PE, Adamson U: A high concentration of circulating insulin suppresses the glucagon response to hypoglycemia in normal man. *J Clin Endocrinol Metab* 73:1123–1128, 1991
6. Rizza RA, Cryer PE, Gerich JE: Role of glucagon, catecholamines, and growth hormone in human glucose counterregulation. *J Clin Invest* 64:62–71, 1979
7. Liu DT, Adamson UC, Lins PE, Kollind ME, Moberg EA, Andreasson K: Inhibitory effect of circulating insulin on glucagon secretion during hypoglycemia in type 1 diabetic patients. *Diabetes Care* 15:59–65, 1992
8. Mellman MJ, Davis MR, Shamoon H: Effect of physiological hyperinsulinemia on counterregulatory hormone responses during hypoglycemia in humans. *J Clin Endocrinol Metab* 75:1293–1297, 1992
9. Davis MR, Mellman M, Shamoon H: Physiologic hyperinsulinemia enhances counterregulatory hormone responses to hypoglycemia in IDDM. *J Clin Endocrinol Metab* 76:1383–1385, 1993
10. Davis SN, Goldstein RE, Jacobs J, Price L, Wolfe R, Cherrington AD: The effects of differing insulin levels on the hormonal and metabolic response to equivalent hypoglycemia in normal humans. *Diabetes* 42:263–272, 1993
11. Davis SN, Goldstein RE, Price L, Jacobs J, Cherrington AD: The effects of insulin on the counterregulatory response to equivalent hypoglycemia in patients with insulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 77:1300–1307, 1993
12. Peacey SR, Rostami-Hodjegan A, George E, Tucker GT, Heller SR: The use of tolbutamide-induced hypoglycemia to examine the intraislet role of insulin in mediating glucagon release in normal humans. *J Clin Endocrinol Metab* 82:1458–1461, 1997
13. Landstedt-Hallin L, Adamson U, Lins PE: Oral glibenclamide suppresses glucagon secretion during insulin-induced hypoglycemia in patients with type 2 diabetes. *J Clin Endocrinol Metab* 84:3140–3145, 1999
14. Segel SA, Paramore DS, Cryer PE: Hypoglycemia-associated autonomic failure in advanced type 2 diabetes. *Diabetes* 51:724–733, 2002
15. Banarer S, McGregor VP, Cryer PE: Intraislet hyperinsulinemia prevents the glucagon response to hypoglycemia despite an intact autonomic response. *Diabetes* 51:958–965, 2002
16. Zhou H, Tran POT, Yang S, Zhang T, LeRoy E, Oseid E, Robertson RP: Regulation of α-cell function by the β-cell during hypoglycemia in Wistar rats: the "switch-off" hypothesis. *Diabetes* 53:1482–1487, 2004
17. Lacy PE, Kostianovsky M: Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* 16:35–39, 1969

18. Morgan CR, Lazarow A: Immunoassay of insulin: two antibody system: plasma insulin levels of normal, subdiabetic, and diabetic rats. *Diabetes* 12:115–126, 1963
19. Harris, V Faloona GR, Unger RH: Glucagon. In *Methods of Hormone Radioimmunoassay*. Jaffe BM, Behrman HR, Eds. New York, Academic Press, 1979, p. 643
20. Weir GC, Knowlton SD, Atkins RF, McKennan KX, Martin DB: Glucagon secretion from the perfused pancreas of streptozotocin-treated rats. *Diabetes* 25:275–282, 1976
21. Stagner JI, Samols E: Retrograde perfusion as a model for testing the relative effects of glucose versus insulin on the A cell. *J Clin Invest* 77:1034–1037, 1986
22. Maruyama H, Hisatomi A, Orci L, Grodsky GM, Unger RH: Insulin within islets is a physiologic glucagon release inhibitor. *J Clin Invest* 74:2296–2299, 1984
23. Asplin CM, Paquette TL, Palmer JP: In vivo inhibition of glucagon secretion by paracrine beta cell activity in man. *J Clin Invest* 68:314–318, 1981
24. Gorus FK, Malaisse WJ, Pipeleers DG: Differences in glucose handling by pancreatic A- and B-cells. *J Biol Chem* 259:1196–1200, 1984
25. Van Schravendijk CF, Foriers A, Hooghe-Peters EL, Rogiers V, De Meyts P, Sodoyez JC, Pipeleers DG: Pancreatic hormone receptors on islet cells. *Endocrinology* 117:841–848, 1985
26. Starks A, Imamura T, Unger RH: Relationship of glucagon suppression by insulin and somatostatin to the ambient glucose concentration. *J Clin Invest* 79:20–24, 1987
27. Shi ZQ, Rastogi KS, Lekas M, Efendic S, Drucker DJ, Vranic M: Glucagon response to hypoglycemia is improved by insulin-independent restoration of normoglycemia in diabetic rats. *Endocrinology* 137:3193–3199, 1996
28. Heimberg H, De Vos A, Moens K, Quartier E, Bouwens L, Pipeleers D, Van Schaftingen E, Madsen O, Schuit F: The glucose sensor protein glucokinase is expressed in glucagon-producing alpha-cells. *Proc Natl Acad Sci U S A* 93:7036–7041, 1996
29. Burcelin R, Thorens B: Evidence that extrapancreatic GLUT2-dependent glucose sensors control glucagon secretion. *Diabetes* 50:1282–1289, 2001
30. Gustavson SM, Nishizawa M, Farmer B, Neal D, Brissova M, Powers AC, Cherrington AD: A fall in portal vein insulin does not cause the alpha-cell response to mild, non-insulin-induced hypoglycemia in conscious dogs. *Metabolism* 52:1418–1425, 2003
31. McCrimmon RJ, Evans ML, Jacob RJ, Fan X, Zhu Y, Shulman GI, Sherwin RS: AICAR and phlorizin reverse the hypoglycemia-specific defect in glucagon secretion in the diabetic BB rat. *Am J Physiol Endocrinol Metab* 283:E1076–E1083, 2002
32. Hisatomi A, Maruyama H, Orci L, Vasko M, Unger RH: Adrenergically mediated intrapancreatic control of the glucagon response to glucopenia in the isolated rat pancreas. *J Clin Invest* 75:420–426, 1985
33. Biggers DW, Myers SR, Neal D, Stinson R, Cooper NB, Jaspan JB, Williams PE, Cherrington AD, Frizzell RT: Role of brain in counterregulation of insulin-induced hypoglycemia in dogs. *Diabetes* 38:7–16, 1989
34. Havel PJ, Veith RC, Dunning BE, Taborsky GJ Jr: Role for autonomic nervous system to increase pancreatic glucagon secretion during marked insulin-induced hypoglycemia in dogs. *Diabetes* 40:1107–1114, 1991
35. Davis SN, Colburn C, Dobbins R, Nadeau S, Neal D, Williams P, Cherrington AD: Evidence that the brain of the conscious dog is insulin sensitive. *J Clin Invest* 95:593–602, 1995
36. Havel PJ, Valverde C: Autonomic mediation of glucagon secretion during insulin-induced hypoglycemia in rhesus monkeys. *Diabetes* 45:960–966, 1996
37. Davis SN, Dunham B, Walmsley K, Shavers C, Neal D, Williams P, Cherrington AD: Brain of the conscious dog is sensitive to physiological changes in circulating insulin. *Am J Physiol* 272:E567–E575, 1997
38. Diem P, Redmon JB, Abid M, Moran A, Sutherland DE, Halter JB, Robertson RP: Glucagon, catecholamine and pancreatic polypeptide secretion in type I diabetic recipients of pancreas allografts. *J Clin Invest* 86:2008–2013, 1990