Glucagon-Like Peptide-1 Stimulates Insulin Secretion by a Ca^{2+}-Independent Mechanism in Zucker Diabetic Fatty Rat Islets of Langerhans

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This study investigates the mechanisms responsible for glucagon-like peptide-1 (GLP-1)-induced insulin secretion in Zucker diabetic fatty (ZDF) rats and their lean control (ZLC) littermates. Glucose, and 100 nmol/L GLP-1 (7-37 hydroxide) in the presence of stimulatory glucose concentrations, induced insulin secretion in islets from ZLC animals. In contrast, ZDF islets hypersecreted insulin at low glucose (5 mmol/L) and were poorly responsive to 15 mmol/L glucose stimulation, but increased insulin secretion following exposure to GLP-1. The insulin secretory response to 100 nmol/L GLP-1 was reduced by 88% in ZLC islets exposed to exendin 9-39. The intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) increased in fura-2-loaded ZLC islets following stimulation with 12 mmol/L glucose alone or GLP-1 in the presence of 12 mmol/L glucose. The increases in $[Ca^{2+}]_i$ and insulin secretion in ZLC islets induced by GLP-1 were attenuated by 1 μmol/L nitrendipine. In contrast, neither glucose nor GLP-1 substantially increased $[Ca^{2+}]_i$ in ZDF islets. Furthermore, insulin secretory responses to GLP-1 were not significantly inhibited in ZDF islets by nitrendipine. However, the insulin secretory response to GLP-1 in both ZLC and ZDF islets was ablated by cholera toxin. Our findings indicate that in ZLC islets, GLP-1 induces insulin secretion by a mechanism that depends on Ca^{2+} influx through voltage-dependent Ca^{2+} channels, whereas in ZDF islets, the action of GLP-1 is mediated by Ca^{2+}-independent signaling pathways.

INCRETIN PEPTIDE HORMONES are released by intestinal endocrine cells and potentiate glucose-induced insulin secretion from pancreatic β cells. Two glucagon-like incretin peptides, GLP-1 [7-37] and GLP-1 [7-36]-amide, are synthesized in the L cells of the distal ileum and proximal colon and are potent insulin secretagogues.[1,2] Secreted postprandially, GLP-1 increases insulin biosynthesis and proinsulin gene expression,[3] augments insulin[1,4] and somatostatin[4] secretion, and inhibits glucagon[1] and gastric acid[5] release. Other effects of GLP-1 may include a role in the differentiation of pancreatic precursors into islet cells,[6] and the hormone also appears to be an important physiologic regulator of satiety.[7] Some of the effects of GLP-1 may be mediated through upregulation of the transcription factor PDX-1, an effect that has been observed in insulinoma cells.[8,9]

GLP-1 has been proposed as an insulin secretagogue for use in the treatment of non–insulin-dependent diabetes mellitus. Studies in animal models of type 2 diabetes and in humans with type 2 diabetes have shown that exogenous GLP-1 infusion reduces hyperglycemia by stimulating insulin release from the pancreatic β cell.[10-12] However, despite the documented ability of GLP-1 to increase insulin secretion in type 2 diabetes, when insulin secretory responses to glucose are lost or attenuated, the underlying mechanisms responsible for this effect have not been characterized.

The effects of GLP-1 on insulin secretion have been studied in vivo in humans and laboratory animals[10,12] and in vitro in insulinoma cell lines, primary β-cell cultures, whole islets of Langerhans, and perfused pancreata.[3,13-16] GLP-1-stimulated insulin release is glucose-dependent[17] and requires the binding of GLP-1 to a G-protein–coupled cell-surface receptor containing 7 membrane-spanning domains.[18-20] Although the effector domains of the receptor are linked to both the adenylate cyclase[21-24] and phospholipase C signaling pathways,[18,23] the relative contribution of these two signal transduction systems in the regulation of GLP-1 stimulus-secretion coupling in β cells remains unclear. Several studies report increases in the cytoplasmic levels of cyclic adenosine monophosphate (cAMP) following GLP-1 stimulation[22-24] and suggest that this step is necessary for increasing insulin secretion. Other evidence indicates an important role of increases in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in mediating GLP-1 responses.[21,25-27]

In this study, we present evidence that GLP-1 elicits insulin secretory responses from nondiabetic Zucker lean control (ZLC) rat islets by a Ca^{2+}-dependent mechanism involving the influx of extracellular Ca^{2+} through L-type voltage-dependent Ca^{2+} channels. However, in islets isolated from Zucker diabetic fatty (ZDF) rats, an animal model of type 2 diabetes in which the expression of voltage-dependent calcium channels is reduced, GLP-1-stimulated insulin secretion is mediated by pathways that are not critically dependent on increases in $[Ca^{2+}]_i$.

MATERIALS AND METHODS

Animals

Experiments were performed in perifused islets isolated from 10- to 14-week-old ZLC and ZDF rats (GMI, Indianapolis, IN).

Isolation of Islets of Langerhans

Islets were isolated by collagenase digestion and differential centrifugation through Ficoll (Pharmacia, Piscataway, NJ) gradients as described previously.[28] Islets were placed into tissue culture plates containing RPMI 1640 supplemented with 10% fetal calf serum, 100 μU/mL penicillin, and 100 μg/mL streptomycin and incubated with 95% air:5% CO_2 at 37°C in a humidified incubator for 2 to 4 hours prior to the experiments.

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Determination of Insulin Release From Isolated Islets

Secretion of insulin from perifused islets was measured using a temperature-controlled multichamber perifusion system (ACUSYST-S; Cellex Biosciences, Minneapolis, MN). Groups of 25 islets were suspended in Bio-Gel P2 beads and Krebs-Ringer buffer (KRB) containing 2 mmol/L glucose and 5 mg/mL bovine serum albumin (BSA). Following an equilibration period during which the islets were perifused for 30 minutes with KRB containing 2 mmol/L glucose, sampling of the effluent perifusate was initiated at 1-minute intervals. After the baseline period, KRB containing 12 mmol/L glucose, 12 mmol/L glucose and 100 nmol/L GLP-1 was perifused successively. In some experiments, potential inhibitors of GLP-1-induced insulin secretion were perifused for 2 to 5 minutes in 12 mmol/L glucose prior to the introduction of GLP-1. The insulin concentration of the effluent perifusate was measured by radioimmunoassay and is expressed as pmol/L per 25 islets.

The concentration of GLP-1, which is higher than that used in some other studies, was the level that produced the maximum increase in $[Ca^{2+}]_i$ in ZLC islets in preliminary experiments. Since the concentration of GLP-1 used in this study could potentially lead to activation of the glucagon receptor, additional experiments were performed on ZLC islets to determine whether the response to GLP-1 could be inhibited by the specific GLP-1 inhibitor, exendin 9-39. Batch incubations were performed on groups of 25 islets from 5 ZLC rats. The islets were preincubated in KRB containing 5 mmol/L HEPES, 5 mg/mL BSA, and 2 mmol/L glucose for 30 minutes at 37°C, after which they were transferred to fresh tubes containing KRB with either 2 mmol/L glucose, 12 mmol/L glucose, 12 mmol/L glucose and 100 nmol/L GLP-1, or 12 mmol/L glucose, GLP-1 and 100 nmol/L exendin 9-39 (Sigma, St Louis, MO) and incubated for 1 hour in a shaking water bath at 37°C. Following the incubation, the islets were placed on ice and an aliquot of KRB was removed for measurement of the insulin concentration as already described. The islets were washed twice in PBS, and the DNA content of the islets was measured as described previously.[29] Results are expressed as picomoles of insulin per liter per nanogram of DNA.

Islet $[Ca^{2+}]_i$ Determination

Islets from ZLC and ZDF rats were isolated, established in primary culture, and loaded with fura-2 acetoxymethylester (Molecular Probes, Eugene, OR) for 25 minutes as described previously.[30] Islets were perifused at a flow rate of 2.5 mL/min (37°C) with KRB (without BSA) in a temperature-controlled microperifusion chamber (Medical Systems, Greenvale, NY) mounted on an inverted microscope (Nikon, Melville, NY) equipped for epifluorescence. Fura-2 dual-wavelength excitation photometry was used to measure individual islet $[Ca^{2+}]_i$. Results are expressed as the ratio of the emission light intensity (detected at 510 nm) following excitation at 340 nm and 380 nm (ratio 340/380).

Statistics

The mean value for insulin secretion by islets from ZLC and ZDF rats under basal conditions (low glucose) and in response to stimulation by glucose and other secretagogues was calculated. The response to each secretagogue was expressed as a ratio of the insulin secretion during administration of the secretagogue to the mean measured during ''steady-state'' conditions (ie, 5 minutes prior to the addition of secretagogues). The results were analyzed with paired *t* tests or the nonparametric Wilcoxon rank sum test depending on whether the data were normally distributed. *P* values less than .05 indicated statistical significance. Results are expressed as the mean ± SEM.

RESULTS

Animal Weight and Glucose Concentration

The mean age of the animals used in this study was 12.5 weeks. ZDF rat weight was 15% greater and ZDF nonfasted blood glucose was 3.6-fold higher versus age-matched ZLC rats (Table 1).

Effect of GLP-1 on Insulin Secretion by Perifused Islets

Relative to control islets, mean basal insulin secretion (2 mmol/L glucose in perifusate) was 5-fold higher in ZDF islets (139.8 ± 84 *v* 28.2 ± 4.8 pmol/L/25 islets, $P < .05$) and they were poorly responsive to a step-increase in glucose from 2 to 12 mmol/L (the mean increase was only 1.3 ± 0.2-fold, *v* a 5.3 ± 0.5-fold increase in insulin secretion in ZLC islets, $P < .05$; Fig 1). The application of 100 nmol/L GLP-1 in the presence of 12 mmol/L glucose stimulated a 1.8 ± 0.3-fold increase in insulin secretion from ZDF islets ($P < .05$ for mean perifusate insulin secretion in response to GLP-1 *v* prior to addition of the incretin). Although the absolute insulin values in response to GLP-1 tended to be lower in ZDF islets versus ZLC islets, they were not statistically different. The increase over basal conditions therefore was not significantly different from that observed in ZLC islets (2.5-fold increase in insulin secretion in response to GLP-1, $P > .05$).

Static incubations were performed in islets isolated from 5 ZLC rats to determine if the insulin secretory response to 100 nmol/L GLP-1 could be inhibited by exendin 9-39. The mean insulin secretion in the presence of 2 mmol/L glucose was 18.6 ± 5.4 pmol/L/ng DNA. In the presence of 12 mmol/L glucose, the mean insulin secretion was 285.6 ± 45.0 pmol/L/ng DNA, and this was further increased in the presence of 100 nmol/L GLP-1 to 582 ± 71.4 pmol/L/ng DNA ($P < .05$ *v* islets incubated in the presence of 12 mmol/L glucose alone). The addition of exendin 9-39 to the incubation medium reduced insulin secretion by 88% to 321.6 ± 86.4 pmol/L/ng DNA ($P < .05$), which was not significantly different from the amount of insulin secreted in the presence of 12 mmol/L glucose alone. Thus, exendin 9-39 inhibited the additional insulin secretory response induced by 100 nmol/L GLP-1, suggesting that even at this high concentration it acts through its own receptor.

The role of Ca^{2+} in mediating GLP-1-induced insulin secretion from perifused ZLC islets was investigated by reducing the extracellular Ca^{2+} concentration with EGTA and inhibiting Ca^{2+} influx through voltage-dependent calcium channels with nitrendipine. The insulin secretory response of ZLC and ZDF islets to GLP-1 was measured in the presence and absence of 2.5 mmol/L EGTA. GLP-1 alone induced a 3.6 ± 0.01-fold increase in insulin secretion in ZLC islets. The addition of EGTA to the perifusate completely ablated the GLP-1-induced

Table 1. Age, Weight, and Blood Glucose in ZDF and ZLC Rats

Parameter	ZLC (n = 19)	ZDF (n = 18)
Age (d)	88.8 ± 4.3	89.5 ± 4.1
Weight (g)	340.6 ± 10.8	391.4 ± 9.6*
Blood glucose (mmol/L)	8.5 ± 0.6	30.5 ± 2.3†

*$P = .002$ *v* ZLC.
†$P = .0001$ *v* ZLC.

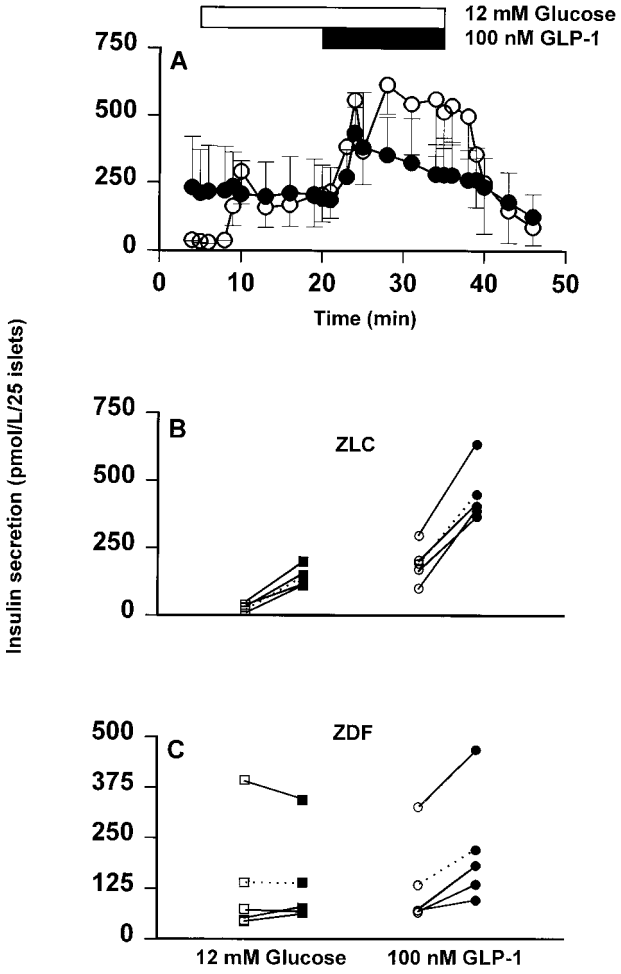

Fig 1. (A) Insulin secretion from perifused ZLC (○) and ZDF (●) islets in response to 12 mmol/L glucose (6-20 min) and 100 nmol/L GLP-1 (21-35 min) in the continued presence of 12 mmol/L glucose. Perifusate contained 2 mmol/L glucose unless otherwise indicated. Data are the mean ± SEM of 4 experiments in each group. (B and C) Insulin secretion from perifused islets of ZLC and ZDF rats under basal conditions (□, ○), in response to an increase in perifusate glucose from 2 to 12 mmol/L (■), and in response to stimulation by 100 nmol/L GLP-1 in the presence of 12 mmol/L glucose (●). (——) Individual data; (----) group means.

increase in secretion and was associated with a 27% reduction in secretion as compared with basal levels ($P < .05$, n = 3 per group). Insulin secretion increased 2.6 ± 1.3-fold in response to GLP-1 in the absence of EGTA in ZDF islets. In contrast to the complete ablation of the insulin secretory response to GLP-1 in the presence of EGTA, there remained a small but significant increase in insulin secretion in ZDF islets (1.3 ± 0.3-fold increase, n = 4 per group, $P > .05$ *v* islets incubated in the absence of EGTA). In ZLC islets, the mean absolute perifusate insulin concentration increased 2.2-fold in response to GLP-1 in the absence of nitrendipine (Fig 2). In contrast, in the presence of 1 μmol/L nitrendipine, the insulin secretory response to GLP-1 was completely ablated (in fact, there was a 22% reduction in secretion *v* basal levels, $P < .03$). Comparing the relative change in insulin secretion in response to GLP-1 in ZDF rats, there was a mean 2.2 ± 0.5-fold increase over basal in the presence of nitrendipine as compared with a 3.1 ± 0.9-fold increase in the absence of nitrendipine ($P > .05$). Therefore, our findings suggest that GLP-1-dependent stimulus-secretion coupling mechanisms are different in ZDF islets versus ZLC islets: GLP-1-stimulated insulin secretion from ZLC islets is critically dependent on an increase in $[Ca^{2+}]_i$ consequent to Ca^{2+} influx through L-type voltage-dependent Ca^{2+} channels, whereas GLP-1 effects on ZDF islet insulin secretion are mediated predominantly by Ca^{2+}-independent mechanisms.

Effect of GLP-1 on Islet $[Ca^{2+}]_i$

To determine more directly if GLP-1-induced insulin secretion from ZDF islets is Ca^{2+}-independent, fluorescence microspectrophotometry was used to measure the effect of GLP-1 on islet $[Ca^{2+}]_i$. Glucose stimulation (12 mmol/L) alone caused an increase in $[Ca^{2+}]_i$ in all ZLC islets studied (n = 30). In the presence of 12 mmol/L glucose, 100 nmol/L GLP-1 also caused an additional increase in $[Ca^{2+}]_i$ in 85% of the ZLC islets (Fig 3A). The addition of GLP-1 in low glucose (2 mmol/L) was without effect (data not shown). In ZDF islets, glucose-induced increases in $[Ca^{2+}]_i$ were attenuated (mean peak increase of 340/380 ratio, 0.64 ± 0.1 in ZLC islets *v* 0.12 ± 0.03 in ZDF islets) and GLP-1 stimulated a detectable increase in $[Ca^{2+}]_i$ in only 8% of the ZDF islets examined (n = 20, peak increase, 0.06 ± 0.02 *v* 0.26 ± 0.08 in ZLC islets, $P < .05$; Fig 3B). In agreement with our previous findings,[31] ZLC and ZDF islets responded similarly following stimulation with carbachol (data not shown). These results clearly demonstrate that GLP-1 stimulation of ZLC rat islets increases $[Ca^{2+}]_i$, whereas in ZDF islets, it causes little or no elevation in $[Ca^{2+}]_i$.

We investigated the mechanisms responsible for GLP-1-induced increases in ZLC islets $[Ca^{2+}]_i$. The GLP-1 response was eliminated by nitrendipine (1 μmol/L; Fig 3C). Consistent with the results of our insulin secretion studies, the fura-2 experiments indicate that the GLP-1-induced increase in islet $[Ca^{2+}]_i$ is mediated by Ca^{2+} influx through L-type voltage-dependent Ca^{2+} channels, and does not occur by the release of intracellular Ca^{2+} stores.

Effect of Cholera Toxin on Glucose- and GLP-1-Induced Insulin Secretion

Following incubation of ZDF islets overnight at 11.6 mmol/L glucose, there was a more robust stimulation of insulin secretion in response to glucose stimulation (2.9 ± 1.0-fold; Fig 4) versus that observed in freshly isolated islets (1.3 ± 0.2; Fig 1), largely as a result of lower basal insulin secretion. This is in contrast to the situation in ZLC islets, in which the responsiveness to glucose was similar in fresh (5.3 ± 0.5-fold increase; Fig 1) and cultured islets (6.4 ± 1.2-fold increase; Fig 4). However, the absolute levels of insulin secretion in response to glucose were significantly lower in cultured ZLC islets (mean insulin secretion during exposure to 12 mmol/L glucose, 29.58 ± 0.3 *v* 146.88 ± 19.2 pmol/L/25 islets, $P < .05$). In cholera toxin–treated ZLC islets, insulin secretion increased 22.2 ± 10.7-fold in response to 12 mmol/L glucose ($P < .05$ *v* control untreated islets). The insulin secretory response to glucose was unaffected by exposure to cholera toxin in ZDF islets. Exposure of ZLC and ZDF islets to cholera toxin (1 μg/mL) completely ablated GLP-1-stimulated insulin secretion (Fig 4). Following an

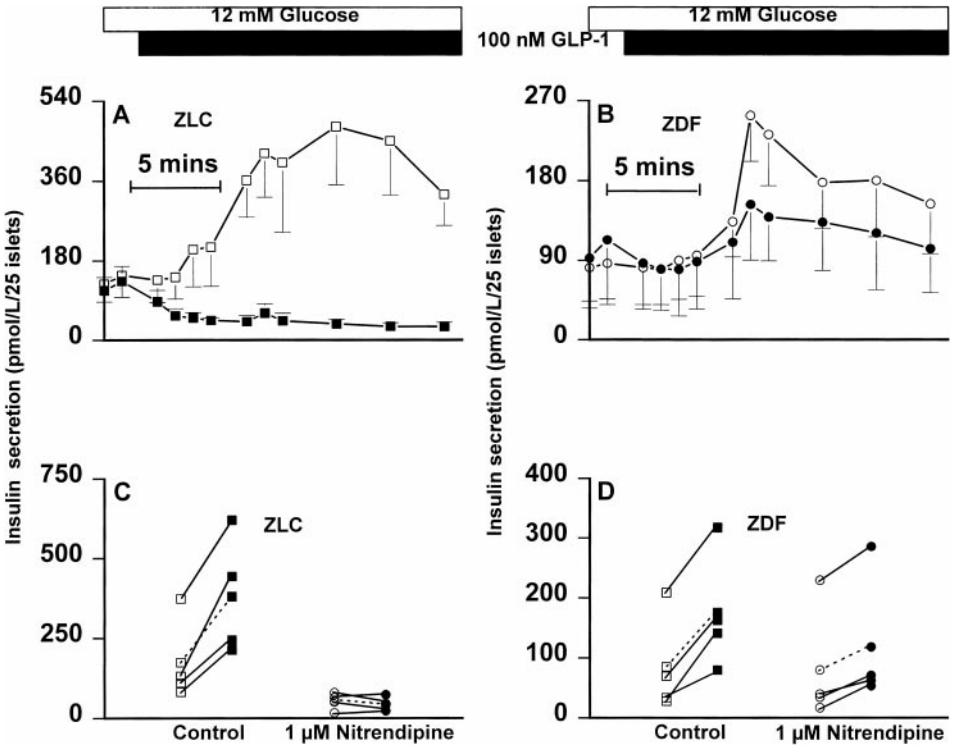

Fig 2. Effect of Ca^{2+} channel blockade on GLP-1-induced insulin secretion by perifused islets. (A and B) Insulin secretion from ZLC and ZDF islets in response to 100 nmol/L GLP-1 (■ in the presence of 12 mmol/L glucose. GLP-1 was administered either in the absence (□,○) or presence (■,●) of 1 μmol/L nitrendipine. Data represent the mean ± SEM of 4 experiments per group. (C and D) Insulin secretion in groups of islets from ZLC and ZDF rats before (□,○) and after (■,●) stimulation with 100 nmol/L GLP-1 and in the absence (□,■) and presence (○,●) of 1 μmol/L nitrendipine. (——) Individual data; (----) group means.

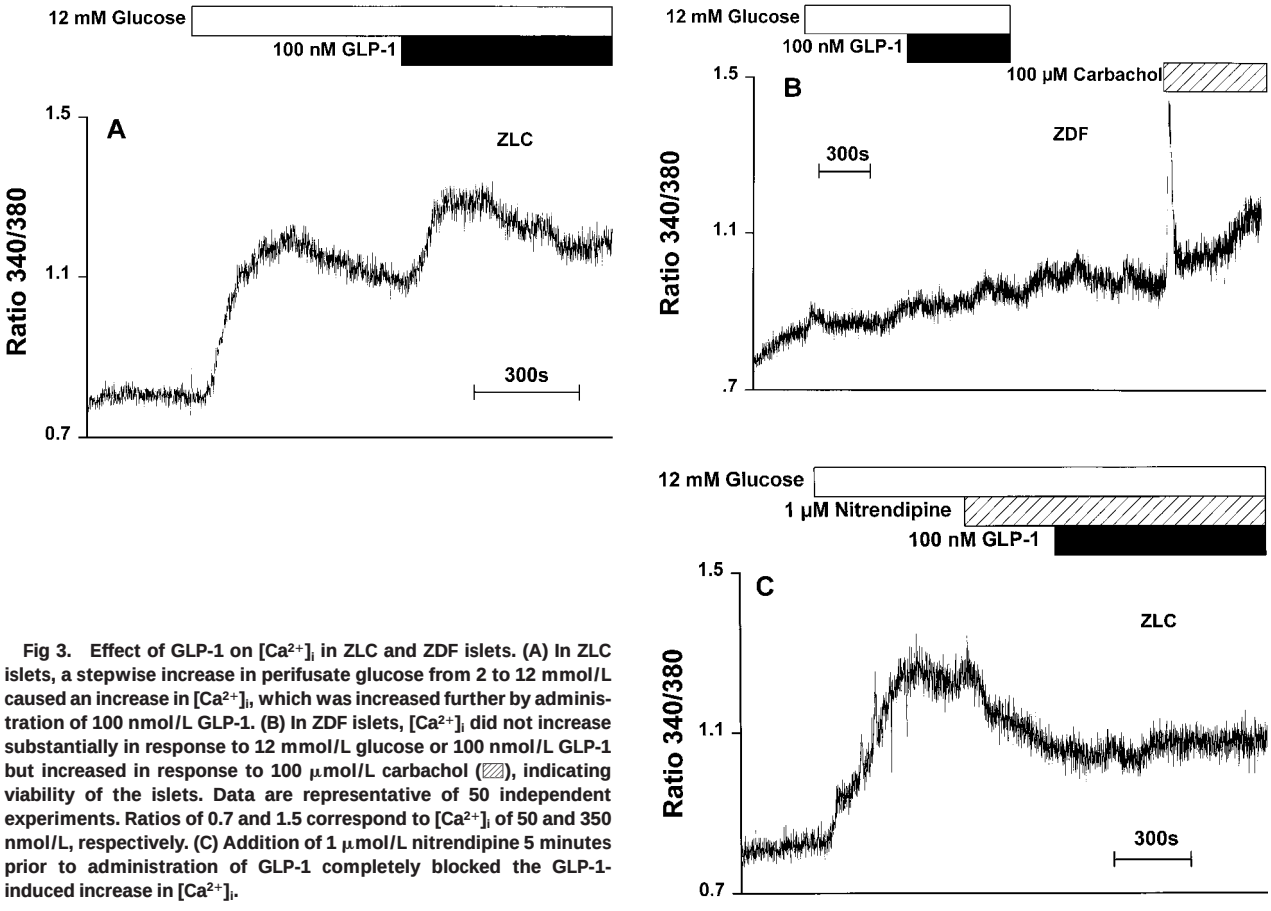

Fig 3. Effect of GLP-1 on $[Ca^{2+}]_i$ in ZLC and ZDF islets. (A) In ZLC islets, a stepwise increase in perifusate glucose from 2 to 12 mmol/L caused an increase in $[Ca^{2+}]_i$, which was increased further by administration of 100 nmol/L GLP-1. (B) In ZDF islets, $[Ca^{2+}]_i$ did not increase substantially in response to 12 mmol/L glucose or 100 nmol/L GLP-1 but increased in response to 100 μmol/L carbachol (▨), indicating viability of the islets. Data are representative of 50 independent experiments. Ratios of 0.7 and 1.5 correspond to $[Ca^{2+}]_i$ of 50 and 350 nmol/L, respectively. (C) Addition of 1 μmol/L nitrendipine 5 minutes prior to administration of GLP-1 completely blocked the GLP-1-induced increase in $[Ca^{2+}]_i$.

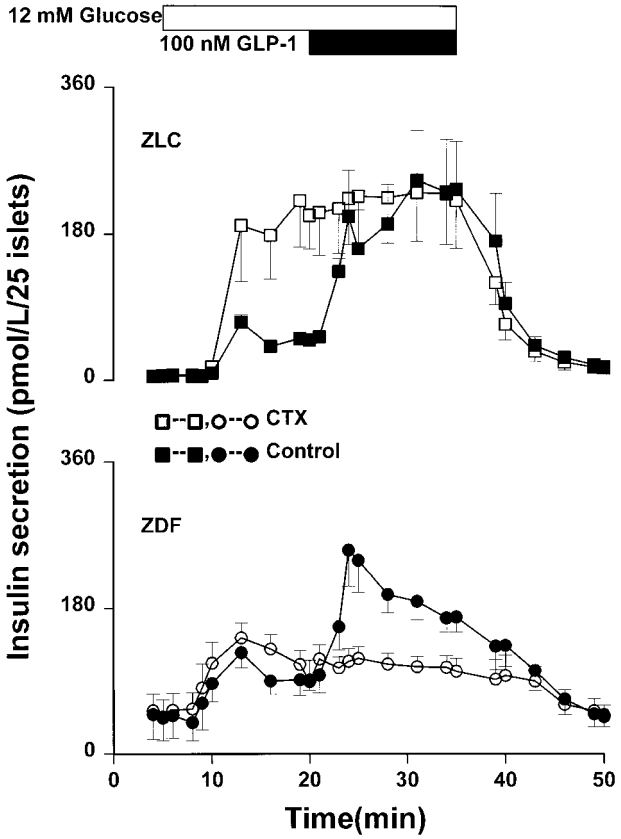

Fig 4. Effect of cholera toxin (CTX) on GLP-1-induced insulin secretion by ZDF and ZLC islets. Prior to the experiments, islets were incubated overnight (18 hours) in RPMI medium supplemented with 10% fetal calf serum and 11.6 mmol/L glucose in the presence or absence of 1 μg/mL CTX. Insulin secretion by islets from ZLC rats (n = 3, top) and ZDF rats (n = 4, bottom) was measured under basal conditions (2 mmol/L glucose) and in response to 12 mmol/L glucose and 100 nmol/L GLP-1.

overnight incubation of ZLC islets with cholera toxin, GLP-1 elicited only a 1.1 ± 0.01-fold increase in insulin secretion as compared with a 4.0 ± 1.0-fold increase in islets incubated in the absence of cholera toxin ($P < .05$). In ZDF islets cultured overnight in the absence of cholera toxin, there was 2.4 ± 0.4-fold increase in insulin secretion in response to GLP-1. However, the response to GLP-1 was inhibited by exposure to cholera toxin, which resulted in only a 1.04 ± 0.03-fold increase in insulin secretion in response to GLP-1 ($P < .05$ *v* islets cultured in the absence of cholera toxin). Cholera toxin did not affect the responses to 100 μmol/L carbachol in ZDF islets, thereby eliminating the possibility that the impairment of the response to GLP-1 could be explained by a nonspecific toxic effect of cholera toxin (data not shown).

One possible explanation for the inhibitory effect of cholera toxin on the insulin secretory response to GLP-1 is that the prolonged incubation caused a desensitization of the secretory pathways consequent to a tonic elevation of cyclic adenosine monophosphate (cAMP). Nonetheless, insulin secretion stimulated by increasing intracellular levels of cAMP with 1 mmol/L dibutyryl cAMP (DBcAMP), a membrane-permeant analog of cAMP, was unimpaired in ZDF islets treated with cholera toxin (Fig 5). There was a trend for greater insulin secretion in cholera toxin–treated islets, but no significant difference in the insulin secretory response to DBcAMP ($P > .05$).

DISCUSSION

GLP-1, a potent insulin secretagogue secreted postprandially by intestinal L cells, may prove effective in the treatment of type 2 diabetes.[2,32] While the mechanisms underlying GLP-1 stimulus-secretion coupling in nondiabetic islets and in insulinoma cell lines involve cAMP- and Ca^{2+}-dependent signaling pathways,[33] little is known about GLP-1 signal transduction in diabetic islets. In agreement with previous studies,[21,25-27,34-36] the evidence presented in this report indicates that the increase in glucose-dependent insulin secretion from nondiabetic ZLC rat islets of Langerhans stimulated with GLP-1 is linked to an increase in $[Ca^{2+}]_i$. However, in islets isolated from overtly diabetic ZDF rats, a well-characterized animal model of type 2 diabetes, we found that GLP-1 induced insulin secretion in the absence of increased $[Ca^{2+}]_i$. We have also demonstrated that stimulation of insulin secretion by GLP-1 involves activation of a cholera toxin–sensitive mechanism.

The concentration of GLP-1 used in this study is higher than the level used in many other studies. This concentration was chosen on the basis of preliminary experiments designed to determine what concentration of GLP-1 produces the maximal increase in $[Ca^{2+}]_i$ in ZLC islets. A similar concentration was used in a recent study with human pancreatic islets in which the insulin secretory response to GLP-1 was completely blocked by exendin 9-39.[37] Therefore, together with our data demonstrating a significant attenuation of the insulin secretory response to GLP-1 by exendin 9-39 in ZLC islets, we believe it is likely that even at such high concentrations GLP-1 exerts its insulin stimulatory effect through binding to its own receptor.

There is much evidence to suggest that GLP-1 stimulates insulin secretion through activation of cAMP- and Ca^{2+}-dependent pathways.[38] Our findings in ZLC rat islets strongly

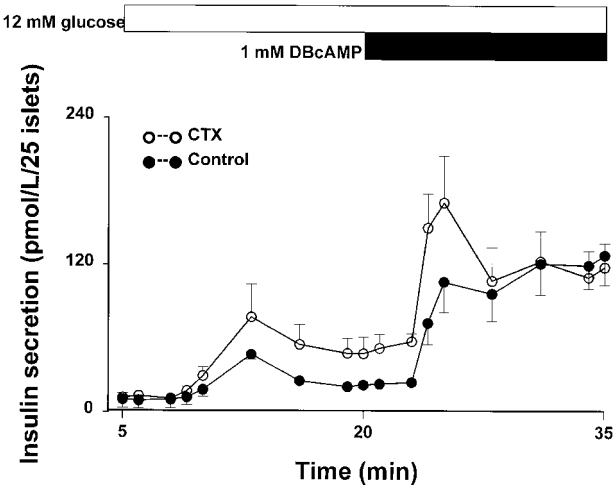

Fig 5. Effect of cholera toxin on DBcAMP-induced insulin secretion by ZDF islets. Islets were incubated overnight in the presence or absence of 1 μg/mL cholera toxin. Insulin secretion by islets from ZDF rats (n = 3) was measured under basal conditions (2 mmol/L glucose, 0-5 min) and in response to 12 mmol/L glucose and 1 mmol/L DBcAMP.

support the hypothesis that GLP-1-stimulated increases in $[Ca^{2+}]_i$ are necessary for insulin secretion. GLP-1-induced elevations in $[Ca^{2+}]_i$ have been reported in COS-7[18,23,24] and human embryonic kidney 293[39] cells transfected with the GLP-1 receptor, as well as cells that express endogenous GLP-1 receptors such as RIN1046-38 cells,[36] HIT cells,[26,35] βTC3[34] and βTC6[26] cells, and rat[25-27,40] and mouse[41] β cells. In sharp contrast to these findings, in COS-7 cells transfected with the GLP-1 receptor[22] and in RINm5F cells,[42] GLP-1 stimulation did not increase $[Ca^{2+}]_i$. GLP-1 (7-36) amide stimulated an increase in cytosolic cAMP levels without affecting either the plasma membrane potential or $[Ca^{2+}]_i$ in RINm5F cells,[42] whereas other investigations in the same cell line demonstrated that GLP-1 stimulation evoked a brisk biphasic increase in insulin secretion in which the early first phase of the secretory response did not depend on the presence of extracellular Ca^{2+}, while the second sustained phase did.[43] Disparities have also appeared in the identification of signal transduction mechanisms in cells transfected with the GLP-1 receptor. Although Chinese hamster lung fibroblasts stably transfected with a cDNA encoding human GLP-1 receptor showed increased cAMP production and $[Ca^{2+}]_i$ following GLP-1 stimulation, similarly transfected COS-7 cells responded with an increase cAMP and no change in $[Ca^{2+}]_i$.[22] A recent study using mouse islets and INS-1 cells suggests that GLP-1 increases $[Ca^{2+}]_i$ by a mechanism that is independent of protein kinase A.[44] These results support the likelihood that the GLP-1 receptor may be coupled to distinct signaling pathways in different cell types.

Although the majority of published data indicate no consistent dissociation between GLP-1 receptor activation and the consequent increase in $[Ca^{2+}]_i$, the underlying mechanisms regulating the increase in $[Ca^{2+}]_i$ remain unclear. Two distinct sources of Ca^{2+} mediate signaling in insulin-secreting cells: extracellular Ca^{2+} and mobilizable Ca^{2+} pools sequestered within the endoplasmic reticulum.[45] GLP-1-induced increases in $[Ca^{2+}]_i$ have been reported to originate from one or both of these sources.[26,33,35,46-48] Evidence suggesting an important role of extracellular Ca^{2+} in GLP-1 stimulus-response coupling was first reported in normal rat islets.[25,43] GLP-1-stimulated increases in rat islet $[Ca^{2+}]_i$ were inhibited by methoxyverapamil, an inhibitor of L-type voltage-dependent Ca^{2+} channels, and by exclusion of Na^+ from the extracellular medium.[25] GLP-1 stimulation of Ca^{2+} influx through voltage-dependent Ca^{2+} channels also has been observed in HIT cells[35] and in single rat pancreatic β cells.[27] Decreasing the extracellular Ca^{2+} concentration with EGTA or exposing HIT cells to nimodipine[35] or rat β cells to nitrendipine[27] completely blocked GLP-1-induced elevations in $[Ca^{2+}]_i$. Different models of GLP-1-induced Ca^{2+} signaling have developed from more recent studies of insulinoma cells[26,34] and rat β cells.[26,40] In these studies, GLP-1 stimulation caused increases in $[Ca^{2+}]_i$ by a combination of increasing plasma membrane conductance and membrane depolarization and release of Ca^{2+} from intracellular stores. However, in accordance with studies demonstrating the dependence of GLP-1 signaling on increases in $[Ca^{2+}]_i$ stemming from Ca^{2+} influx through L-type voltage-dependent Ca^{2+} channels, our findings in the normal ZLC rat islets suggest that this is the predominant source of the increase in $[Ca^{2+}]_i$ induced by GLP-1 in this model, although it is possible that calcium influx via L-type calcium channels could mediate the mobilization of calcium from intracellular stores as previously suggested.[48] The precise reason for this inconsistency remains to be determined, but might be related to the use of different β-cell systems to study GLP-1 signaling.

In islets isolated from diabetic ZDF rats, a different picture of GLP-1 signaling emerged. GLP-1 induced insulin secretion in the absence of an increase in $[Ca^{2+}]_i$. This is consistent with our previous studies of this animal model of type 2 diabetes that showed a loss of L-type voltage-dependent Ca^{2+} channel activity in β cells.[31] Insulin secretion by ZDF islets in the presence of the Ca^{2+} channel blocker, although slightly less, was not significantly attenuated as in ZLC islets, providing further evidence against a critical role for Ca^{2+} in GLP-1 stimulus-secretion coupling in diabetic islets. This difference between ZLC and ZDF islets is unlikely a quantitative one, based purely on islet size, since insulin secretory responses to GLP-1 in islets from nondiabetic Zucker fatty rats, which are of a size similar to or larger than those of ZDF rats, were also completely attenuated by 1 μmol/L nitrendipine (data not shown). It has been suggested that the ability of the increase in cAMP induced by the binding of GLP-1 to its receptor to accelerate insulin granule mobilization may account for as much as 70% of the incretin's stimulatory action on insulin secretion.[38] It is possible that in the absence of L-type calcium channels in ZDF islets,[31] this mechanism of action accounts for an even greater component of the GLP-1-induced insulin secretory response.

A previous study demonstrated a role for nonselective cation channels in the signal transduction pathways of GLP-1 in βTC6 cells.[26] The influx of Na^+ and possibly Ca^{2+} through these channels was associated with increases in $[Ca^{2+}]_i$. Although it is unlikely that such channels play a role in GLP-1 responses in ZLC islets since these are completely inhibited by nitrendipine, it is theoretically possible that such channels could be important in ZDF islets. However, since significant elevations in $[Ca^{2+}]_i$ in ZDF islets were not found in response to GLP-1 using fura-2 to indirectly measure $[Ca^{2+}]_i$ levels, this appears unlikely.

The observation that overnight incubation of diabetic and nondiabetic islets with cholera toxin completely blocked the insulin secretory response to GLP-1 is novel and interesting. This suggests that the coupling of GLP-1 receptor activation to the insulin secretory apparatus is mediated by guanine nucleotide-binding proteins (G proteins), possibly the α-subunit of the stimulatory guanosine triphosphate (GTP)-binding protein, G_S. The mechanisms mediating the attenuation of GLP-1-induced insulin secretion by cholera toxin are uncertain. Cholera toxin causes adenosine diphosphate (ADP)-ribosylation of several membrane-bound G proteins in islets and insulin-secreting cells[49-51] and activates adenylate cyclase in many cell types.[52] However, since DBcAMP elicited considerable increases in insulin secretion from cholera toxin–treated ZDF islets, it is unlikely that the inhibition of the GLP-1 secretory response was caused by persistent cAMP elevation and consequent desensitization of the exocytotic pathway activated by protein kinase A. This conclusion accords with the observation in a transgenic mouse model that expression of the cholera toxin A1 subunit

under the control of the insulin promoter did not augment cAMP production in islet cells but diminished glucose-induced insulin secretion from perfused pancreata.[53] Inhibitory effects of cholera toxin independent of cAMP signaling pathways have been reported in many other cell types, including human T cells,[54] Nb2 T lymphoma cells,[55] rat peritoneal macrophages,[56] and human small-cell carcinoma cell lines,[57] and more recently in the norepinephrine-secreting PC12 cell line.[58] These data could be interpreted as demonstrating that cholera toxin effects on GLP-1-induced insulin secretion either involve G proteins other than G_S or are mediated by direct actions of the toxin on the GLP-1 receptor. In support of the former mechanism, the pancreatic GLP-1 receptor expressed in Chinese hamster ovary cells has been shown to couple to the α-subunits of G_S, G_q, and $G_{i1,2}$ but not G_{i3}.[59] However, it has been shown that long-term exposure to cholera toxin is associated with markedly reduced ADP-ribosylation of $G_{S\alpha}$ and impaired norepinephrine secretion in PC12 cells.[58] In addition, long-term exposure of chick sympathetic neurons to cholera toxin, but not to forskolin or 8-bromo-AMP, was shown to result in translocation of $G_{S\alpha}$ from the cell membrane to the cytosol and ultimately a complete loss of $G_{S\alpha}$ from the neurons.[60] It seems likely that the lack of stimulation of insulin secretion in response to GLP-1 in cholera toxin–treated islets is also secondary to reduced ADP-ribosylation of $G_{S\alpha}$.

The difference in the response of fresh and cultured islets to glucose is an interesting observation in this study. It has been shown previously that normal rat islets cultured for 6 hours demonstrated significantly reduced peak and reduced total insulin secretion in response to glucose,[61] and other studies have also demonstrated differences in the function of cultured islets compared with fresh islets.[62] Similar findings were demonstrated in the ZLC islets in this study, although glucose responsiveness (fold-increase) remained the same. However, in ZDF islets, the absolute insulin secretory response to glucose in islets cultured overnight tended to be but was not significantly lower in ZLC islets, and the responsiveness to glucose improved. This observation may be explained by the fact that the islets were removed from an environment of ambient hyperglycemia (30.5 mmol/L) and cultured at 11.6 mmol/L glucose overnight (a glucose level closer to the value in ZLC rats, 8.5 mmol/L). Thus, the islets recovered from hyperglycemia-induced desensitization to glucose or ''glucose toxicity.''

In summary, we have investigated signal transduction pathways activated by GLP-1 in normal ZLC and diabetic ZDF rat islets. Consistent with the hypothesis that the increase in β-cell $[Ca^{2+}]_i$ is the final signaling pathway common to nutrient and nonnutrient secretagogues, GLP-1 stimulus-secretion coupling was shown to depend on an increase in ZLC islet $[Ca^{2+}]_i$ originating from Ca^{2+} influx through L-type voltage-dependent Ca^{2+} channels. On the other hand, while the insulin secretory responses to GLP-1 were retained in perifused islets from ZDF rats, these responses were mediated by a signaling pathway distinct from the Ca^{2+}-dependent pathway found in ZLC islets. Although we are not aware of any evidence specifically relating these different signaling mechanisms of GLP-1-induced insulin secretion to the presence of leptin receptor mutations in the ZDF rat, this possibility has not been specifically excluded. Identification and characterization of the mechanisms that regulate this pathway should prove useful in the development of new therapies for the treatment of insulin secretory defects associated with type 2 diabetes.

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