Glucagon-Like Peptide-1 Stimulates Insulin Secretion But Not Phosphoinositide Hydrolysis From Islets Desensitized by Prior Exposure to High Glucose or the Muscarinic Agonist Carbachol

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In the present series of experiments, the ability of the postulated incretin factor, glucagon-like peptide-1 (GLP-1), to stimulate insulin release from desensitized islets was determined. Compared with responses observed from control islets incubated for 3.5 hours with 5.6 mmol/L glucose alone, prior exposure to 10 mmol/L glucose, 20 mmol/L glucose, or 10 μmol/L carbachol reduced peak second-phase insulin release rates to a subsequent 20-mmol/L glucose stimulus by 63%, 81%, or 70%, respectively. Efflux of ^{3}H-inositol from prior high-glucose– or carbachol-exposed islets was abolished and accumulation of inositol phosphates (IPs) in response to 20 mmol/L glucose was reduced. Further addition of 10 nmol/L GLP-1 together with 20 mmol/L glucose significantly increased insulin output from desensitized islets. Carbachol (10 μmol/L) preexposure also abolished the subsequent insulin secretory and ^{3}H-inositol efflux responses to 8 mmol/L glucose plus 10 μmol/L carbachol. Inclusion of 10 nmol/L GLP-1 together with 8 mmol/L glucose plus 10 μmol/L carbachol improved but did not normalize secretion from these islets. These improvements in secretory responsiveness from high-glucose– or carbachol-desensitized islets occurred despite the lack of any apparent restorative effect of GLP-1 on agonist-induced increases in phosphoinositide (PI) hydrolysis. Finally, unlike the situation observed with carbachol or high-glucose preexposure, chronic exposure of islets to GLP-1 (100 nmol/L) did not desensitize islets to a subsequent 20-mmol/L glucose stimulus. We conclude from these studies that the incretin factor GLP-1 may play an important role in maintaining insulin output from islets in which phospholipase C (PLC)-mediated hydrolysis of islet PI pools is impaired. GLP-1 may prevent a further decline in β-cell function and the associated deterioration in glucose tolerance that accompanies chronic exposure of islets to one of several agonists, including high glucose.

INSULIN RELEASE from the pancreatic β cell is acutely regulated by metabolic fuels such as glucose and by a variety of neurohumoral agonists and circulating incretin factors such as acetylcholine, cholecystokinin, and glucagon-like peptide-1 (GLP-1). These latter agonists increase release by generating a host of second-messenger molecules including the calcium-mobilizing inositol phosphates (IPs), the protein kinase C activator diacylglycerol, and cyclic adenosine monophosphate (cAMP).[1-3] It has previously been observed that chronic exposure to high glucose or carbachol desensitizes β cells to subsequent stimulation.[4-6] This process of desensitization, also referred to as third-phase release, appears to be the result of impaired information flow in the phospholipase C (PLC)-phosphoinositide (PI) pathway.[7-9] For example, parallel decreases in both PLC-mediated PI hydrolysis and insulin secretion characterize desensitized islet responses to subsequent stimulation.[7,8] In the present series of studies, effects of the incretin factor GLP-1 on insulin release from high (10 or 20 mmol/L)-glucose– or carbachol-desensitized islets were determined. The effects of chronic sustained islet exposure to this peptide were also assessed. These studies demonstrate that 10 nmol/L GLP-1 is capable of significantly increasing insulin output from control and desensitized islets. In addition, in contrast to the situation observed with high glucose or carbachol, chronic exposure to 100 nmol/L GLP-1 did not impair islet responsiveness to subsequent stimulation with 20 mmol/L glucose. These findings suggest that by maintaining insulin release from desensitized islets, GLP-1 may prevent further deterioration of β-cell secretory responsiveness and worsening of glucose tolerance.

MATERIALS AND METHODS

The detailed methodologies used to assess insulin output and PI hydrolysis from collagenase-isolated islets have been previously described.[10,11] Male Sprague-Dawley rats were used in all studies. All animals were treated in a manner that complied with National Institutes of Health guidelines for the care and use of laboratory animals. The animals were fed ad libitum and weighed 300 to 450 g. After Nembutal (pentobarbital sodium 50 mg/kg; Abbott, North Chicago, IL)-induced anesthesia, islets were isolated by collagenase digestion and handpicked using a glass-loop pipette under a stereomicroscope. They were free of exocrine contamination. To label PI pools, groups of freshly isolated islets were placed in small glass vials and incubated for 3 to 3.5 hours in a ^{3}H-inositol–containing Krebs-Ringer bicarbonate (KRB) solution formulated as follows: 10 μCi ^{3}H-inositol (specific activity, 16 to 23 Ci/mmol) was placed in a 10 × 75-mm culture tube. To this aliquot of label, 260 μL warmed (to 37°C) and oxygenated KRB medium was added with varying glucose levels as indicated in the results. In several studies, in addition to 5.6 mmol/L glucose, 10 μmol/L carbachol or 100 nmol/L GLP-1 was included during the labeling period. After mixing, 240 μL of this solution was gently added to the vial with islets. The vial was capped with a rubber stopper, gassed for 10 seconds with 95% O_2/5% CO_2, and placed in a metabolic shaker at 37°C. After 90 to 120 minutes, the vials were again gently oxygenated. After the labeling period, islets were washed with 5 mL fresh KRB. Groups of 3-hour–labeled islets were batch-incubated or perifused in the presence of 10 mmol/L LiCl to measure IP accumulation. LiCl is useful because it prevents degradation of IPs, allows them to accumulate intracellularly, and facilitates their measurement.[12] Because LiCl inhibits both ^{3}H-

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Submitted April 17, 1995; accepted August 4, 1995.

Supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants No. 41230 (W.S.Z.) and 45735 (the Yale Diabetes Endocrinology Research Center).

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0026-0495/96/4502-0024$03.00/0

inositol efflux from islets (labeled IPs cannot efflux from the cell only free inositol can) and insulin secretion,[7] additional experiments were conducted with islets that were perifused in the absence of LiCl to measure ^{3}H-inositol efflux rates and insulin secretion profiles.

IP Measurements in Batch-Incubated Islets

In these experiments, islets were incubated with ^{3}H-inositol and the indicated additions for 3 hours. After washing to remove unincorporated label, the islets on nylon filters were placed in small glass vials. Added gently to each vial was 400 μL KRB supplemented with 10 mmol/L LiCl to prevent IP degradation[12] and the appropriate agonists as indicated. The vials were capped and gently gassed for 5 seconds with 95% O_2/5% CO_2. After 30 minutes, generation of IPs was stopped by adding 400 μL 20% perchloric acid. Total IPs formed were then measured using Dowex columns as described previously.[13,14]

IP Measurements in Perifused Islets

In these experiments, islets were incubated with ^{3}H-inositol and the indicated additions for 3 hours. After washing to remove unincorporated label, the islets on nylon filters were perifused for 30 minutes with 5.6 mmol/L glucose alone and for an additional 30 minutes with 20 mmol/L glucose plus 10 mmol/L LiCl. At this point, islets were rapidly retrieved and placed in small glass vials. Perchloric acid was added, and labeled IPs formed during perifusion were measured as indicated earlier.

^{3}H-inositol Efflux and Insulin Secretory Responses

After the labeling period, islets were washed and then perifused in KRB buffer at a flow rate of 1 mL/min for 30 minutes to establish basal and stable ^{3}H-inositol efflux and insulin secretory rates. After this 30-minute stabilization period, they were then perifused for an additional 30 minutes with the appropriate agonist or agonist combinations as indicated in figure legends and the results. Perifusate solutions were gassed with 95% O_2/5% CO_2 and maintained at 37°C. Two-minute perifusate samples were collected and counted for ^{3}H-inositol content and efflux calculated as fractional efflux rates.[15] Insulin released into the medium was measured by radioimmunoassay.

Reagents

Hank's solution was used for islet isolation. The perifusion medium consisted of 115 mmol/L NaCl, 5 mmol/L KCl, 2.2 mmol/L $CaCl_2$, 1 mmol/L, $MgCl_2$, 24 mmol/L $NaHCO_3$, and 0.17 g/dL bovine serum albumin. Other compounds were added where indicated, and the solution was gassed with a mixture of 95% O_2/5% CO_2. ^{125}I-labeled insulin used for insulin assay was purchased from New England Nuclear (Boston, MA) and the *myo*-[2-^{3}H]inositol from Amersham (Arlington Heights, IL). Bovine serum albumin (radioimmunoassay grade), glucose, carbachol, and the salts used to make Hanks solution and perifusion medium were purchased from Sigma (St Louis, MO). GLP-1 (Insulinotropin, GLP-1,7-37) was a generous gift from Dr David Kreutter (Pzifer, Groton, CT). Rat insulin standard (lot #615-ZS-157) was a generous gift from Dr Gerald Gold (Eli Lilly & Co, Indianapolis, IN). Collagenase (type P) was obtained from Boehringer-Mannheim Biochemicals (Indianapolis, IN).

Statistics

Statistical significance was determined using Student's *t* test for unpaired data or ANOVA in conjunction with the Neuman-Keuls test for unpaired data. $P < .05$ was taken as significant. Results represent the mean ± SE of at least three observations.

RESULTS

After a 3.5-hour incubation period in KRB supplemented with 5.6 mmol/L glucose plus ^{3}H-inositol, secretory integrity of control islets was determined. In response to a subsequent 20-mmol/L glucose stimulus, insulin release measured during a dynamic perifusion increased in a biphasic pattern (Fig 1). Peak first-phase release was 161 ± 17 (mean ± SE) pg/islet/min, and release rates recorded 28 to 30 minutes after the onset of stimulation were 589 ± 63 pg/islet/min. This second-phase response represents a 24-fold increase in insulin release rates compared with the responses of control islets maintained for the entire 60 minutes at 5.6 mmol/L glucose (25 ± 5 pg/islet/min, n = 4; results not shown). Inclusion of 10 nmol/L GLP-1 together with 20 mmol/L glucose amplified both phases of release from control islets (Fig 1). Peak first- and second-phase secretion was now 279 ± 59 and 875 ± 105 pg/islet/min, respectively.

A different picture emerged if islets were incubated for the 3.5-hour period with 10 mmol/L glucose, 20 mmol/L glucose, or 5.6 mmol/L glucose plus 10 μmol/L of the muscarinic agonist carbachol. In all circumstances, dramatic reductions in peak second-phase release were noted (Figs 2, 3, and 4). For example, whereas peak second-phase release rates were 589 ± 63 pg/islet/min from control islets, this response decreased to 222 ± 43, 109 ± 19, or 178 ± 39

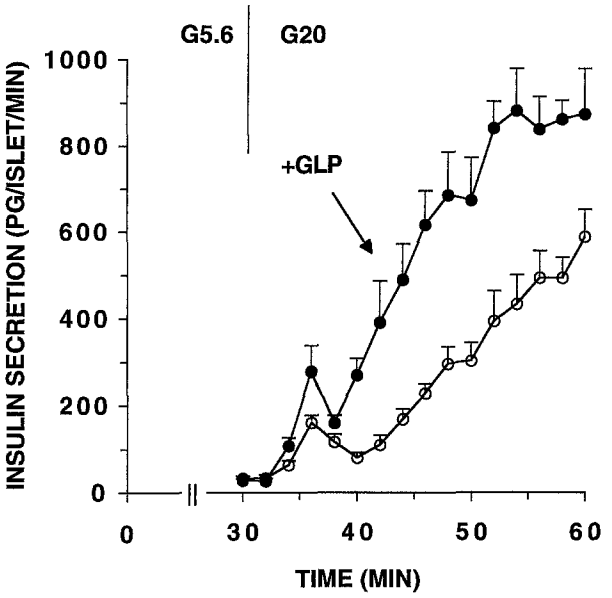

Fig 1. Stimulatory effect of GLP-1 on insulin release from isolated perifused islets. Two groups of islets were incubated for 3.5 hours in KRB medium supplemented with 5.6 mmol/L glucose. After washing, they were then perifused for 30 minutes with 5.6 mmol/L glucose alone to establish basal stable insulin secretory rates. Then they were stimulated for 30 minutes with 20 mmol/L glucose alone (○) or 20 mmol/L glucose plus 10 nmol/L GLP-1 (●). The mean ± SE of ≥3 experiments are indicated. This and subsequent figures were not corrected for the dead space in the perifusion apparatus, 2.5 mL, or 2.5 minutes with a flow rate of 1 mL/min.

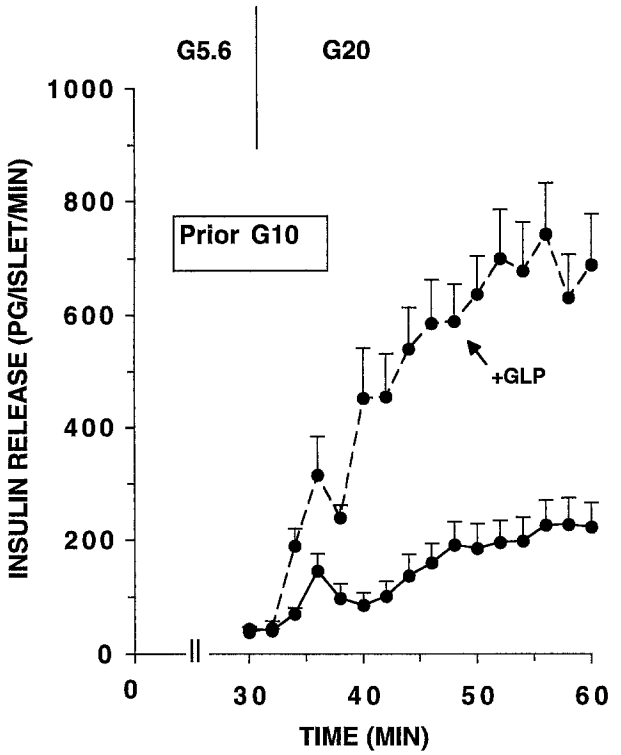

Fig 2. Influence of GLP-1 on insulin release from islets previously exposed to 10 mmol/L glucose. Two groups of islets were incubated for 3.5 hours in KRB medium supplemented with 10 mmol/L glucose. After washing, they were then perifused for 30 minutes with 5.6 mmol/L glucose alone to establish basal stable insulin secretory rates. Then they were stimulated with 20 mmol/L glucose alone (—) or 20 mmol/L glucose plus 10 nmol/L GLP-1 (---) for 30 minutes. The mean ± SE of ≥3 experiments are indicated.

pg/islet/min from islets incubated in 10 mmol/L glucose, 20 mmol/L glucose, or 5.6 mmol/L glucose plus 10 μmol/L carbachol, respectively. Inclusion of the incretin factor GLP-1 (10 nmol/L) together with 20 mmol/L glucose resulted in an amplified secretory response from islets desensitized by prior exposure to high glucose or carbachol (Figs 2, 3, and 4). Although the restorative effect of 10 nmol/L GLP-1 on secretory output from 10-mmol/L glucose–preexposed islets was significant, secretion from high-glucose– or carbachol-pretreated islets to the combination of 20 mmol/L glucose plus GLP-1 was less than that observed from control islets (previously exposed to 5.6 mmol/L glucose alone) stimulated with the same stimulant combination (Fig 1).

The restorative effect of GLP-1 could be duplicated by forskolin, a compound that elevates cAMP levels in islets.[16] For example, 30 minutes after the onset of stimulation, insulin release rates in response to stimulation with 20 mmol/L glucose plus 1 μmol/L forskolin were 996 ± 64 pg/islet/min from islets previously exposed to 20 mmol/L glucose for 3.5 hours. In the absence of forskolin, the response from 20-mmol/L glucose–desensitized islets was 109 ± 19 pg/islet/min.

High-glucose– or carbachol-preexposed islets displayed reduced fractional efflux rates of ^{3}H-inositol as compared with control islets (results not shown). For example, when stimulated with 20 mmol/L glucose, control islets previously incubated for 3.5 hours with 5.6 mmol/L glucose alone responded with slowly increasing but significant increments in ^{3}H-inositol efflux, an indirect measure of islet PI hydrolysis. Thirty minutes poststimulation, ^{3}H-inositol efflux rates were 0.26 ± 0.04%/min (n = 9). In contrast, prior exposure to 20 mmol/L glucose or 10 μmol/L carbachol reduced the responses at this time point to 0.088 ± 0.016 (n = 5) or 0.092 ± 0.015 (n = 6) %/min, respectively. An analysis of IP accumulation yielded parallel findings (Table 1). The responses of desensitized islets to stimulation were decreased. Further addition of GLP-1, a maneuver that dramatically increases the insulin secretory response to a subsequent 20-mmol/L glucose stimulus (Fig 3), had no restorative effect on either IP accumulation (Table 1) or efflux of ^{3}H-inositol (results not shown).

To achieve basal and stable rates of insulin release and ^{3}H-inositol efflux from control or desensitized islets, a 30-minute perifusion period with 5.6 mmol/L glucose alone was used before 20 mmol/L glucose stimulation. This 30-minute period in low glucose might result in differences in IP accumulation in these islets compared with batch-incubated islets (Table 1). To examine this issue, islets were incubated for 3 hours in 5.6 or 20 mmol/L glucose together with ^{3}H-inositol. They were then washed and perifused for 30 minutes with 5.6 mmol/L glucose and for an additional

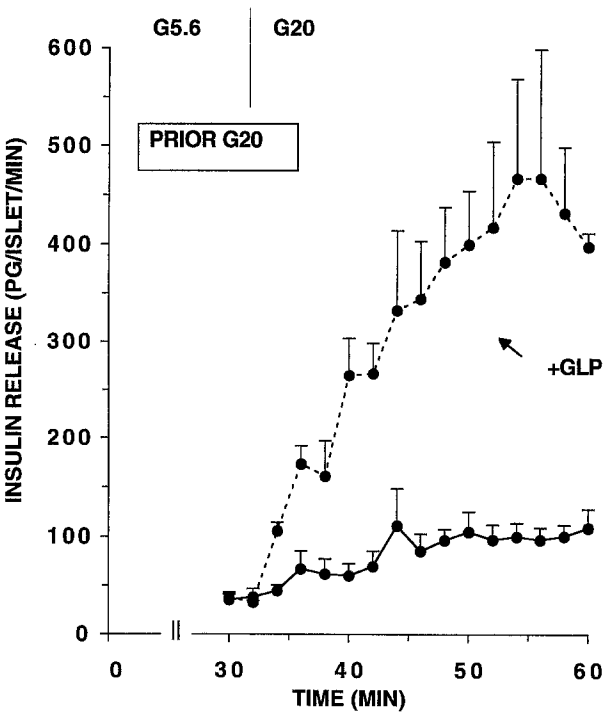

Fig 3. Influence of GLP-1 on insulin release from islets previously exposed to 20 mmol/L glucose. Two groups of islets were incubated for 3.5 hours in KRB medium supplemented with 20 mmol/L glucose. After washing, they were then perifused for 30 minutes with 5.6 mmol/L glucose alone to establish basal stable insulin secretory rates. Then they were stimulated for 30 minutes with 20 mmol/L glucose alone (—) or 20 mmol/L glucose plus 10 nmol/L GLP-1 (---). The mean ± SE of ≥3 experiments are indicated.

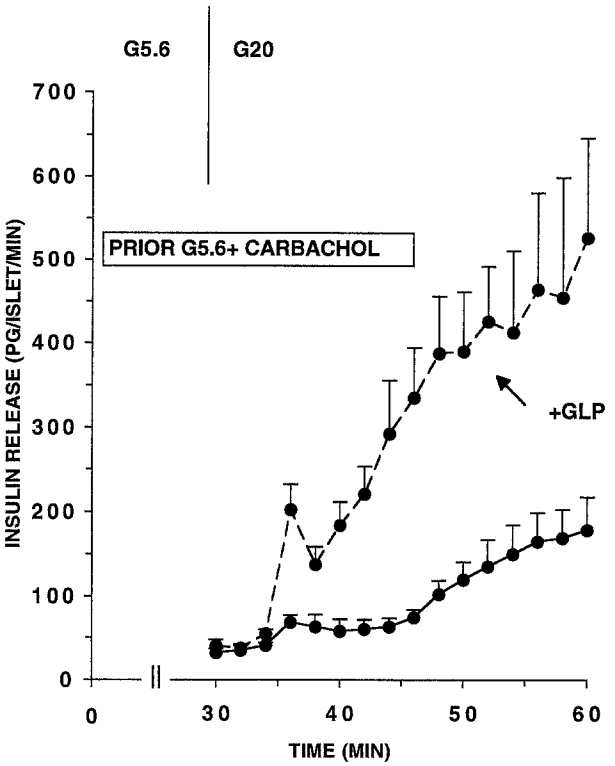

Fig 4. Influence of GLP-1 on insulin release from islets previously exposed to 5.6 mmol/L glucose plus 10 μmol/L carbachol. Two groups of islets were incubated for 3.5 hours in KRB medium supplemented with 5.6 mmol/L glucose plus 10 μmol/L carbachol. After washing, they were then perifused for 30 minutes with 5.6 mmol/L glucose alone to establish basal stable insulin secretory rates. They were then stimulated for 30 minutes with 20 mmol/L glucose alone (—) or 20 mmol/L glucose plus 10 nmol/L GLP-1 (-----). The mean ± SE of ≥3 experiments are indicated.

30 minutes with 20 mmol/L glucose plus 10 mmol/L LiCl to trap the labeled IPs formed. Results similar to those obtained with batch-incubated islets were recorded. For example, control (labeled in 5.6 mmol/L glucose) islet IP levels were 15,966 ± 2,675 cpm/40 islets in response to 30 minutes of 20 mmol/L glucose stimulation. The response of desensitized islets (labeled in 20 mmol/L glucose) was 6,760 ± 1,517 cpm/40 islets. Further addition of 10 nmol/L GLP-1 to desensitized islets did not change this impaired IP response to 20 mmol/L glucose stimulation. It was now 6,317 ± 1,414 cpm/40 islets.

Physiologic regulation of insulin release is achieved by more moderate changes in the level of glucose bathing the islet acting in concert with several incretin factors including GLP-1.[3,17,18] In an additional set of experiments, groups of islets were desensitized by a prior 3.5-hour exposure to 10 μmol/L carbachol. These islets were then perifused, and after a 30-minute stabilization period in 5.6 mmol/L glucose alone, they were stimulated with 8 mmol/L glucose plus 10 μmol/L carbachol or 8 mmol/L glucose plus 10 μmol/L carbachol plus 10 nmol/L GLP-1 (Fig 5). Control islets (incubated for 3.5 hours with 5.6 mmol/L glucose alone) responded to the combination of 8 mmol/L glucose plus 10 μmol/L carbachol plus 10 nmol/L GLP-1 with significant increases in release. Carbachol-desensitized islets failed to respond to 8 mmol/L glucose plus 10 μmol/L carbachol during the perifusion. They did respond to further addition of GLP-1, but the response was significantly less than the control islet response to this combination of agonists.

In the final set of experiments, islets were incubated for 3.5 hours with 5.6 mmol/L glucose plus 100 nmol/L GLP-1. They were then stimulated with 20 mmol/L glucose alone, and insulin responses were assessed (Fig 6). GLP-1, even at 100 nmol/L and in contrast to the findings made with 10-μmol/L carbachol– and high-glucose–preexposed islets, did not impair the subsequent insulin secretory response to 20 mmol/L glucose.

DISCUSSION

The present series of experiments were designed to assess the effects of the incretin factor GLP-1 on insulin release from islets desensitized by prior exposure to 10 mmol/L glucose, 20 mmol/L glucose, or 10 μmol/L carbachol. Previous studies from this laboratory have suggested that events associated with increases in PI hydrolysis during the induction of desensitization and the inability of subsequent stimulation to appropriately activate this important second-messenger system account for impaired secretory responsiveness.[7,8,19-21] Since GLP-1 stimulates release by increasing cAMP levels,[3] we postulated that in glucose- or carbachol-desensitized islets the integrity of the cAMP second-messenger system and processes distal to cAMP may help to sustain β-cell responsiveness. In addition, we undertook experiments to determine if chronic exposure to GLP-1, under conditions identical to those used to induce desensitization with high glucose or carbachol, has any

Table 1. IP Accumulation in Control and Desensitized Islets

Preincubation Period (180 min)	Stimulatory Period (30 min)	IP Response (cpm/40 islets)
1. 5.6 mmol/L glucose	5.6 mmol/L glucose	3,983 ± 197
2. 5.6 mmol/L glucose	20 mmol/L glucose	21,430 ± 2,694
3. 5.6 mmol/L glucose plus 10 μmol/L carbachol	20 mmol/L glucose	10,698 ± 1,737
4. 5.6 mmol/L glucose plus 10 μmol/L carbachol	20 mmol/L glucose plus 10 nmol/L GLP-1	9,006 ± 785
5. 20 mmol/L glucose	20 mmol/L glucose	8,143 ± 513
6. 20 mmol/L glucose	20 mmol/L glucose plus 10 nmol/L GLP-1	7,984 ± 536

NOTE. Groups of 20 to 25 islets were preincubated for 180 minutes in a ^{3}H-inositol–containing medium plus the indicated glucose and/or carbachol concentrations. They were then washed to remove unincorporated label and stimulated for 30 minutes with the indicated additions. Lithium chloride (10 mmol/L) was included during the final stimulatory period to prevent degradation of IPs. At least 4 experiments were conducted under each condition, and the mean ± SE are indicated.

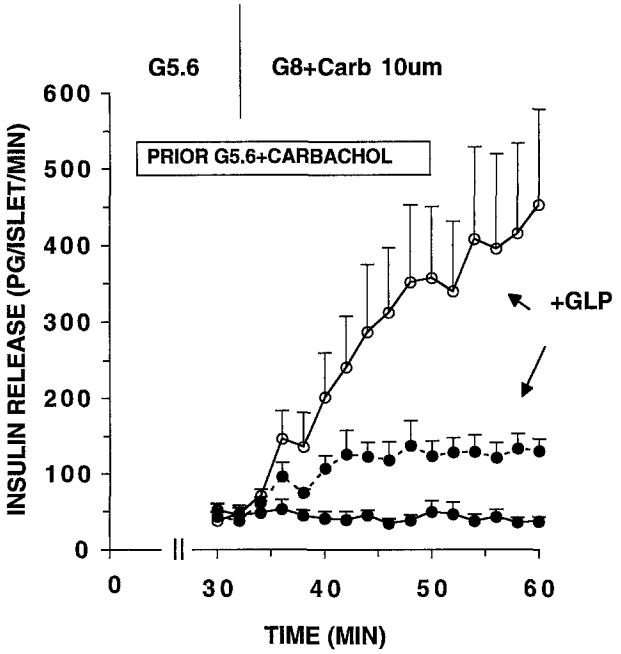

Fig 5. Insulin release from control or carbachol-desensitized islets: responses to the combination of 8 mmol/L glucose, carbachol, and GLP-1. Three groups of islets were incubated for 3.5 hours in KRB medium supplemented with 5.6 mmol/L glucose alone (○) or 5.6 mmol/L glucose plus 10 μmol/L carbachol (●). They were then perifused. After a 30-minute stabilization period in 5.6 mmol/L glucose, one group of carbachol-pretreated islets was stimulated with 8 mmol/L glucose plus 10 μmol/L carbachol (●). The second carbachol-pretreated group was treated in a similar fashion except for further addition of 10 nmol/L GLP-1 during the stimulation period with 8 mmol/L glucose plus carbachol (--●--). The control group pretreated with 5.6 mmol/L glucose alone during the 3.5-hour incubation period was stimulated during the final 30 minutes of the perifusion with 8 mmol/L glucose plus 10 μmol/L carbachol plus 10 nmol/L GLP-1 (○). The mean ± SE of ≥3 experiments are indicated.

adverse impact on the β cell. Our findings suggest that this peptide may be important in sustaining secretory responses from islets characterized by defective agonist-induced increases in PI hydrolysis.

In the first set of experiments we document, in agreement with other reports,[22] that GLP-1 has a dramatic stimulatory effect on insulin release from the β cell. In the simultaneous presence of 20 mmol/L glucose, the peptide amplifies both the first and second phases of release. In further studies, we demonstrated that a prior 3.5-hour exposure to 10 or 20 mmol/L glucose alone or to 10 μmol/L carbachol together with 5.6 mmol/L glucose impaired islet responsiveness to subsequent stimulation. Dramatic and parallel reductions in insulin secretion and ^{3}H-inositol efflux were observed. We have previously described in detail the advantages of monitoring dynamic changes in both PI hydrolysis and insulin release using this efflux methodology.[7] More direct assessment of the activation of this signaling pathway was obtained by measuring labeled IP accumulation in ^{3}H-inositol–prelabeled islets, and these results corroborated the efflux findings.

Of particular significance were studies with desensitized islets subsequently stimulated with glucose plus 10 nmol/L GLP-1. Whether islets were desensitized by high (10 or 20 mmol/L) glucose or carbachol, GLP-1 addition together with 20 mmol/L glucose resulted in amplified insulin secretory responses. This insulin stimulatory effect occurred despite the absence of any apparent restorative effect of the peptide on PI hydrolysis, and could be duplicated by forskolin, a compound that also elevates cAMP in islets.[16] If the biochemical alterations observed in these studies reflect those occurring in vivo with glucose-desensitized islets, these findings suggest that in vivo GLP-1 may be important in maintaining β-cell secretion in the face of impaired information flow in the PLC-PKC signaling pathway.

Although chronic exposure of pancreatic islets to high glucose or carbachol, agonists that activate PLC, desensitized islets, no such deleterious action of chronic GLP-1 exposure could be detected under the conditions used in these studies. Previous studies with forskolin, which, like GLP-1, increased cAMP levels in islets,[16] demonstrated that forskolin is capable of inducing alterations in PLC-mediated PI hydrolysis.[20] We did not measure cAMP levels in these studies. However, it might be proposed that the failure of GLP-1 to duplicate the effect of forskolin may be

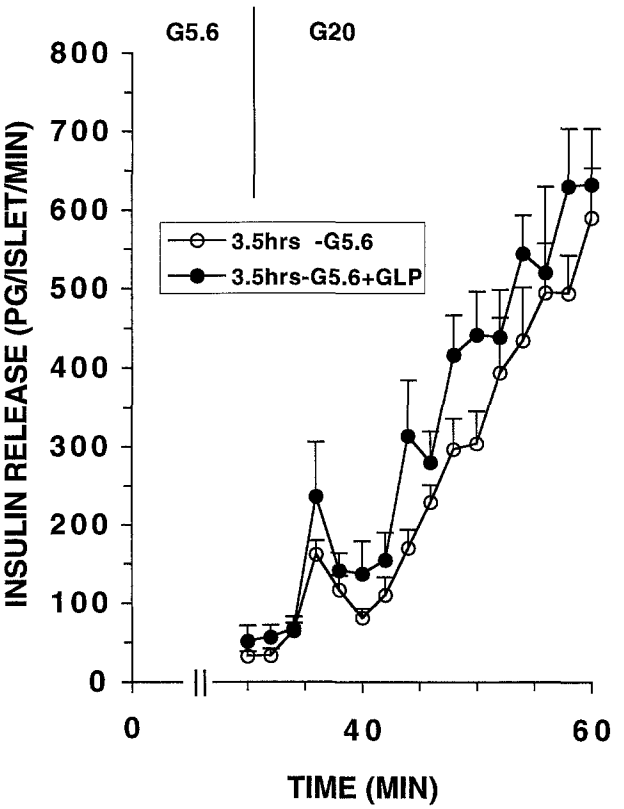

Fig 6. Chronic exposure to GLP-1 fails to desensitize islet responses to 20 mmol/L glucose. Two groups of islets were studied. The first group (○) was incubated for 3.5 hours in 5.6 mmol/L glucose alone before perifusion. The second group (●) was similarly treated during the 3.5-hour incubation period, except for further addition of 100 nmol/L GLP-1 to 5.6 mmol/L glucose. Both groups of islets were subsequently perifused for 30 minutes with 5.6 mmol/L glucose and for an additional 30 minutes with 20 mmol/L glucose. The mean ± SE of ≥4 experiments are indicated.

the result of its inability to induce comparable increments in cAMP. Alternatively, the effects of forskolin may be mediated by other cellular events.

In conclusion, we have demonstrated in agreement with previous studies that chronic exposure of islets to high glucose impairs release and PI hydrolysis. These adverse events cannot be duplicated by chronic exposure to GLP-1. Most importantly, in islets desensitized by high-glucose or carbachol preexposure, GLP-1 remains an effective insulin secretagogue. These results suggest that GLP-1 may help to maintain insulin release from islets in which PLC-mediated hydrolysis of islet PIs is impaired. This effect would serve to facilitate the maintenance of glucose homeostasis despite the existence of a significant biochemical lesion in the islet.

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