Altered cAMP and Ca^{2+} Signaling in Mouse Pancreatic Islets With Glucagon-Like Peptide-1 Receptor Null Phenotype

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β-Cells from rodents and humans express different receptors recognizing hormones of the secretin-glucagon family, which—when activated—synergize with glucose in the control of insulin release. We have recently reported that isolated islets from mice homozygous for a GLP-1 receptor null mutation (GLP-1R–/–) exhibit a well-preserved insulin-secretory response to glucose. This observation can be interpreted in two different ways: *1*) the presence of GLP-1R is not essential for the secretory response of isolated islets to glucose alone; *2*) β-cells in GLP-1R–/– pancreases underwent compensatory changes in response to the null mutation. To explore these possibilities, we studied islets from control GLP-1R+/+ mice in the absence or presence of 1 µmol/l exendin (9–39)amide, a specific and potent GLP-1R antagonist. Exendin (9–39)amide (15-min exposure) reduced glucose-induced insulin secretion from both perifused and statically incubated GLP-1R+/+ islets by 50% ($P < 0.05$), and reduced islet cAMP production in parallel ($P < 0.001$). Furthermore, GLP-1R–/– islets exhibited: *1*) reduced cAMP accumulation in the presence of 20 mmol/l glucose (knockout islets versus control islets, 12 ± 1 vs. 27 ± 3 fmol · islet^{-1} · 15 min^{-1}; $P < 0.001$) and exaggerated acceleration of cAMP production by 10 nmol/l glucose-dependent insulinotropic peptide (GIP) (increase over 20 mmol/l glucose by GIP in knockout islets versus control islets: 66 ± 5 vs. 14 ± 3 fmol · islet^{-1} · 15 min^{-1}; $P < 0.001$); *2*) increased mean cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_c$) at 7, 10, and 15 mmol/l glucose in knockout islets versus control islets; and *3*) signs of asynchrony of $[Ca^{2+}]_c$ oscillations between different islet subregions. In conclusion, disruption of GLP-1R signaling is associated with reduced basal but enhanced GIP-stimulated cAMP production and abnormalities in basal and glucose-stimulated $[Ca^{2+}]_c$. These abnormalities suggest that GLP-1R signaling is an essential upstream component of multiple β-cell signaling pathways. *Diabetes* 48:1979–1986, 1999

Oral glucose administration stimulates insulin secretion more than intravenous glucose infusion (1–3) does. This so-called incretin effect of oral glucose results from the secretion of gut hormones that potentiate the effect of glucose on insulin secretion (4). Two intestinal peptides released during a meal are important in mediating the incretin effect: GIP (glucose-dependent insulinotropic peptide) and GLP-1 [glucagon-like peptide-1-(7–36) amide] (4,5). Evidence for the functional importance of GIP and GLP-1 as physiologically relevant incretins derives from a combination of experimental approaches including immunoneutralization of GIP action (6), blockade of GLP-1 action with the specific antagonist exendin (9–39)amide (7), and studies of GLP-1 receptor (GLP-1R) –/– mice (8). Because both peptides exert their potentiating effects on insulin release only at elevated glucose concentrations, they have been considered as potential therapeutic agents for patients with type 2 diabetes (4,9).

It has also been suggested that regular exposure of β-cells to GLP-1 is important to maintain normal insulin secretory responsiveness to glucose (10,11). However, we have recently shown that isolated islets from mice homozygous for a GLP-1 receptor null mutation (GLP-1R–/–) exhibit a well-preserved insulin response to glucose (12). These observations suggest that either the presence of GLP-1R is not required for glucose competence in isolated islets or that mice with GLP-1R–/– have compensated for their genetic defect. One element of compensation appears to involve the GIP component of the enteroinsular axis, inasmuch as both GIP secretion and GIP action are increased in GLP-1R–/– mice (13).

In this study we have explored the possibility that additional compensatory changes occur at the level of GLP-1R–/– islets following disruption of GLP-1 signaling. Previous in vitro experiments showed that both GLP-1 and GIP stimulate insulin secretion mainly by increasing cellular cAMP levels in β-cells (14,15) or β-cell lines (16,17) but other signaling pathways may also be involved (18). Compensation might therefore occur at the level of intracellular cAMP, which is critical for insulin release (19–22) as well as cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_c$), which is generally accepted to be one of the main regulators of exocytosis (23,24). Our data

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ACh, acetylcholine; BSA, bovine serum albumin; $[Ca^{2+}]_c$, cytosolic $[Ca^{2+}]$; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide-1(7–36) amide; GLP-1R, GLP-1 receptor; IBMX, 3-isobutyl-1-methylxanthine; Sp-cAMPS, cAMP Sp-isomer.

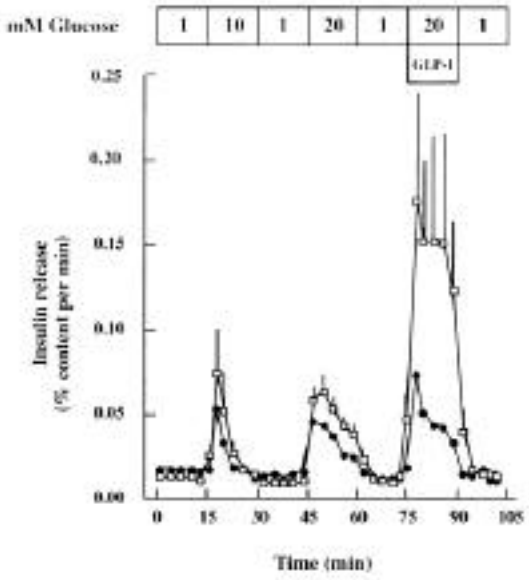

FIG. 1. Effect of exendin (9–39)amide on glucose-induced insulin release. Islets of female GLP-1R+/+ mice were perifused with 10 mmol/l glucose, 20 mmol/l glucose, and 20 mmol/l glucose with 10 nmol/l GLP-1. One batch of islets was perifused with 1 µmol/l exendin (9–39)amide (●), the other without exendin (9–39)amide (□). Data represent the means ± SE of four experiments. For graphical clarity, mean symbols and error bars are shown once per four consecutive minute fractions.

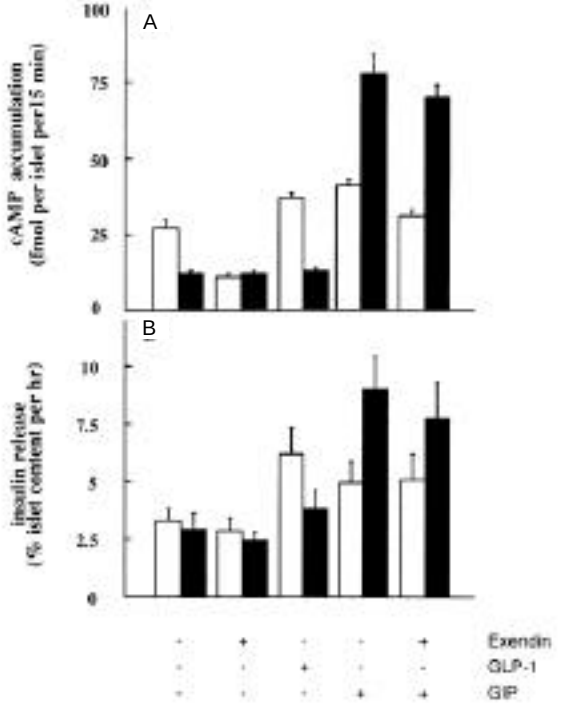

FIG. 2. cAMP content measured in the presence of 250 µmol/l IBMX (*A*) and insulin release measured without IBMX (*B*) from GLP-1R+/+ and GLP-1R–/– islets. The effects of 20 mmol/l glucose alone or 20 mmol/l glucose plus exendin (9–39)amide (1 µmol/l), GLP-1 (10 nmol/l), GIP (10 nmol/l), and GIP plus exendin (9–39)amide were assessed as indicated using parallel incubations of GLP-1R+/+ (□) and GLP-1R–/– islets (■). Data are means ± SE of 13 (GLP-1R+/+ islets) and 7 (GLP-1R–/– islets) independent experiments.

indicate that changes in both cAMP and $[Ca^{2+}]_c$ occur in the GLP-1R–/– model.

RESEARCH DESIGN AND METHODS

Chemicals. Synthetic GLP-1 (7–36)amide and GIP were purchased from Sigma (St. Louis, MO); 3-isobutyl-1-methylxanthine (IBMX) was purchased from Aldrich (Janssen Chimica, Beerse, Belgium). Exendin (9–39)amide was a gift from Dr. J. Vandekerckhove (Department of Physiological Chemistry, University of Ghent, Ghent, Belgium). cAMP Sp-isomer (Sp-cAMPS) was purchased from Biolog Life Science Institute (Bremen, Germany). All other products were of analytical grade and obtained from either Merck (Darmstadt, Germany) or Sigma.

Animals. The study was conducted on 8- to 12-week-old male and female CD1 mice fed ad libitum that were wild type (GLP-1R+/+) or homozygous (GLP-1R–/–) for a targeted null mutation of the *GLP-1R* gene (8). The animals were bred under specific pathogen-free conditions and cared for according to Belgian regulations of animal welfare.

Isolation and culture of mouse pancreatic islets. Islets of Langerhans were isolated as described previously (12) using collagenase P (Boehringer Mannheim, Mannheim, Germany). Isolated islets were then cultured overnight in F10 medium Nutrient Mixture (Ham) (GIBCO BRL, Life Technologies, Strathclyde, U.K.) containing 0.075 mg/ml penicillin, 0.1 mg/ml streptomycin, 0.5% (wt/vol) bovine serum albumin (BSA) (fraction V, radioimmunoassay grade; Sigma), 2 mmol/l glutamine, and 10 mmol/l glucose (25). Insulin release and islet cAMP experiments were performed after overnight culture, and the measurements of $[Ca^{2+}]_c$ were performed after two days of culture. Experiments using control islets which were cultured for one or two days gave similar results.

Insulin release. Perifusion experiments were performed at 37°C with overnight cultured female GLP-1R+/+ control islets using a perifusion system with a multiple microchamber module (Endotronics, Coon Rapids, MN) (12). Islets were divided in equal batches (~200 islets per batch) and perifused in parallel, with various glucose concentrations with or without 10 nmol/l GLP-1. One perifusion column was exposed during the entire experiment to 1 µmol/l exendin (9–39)amide. Preperifusion (15 min) was done in basal medium that consisted of F10 medium Nutrient Mixture supplemented with 0.5% BSA, 2 mmol/l glutamine, 2 mmol/l $CaCl_2$, and 1 mmol/l glucose equilibrated with 95% O_2/5% CO_2, at a flow rate of 0.5 ml/min. Samples were collected every minute and assayed for immunoreactive insulin (19). The dead space was taken into account when expressing results as a function of time. Insulin content of the GLP-1R+/+ islets (63 ± 8 ng/islet) (n = 10) was measured after perifusion by sonicating the Biogel P2 containing the islets in 5 ml of 2 mmol/l acetic acid/0.25% BSA.

The effect of 1 µmol/l exendin (9–39)amide was also studied on 20 mmol/l glucose–induced insulin release from control islets during 15 min static incubations. Furthermore, effects of exendin (9–39)amide, GLP-1, GIP, and Sp-cAMPS were tested during 60-min static incubations in the presence of 20 mmol/l glucose using triplicate batches of 20 islets isolated from GLP-1R+/+ and GLP-1R–/– mice. For this purpose, overnight cultured islets were washed three times in Earle's HEPES medium (124 mmol/l NaCl, 5.4 mmol/l KCl, 1.8 mmol/l $CaCl_2$, 0.8 mmol/l $MgSO_4$, 1 mmol/l NaH_2PO_4, 14 mmol/l $NaHCO_3$, 10 mmol/l HEPES, and 0.5% BSA) (no glucose). Incubation was started by adding fresh Earle's HEPES medium supplemented with test agents at final concentrations that are indicated in Figs. 2*B* and 3. Results were expressed as percentage of the insulin content, measured in each individual batch of islets after finishing the experiment. No difference in insulin content of GLP-1R+/+ and –/– islets was observed after the static incubations: 67 ± 4 vs. 65 ± 3 ng/islet (mean ± SE of 13 experiments).

Measurement of cAMP production. Levels of cAMP were determined in whole islets as was described previously (15,20). Batches of 40 islets were incubated for 15 min at 37°C in Earle's HEPES medium supplemented with 0.5% BSA, 250 µmol/l IBMX, 20 mmol/l glucose, and the peptide hormones as indicated. cAMP content of acetylated samples was measured using a commercially available [^{125}I]- cAMP radioimmunoassay kit (Amersham, Little Chalfont, U.K.).

Measurements of $[Ca^{2+}]_c$. A bicarbonate-buffered medium (pH 7.4) containing 120 mmol/l NaCl, 4.8 mmol/l KCl, 2.5 mmol/l $CaCl_2$, 1.2 mmol/l $MgCl_2$, and 24 mmol/l $NaHCO_3$, supplemented with 1 mg/ml BSA (fraction V; Boehringer Mannheim), gassed with 94% O_2/6% CO_2, was used for Ca^{2+} experiments. In one experimental condition where the KCl concentration was increased to 30 mmol/l, that of NaCl was decreased accordingly to keep the osmolarity constant. After two days of culture, male islets were loaded with 2 µmol/l fura-PE3/AM (Teflabs, Austin, TX) for 90–120 min at 37°C in a bicarbonate-buffered solution containing 10 mmol/l glucose and transferred into a temperature-controlled perifusion chamber (37°C) with a bottom made of a glass coverslip. The flow rate of perifusion was 1.3 ml/min. The islets were excited successively at 340 and 380 nm, the fluorescence emitted at 510 nm was captured by a CCD camera (Photonic Science, Tunbridge Wells, U.K.), and the images were analyzed by the QuantiCell system (Applied Imaging, Sunderland, U.K.). The technique has previously been described in detail (26). In the experiments illustrated in Fig. 6, the 510-nm

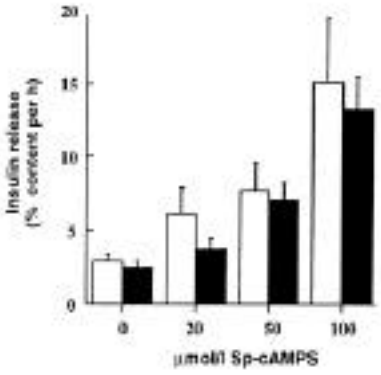

FIG. 3. Effect of the cAMP analog Sp-cAMPS on glucose-induced insulin release in GLP-1R+/+ and GLP-1R–/– islets. Batches of 20 islets isolated from GLP-1R+/+ (□) and GLP-1R–/– mice (■) were challenged with different concentrations of Sp-cAMPS as indicated in the presence of 20 mmol/l glucose. Data are means ± SE of five independent experiments.

fluorescence was monitored by a photomultiplier-based system (Photon Technology International, Princeton, NJ).

Statistical analysis. The results are illustrated either as means ± SE of at least three different experiments or as representative data obtained with the indicated number of islets. The statistical significance of observed differences was assessed by unpaired two-tailed Student's *t* tests.

RESULTS

Effect of exendin (9–39)amide on glucose-induced insulin secretion. Because glucose-induced insulin release is well preserved in GLP-1R–/– islets (12), we investigated the hypothesis that the GLP-1R is not essential for a normal glucose-induced insulin secretion by assessing the effect of pharmacological inhibition of the GLP-1R with 1 μmol/l exendin (9–39)amide in wild-type islets. As is shown in Fig. 1, this treatment almost completely prevented the potentiation by 10 nmol/l GLP-1 (7–36)amide of 20 mmol/l glucose-induced insulin release [3.5 ± 1.5–fold potentiation in controls vs. 1.5 ± 0.2–fold potentiation in the presence of 1 μmol/l exendin (9–39)amide; $P < 0.05$]. Furthermore, glucose-induced insulin release was reduced by 40–50% in exendin (9–39)amide–treated islets: release above basal per 15 min for control and exendin (9–39)amide treatment was 0.34 ± 0.04 vs. 0.14 ± 0.04% (10 mmol/l glucose) and 0.53 ± 0.06 vs. 0.31 ± 0.07% (20 mmol/l glucose) of islet insulin content, respectively ($P < 0.05$). A similar reduction in 20 mmol/l glucose–induced insulin release was observed in statically incubated control islets treated for 15 min with exendin (9–39)amide (glucose alone: 1.7 ± 0.2% vs. glucose plus exendin (9–39)amide: 0.8 ± 0.2% of islet insulin content; mean ± SE, $n = 3$, $P < 0.05$). These observations implicate a role for the GLP-1R in the response of β-cells to glucose stimulation in vitro, and suggest that the preserved glucose-induced insulin secretion in GLP-1R–/– islets is due to adaptive mechanisms that compensate for the loss of GLP-1R activity in β-cells.

Islet cAMP accumulation and insulin release from islets isolated from GLP-1R+/+ and GLP-1R–/– mice. Islet cAMP accumulation, measured in the presence of 20 mmol/l glucose and 250 μmol/l IBMX (Fig. 2*A*), was two times higher in GLP-1R+/+ islets (27 ± 3 fmol · islet^{-1} · 15 min^{-1}) than in GLP-1R–/– islets (12 ± 1 fmol · islet^{-1} · 15 min^{-1}; $P < 0.01$). Exendin (9–39)amide did not affect islet cAMP accumulation in GLP-1R–/– islets, but it decreased cAMP accumulation in the GLP-1R+/+ islets (12 ± 1 fmol · islet^{-1} · 15 min^{-1}; $P < 0.001$), so that in the presence of exendin (9–39)amide, cAMP levels in GLP-1R–/– and GLP-1R+/+ islets were comparable. These findings implicate a role for GLP-1R signaling in the regulation of basal islet cAMP levels in vitro. Paradoxically, exendin (9–39)amide did not affect glucose-induced insulin secretion in GLP-1R+/+ islets after 15 min incubation in the presence of IBMX [3.1 ± 0.5% of insulin content in exendin (9–39)amide–treated islets vs. 3.7 ± 0.6% in control islets, $n = 4$] or after 60 min incubation in the absence of IBMX (Fig. 2*B*). After 15-min incubations without IBMX, however, 20 mmol/l glucose–induced insulin secretion was inhibited by exendin (9–39)amide (0.8 ± 0.2% of insulin content vs. 1.7 ± 0.2% of insulin content in glucose alone, $n = 4$, $P < 0.05$). This short-term inhibition is consistent with the results obtained with the perifused islets (Fig. 1).

GLP-1 slightly stimulated ($P = 0.01$) cAMP accumulation in control islets incubated in the presence of 20 mmol/l glucose and 250 μmol/l IBMX (Fig. 2*A*). The accompanying increase in insulin secretion, however, failed to reach statistical significance (5.1 ± 0.7 vs. 3.7 ± 0.6% of islet content, $n = 4$, $P = 0.12$). A larger effect of GLP-1 on insulin secretion was observed in islets incubated for 60 min in absence of IBMX (Fig. 2*B*).

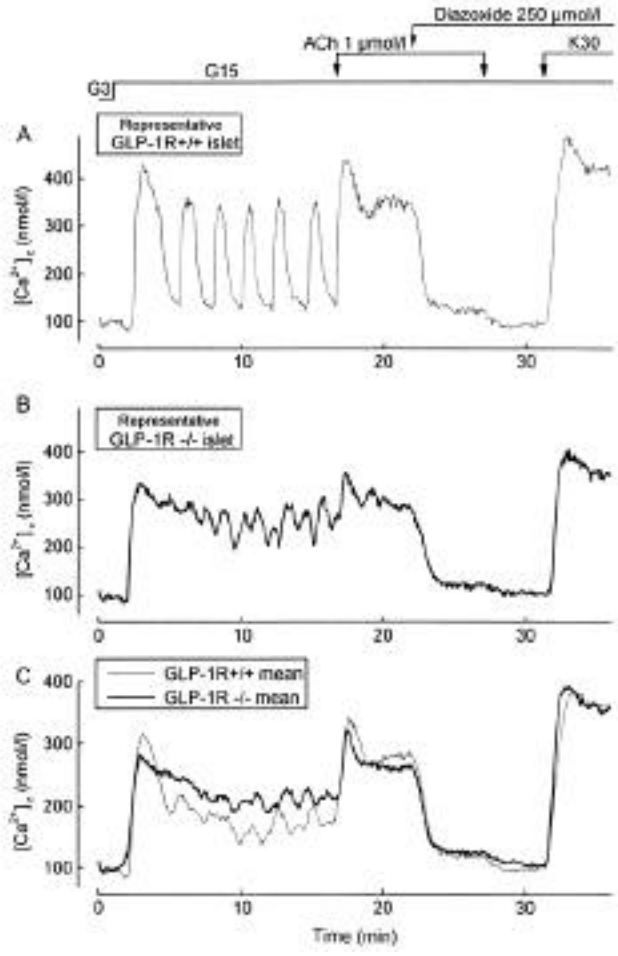

FIG. 4. Effect of glucose, ACh, diazoxide, and high potassium on $[Ca^{2+}]_c$ in GLP-1R+/+ and GLP-1R–/– islets. The glucose concentration of the perifusion medium was increased from 3 (G3) to 15 mmol/l (G15) after 1 min of recording. ACh (1 μmol/l), diazoxide (250 μmol/l), and 30 mmol/l K^+ (K30) were applied as indicated. The traces are representative or mean results from 38 GLP-1R+/+ and 38 GLP-1R–/– islets.

It was previously reported that GLP-1R–/– islets exhibit an increased responsiveness to GIP (13). This behavior is again shown in Fig. 2*B*, where GLP-1R–/– islets respond more to 20 mmol/l glucose plus 10 nmol/l GIP than do control islets (1.5-fold increase above basal in the control islets vs. threefold stimulation in GLP-1R–/– islets, $P < 0.001$). This difference in insulin secretion between GLP-1R–/– and GLP-1R+/+ islets is paralleled by a larger GIP-induced formation of cAMP (1.5-fold increase of basal in control islets [$P = 0.001$] vs. 6.4-fold stimulation above basal in GLP-1R–/– islets, $P = 0.0001$). In the presence of 1 µmol/l exendin (9–39)amide, cAMP production in GIP-treated control mouse islets was slightly lower ($P < 0.01$) than in islets treated with GIP but with no exendin (9–39)amide. This difference can be explained, however, by the effect of 1 µmol/l exendin (9–39)amide on 20 mmol/l glucose–induced cAMP production. Thus, the GIP-induced increase in cAMP production above that with 20 mmol/l glucose alone was 14 ± 3 fmol · islet^{-1} · 15 min^{-1} without exendin (9–39)amide vs. 21 ± 2 fmol · islet^{-1} · 15 min^{-1} in the presence of exendin (9–39)amide. Second, the GIP-induced cAMP accumulation in knockout islets was also unaffected by exendin (9–39)amide, the increase of GIP above 20 mmol/l glucose alone being 66 ± 5 fmol · islet^{-1} · 15 min^{-1} without exendin (9–39)amide vs. 59 ± 3 fmol · islet^{-1} · 15 min^{-1} with exendin (9–39)amide. Third, the increase in insulin secretion induced by GIP in GLP-1R–/– islets was similar in the presence and absence of exendin (9–39)amide. In summary, 1 µmol/l exendin (9–39)amide does not interfere with GIP signaling in mouse islets.

Effect of the cAMP analog Sp-cAMPS on 20 mmol/l glucose–induced insulin release. One possible explanation for the similar secretory response of GLP-1R–/– and GLP-1R+/+ islets in the presence of a twofold difference in islet cAMP content is that the β-cells from GLP-1R–/– mice have augmented the sensitivity of their secretory machinery to cAMP. To investigate this possibility, we exposed batches of islets to different concentrations of the cAMP analog Sp-cAMPS, a potent stimulator of protein kinase A (Fig. 3). Insulin secretion at 20 mmol/l glucose was again comparable in GLP-1R+/+ and GLP-1R–/– islets. Furthermore, Sp-cAMPS produced a concentration-dependent potentiation of insulin secretion without any suggestion of an increased sensitivity of the insulin secretory responsiveness to cAMP in the GLP-1R–/– islets.

Effects of glucose, acetylcholine, and high K^+ on islet $[Ca^{2+}]_c$. Because $[Ca^{2+}]_c$ and cAMP synergize at the level of exocytosis (27), we assessed the possibility that a larger $[Ca^{2+}]_c$ response could compensate for the lower levels of cAMP, resulting in well-preserved glucose-induced insulin secretion in GLP-1R–/– islets. In 3 mmol/l glucose, $[Ca^{2+}]_c$ was low, stable, and similar in control and GLP-1R–/– islets (Fig. 4). Raising the glucose concentration to 15 mmol/l produced a biphasic increase in $[Ca^{2+}]_c$. The kinetics and amplitude of the initial $[Ca^{2+}]_c$ phase were similar in control and GLP-1R–/– islets. In contrast, the characteristics of the second phase were different in the two types of islets. In control islets, $[Ca^{2+}]_c$ displayed large amplitude and regular oscillations above a slightly elevated level (Fig. 4*A*). In GLP-1R–/– islets, $[Ca^{2+}]_c$ oscillations occurred from a higher level, but were of smaller amplitude and poorly organized (Fig. 4*B*). The overall $[Ca^{2+}]_c$ response to glucose was significantly larger in GLP-1R–/– (215 ± 4 nmol/l, $n = 38$) than in control islets (184 ± 4 nmol/l, $n = 38$, $P < 0.01$ for average $[Ca^{2+}]_c$ integrated from 1 to 17 min).

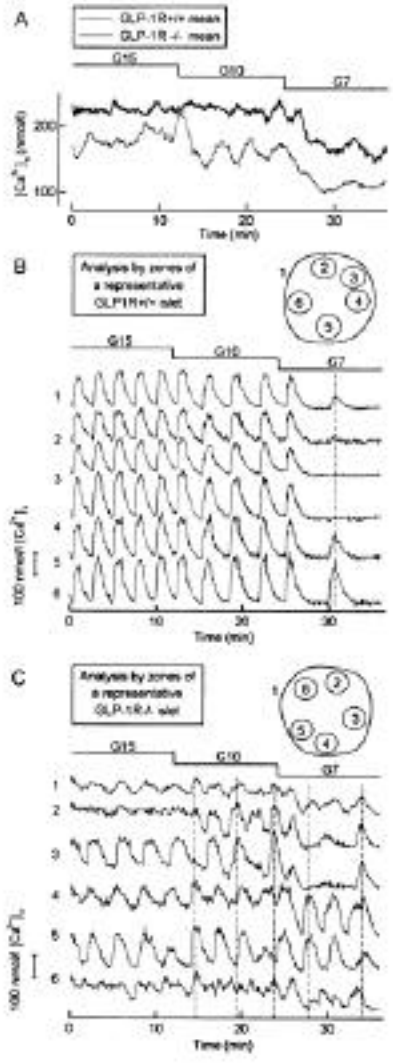

FIG. 5. Concentration-dependency and degree of synchronization of glucose effects on $[Ca^{2+}]_c$ in GLP-1R+/+ and GLP-1R–/– islets. The glucose concentration of the perifusion medium was decreased stepwise from 15 to 7 mmol/l as indicated. *A*: Mean whole islet $[Ca^{2+}]_c$ of 36 GLP-1R–/– and GLP-1R+/+ islets. *B* and *C*: Analysis of $[Ca^{2+}]_c$ in various subregions of islets from GLP-1R+/+ (*B*) and GLP-1R–/– mice (*C*). Traces in panels *B* and *C* represent data from 10 of 36 control islets and 36 of 36 knockout islets. As explained in the text, 20 of 36 control islets did not display $[Ca^{2+}]_c$ oscillations in the presence of 7 mmol/l glucose. Dotted vertical lines were added to facilitate temporal comparison between traces.

We next investigated whether a similar difference in $[Ca^{2+}]_c$ is also seen when the islets are stimulated by secretagogues other than glucose. By activating muscarinic receptors, acetylcholine (ACh) exerts complex effects on β-cell $[Ca^{2+}]_c$, which involve both mobilization of cellular Ca^{2+} and stimulation of Ca^{2+} influx (28). Addition of ACh to the medium containing 15 mmol/l glucose rapidly increased $[Ca^{2+}]_c$ and transformed the glucose-induced $[Ca^{2+}]_c$ oscillations into a sustained elevation that was similar in both types of islets (Fig. 4). Subsequent hyperpolarization of the plasma membrane with diazoxide, a K^+_{ATP}-channel opener, counteracted the effects of glucose and ACh, and lowered $[Ca^{2+}]_c$ to

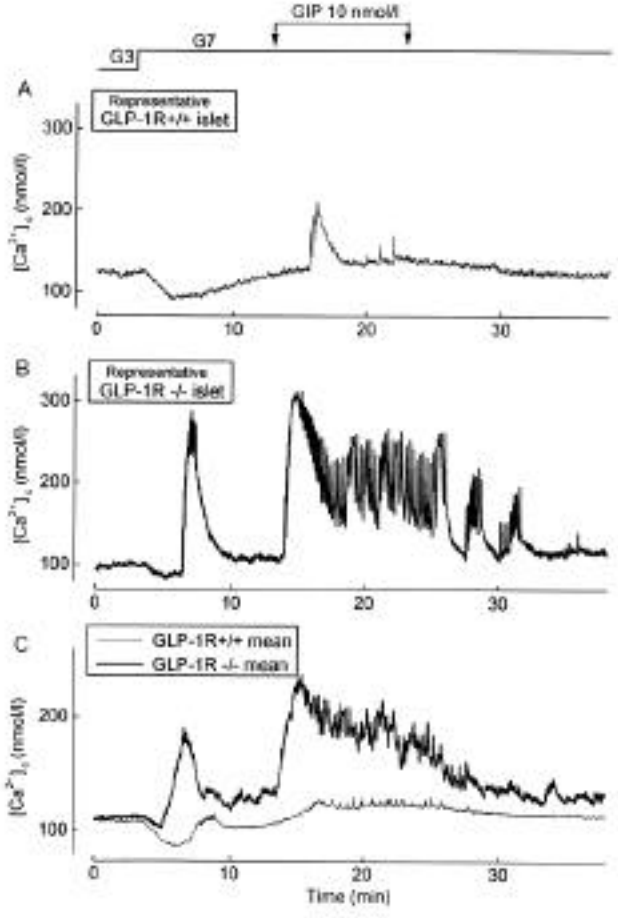

FIG. 6. Effect of GIP on $[Ca^{2+}]_c$ in GLP-1R+/+ and GLP-1R–/– islets. The glucose concentration of the perifusion medium was increased from 3 mmol/l (G3) to 7 mmol/l (G7), and GIP (10 nmol/l) was applied when indicated. The traces are representative (*A* and *B*) or mean (*C*) results from 13 GLP-1R+/+ and 12 GLP-1R–/– islets.

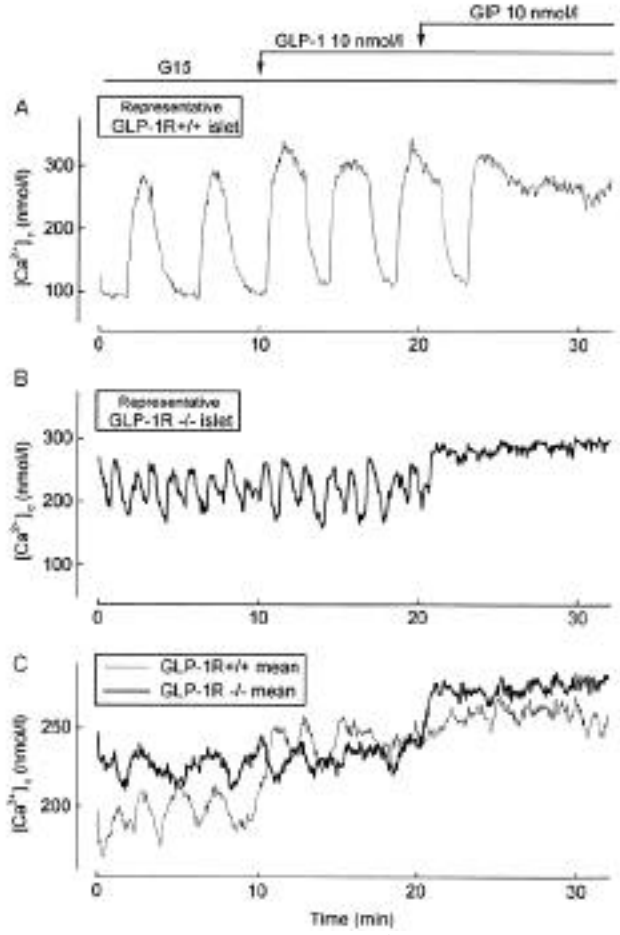

FIG. 7. Effect of GLP-1 and GIP on $[Ca^{2+}]_c$ in GLP-1R+/+ and GLP-1R–/– islets stimulated with 15 mmol/l glucose. The glucose concentration of the perifusion medium was 15 mmol/l (G15) throughout the experiment. GLP-1 (10 nmol/l) and GIP (10 nmol/l) were applied as indicated. The traces are representative (*A* and *B*) or mean (*C*) results from 48 control and 49 knockout islets.

near-basal levels. Finally, depolarization with 30 mmol/l K^+ in the presence of diazoxide and absence of ACh rapidly reactivated voltage-dependent Ca^{2+} channels and raised $[Ca^{2+}]_c$ to a sustained plateau that was similar in control and GLP-1R–/– islets (Fig. 4).

The difference in average $[Ca^{2+}]_c$ observed at 15 mmol/l glucose was even more pronounced at 10 and 7 mmol/l (Fig. 5*A*). When the glucose concentration was raised from 3 to 7 mmol/l, $[Ca^{2+}]_c$ transiently decreased in most control islets whereas it displayed a biphasic rise in GLP-1R–/– islets (Fig. 6). The threshold glucose concentration for induction of a $[Ca^{2+}]_c$ response is thus lower in GLP-1R–/– islets than in control islets.

Digital image analysis was used to test whether the disorganization of glucose-induced $[Ca^{2+}]_c$ oscillations in GLP-1R–/– islets could result from an asynchrony between subregions of the islets. In GLP-1R+/+ islets, glucose-induced $[Ca^{2+}]_c$ changes were well synchronized between all subregions even when their amplitude was variable (Fig. 5*B*). In 10 of 16 GLP-1R+/+ islets that displayed $[Ca^{2+}]_c$ oscillations in the presence of 7 mmol/l glucose, some subregions were silent aside from the oscillating ones (Fig. 5*B*). In contrast to control islets, GLP-1R–/– islets showed poor synchronization of glucose-induced $[Ca^{2+}]_c$ changes between subregions even in 15 mmol/l glucose (Fig. 5*C*). A sustained $[Ca^{2+}]_c$ response in certain subregions was associated with $[Ca^{2+}]_c$ oscillations in others.

Effect of GLP-1 and GIP on $[Ca^{2+}]_c$. Addition of 10 nmol/l GLP-1 to a medium containing 15 mmol/l glucose induced a small increase in $[Ca^{2+}]_c$ accompanied by a lengthening of the duration of $[Ca^{2+}]_c$ oscillations in 37 of 48 control islets (Fig. 7*A*), and a sustained plateau in the other islets (not shown). As expected, GLP-1 was ineffective in GLP-1R–/– islets (Fig. 7*B*). Subsequent addition of GIP raised $[Ca^{2+}]_c$ further and transformed $[Ca^{2+}]_c$ oscillations into a sustained plateau in most control and GLP-1R–/– islets. Average $[Ca^{2+}]_c$ integrated from 24–32 min was not significantly different under these conditions (276 ± 5 nmol/l in 49 GLP-1R–/– islets vs. 259 ± 6 nmol/l in 48 control islets; Fig. 7*C*). However, when GIP was added to a medium containing 7 mmol/l glucose, it was ineffective in 8 of 13 control islets and induced fast but infrequent $[Ca^{2+}]_c$ oscillations in the other islets (Fig. 6*A*). By contrast, GIP increased $[Ca^{2+}]_c$ in 12 of 12 GLP-1R–/– islets (Fig. 6*B*). On average, the GIP-induced $[Ca^{2+}]_c$ rise at 7 mmol/l glucose was much larger in GLP-1R–/– islets than in control islets (Fig. 6*C*) ($P < 0.01$).

DISCUSSION

It is currently accepted that GLP-1 and GIP are the major incretins in animal models and in man (4,29–31). Furthermore,

it has been proposed that GLP-1 may also be important to keep β-cells—at least part of them—in a glucose-competent state (10,11). We previously suggested that the glucose sensitivity and responsiveness of a pancreatic β-cell population can be explained by a model of heterogeneity among the individual cells (32–34). Since the insulin response of β-cells can be modulated by hormones acting via cAMP (19,20), mouse islets with disrupted GLP-1R signaling (8) are a useful model for studying the role of GLP-1 in the glucose competence of β-cells. The observations presented here demonstrate that, although glucose competence is preserved in perifused GLP-1R–/– islet cells, these islet cells show profound changes in intracellular signal transduction, both in terms of glucose-induced calcium signaling and GIP-induced cAMP production.

We have recently reported (12), and confirm in the present study, that isolated islets from mice homozygous for GLP-1R–/– exhibit a well-preserved insulin-secretory response to glucose. This observation can be interpreted in two ways. First, the presence of GLP-1R is not essential for the secretory response of isolated islets to glucose alone. This possibility is contradicted by our demonstration that the GLP-1R antagonist exendin (9–39)amide inhibited glucose-induced insulin release from control islets during 15-min stimulation either in perifusions or during static incubations. Second, functional GLP-1R are important for maintaining a good secretory response to glucose, but their loss as a result of gene disruption can be compensated for via changes in signaling in β-cells. Our present results showing differences in cAMP accumulation and $[Ca^{2+}]_c$ between GLP-1R–/– and GLP-1R+/+ islets support this second hypothesis. cAMP accumulation in the presence of 20 mmol/l glucose was significantly less in GLP-1R–/– islets than in GLP-1R+/+ islets, and this difference disappeared completely in the presence of 1 μmol/l exendin (9–39)amide. This finding suggests that in control islets, even in the absence of ligand, GLP-1R may exhibit a constitutively active role in maintaining the rate of islet cell cAMP production. Consistent with these observations, we previously reported that exendin (9–39)amide lowers basal cAMP accumulation and insulin secretion from rat purified β-cells (14). Others have recently observed reduced basal rate of cAMP formation and decreased insulin secretion from a GLP-1R–/– β-cell line (35). Taken together, these findings suggest that exendin (9–39) amide acts on the "empty" GLP-1R as an inverse agonist. In intact islets of Langerhans and, to a lesser extent, flow-sorted β-cells, paracrine glucagon can accumulate (20). In such conditions, competition between exendin (9–39)amide and glucagon for binding to GLP-1R on β-cells may be an additional mechanism for the effect of exendin (9–39)amide on cAMP accumulation and insulin release (14).

The effect of exendin (9–39)amide on insulin release requires functional GLP-1R and appears cAMP-mediated, because: *1*) no effects of exendin (9–39)amide were observed in GLP-1R–/– islets; and *2*) in control islets, exendin (9–39)amide did not impair the potentiating effect of 10 nmol/l GIP (Fig. 2*B*) or Sp-cAMPS (data not shown) on glucose-induced insulin secretion. We do not know why glucose-induced insulin release from control islets incubated for 60 min was not suppressed by exendin (9–39)amide, whereas the effect of a 15-min stimulation was effectively inhibited. One possibility is that GLP-1R might become desensitized after longer exposure to 1 μmol/l exendin (9–39)-amide, but the results of the continuous perifusion with exendin (Fig. 1) do not support this idea. Alternatively, 1-h static incubation of control islets might allow sufficient accumulation of paracrine glucagon to compete with exendin (9–39)amide for binding on GLP-1R.

A caveat in the direct comparison of the cAMP data and insulin release data is that the former were collected after incubation with the phosphodiesterase inhibitor IBMX, thus allowing to detect differences in cAMP production, while insulin release was measured in the absence of IBMX. In control experiments, in which we added 250 μmol/l IBMX to the 15-min statically incubated islets, the same trends on insulin release after adding GLP-1 and GIP were observed as in the absence of IBMX, but the responses were much smaller, largely because the effect of glucose alone was very high (data not shown). It should not be overlooked, therefore, that the difference in "basal" cAMP concentration of control and knockout β-cells may be smaller in vivo than what was measured in the present conditions, first because the in vitro measurements required addition of a phosphodiesterase inhibitor and, second, because the contribution of non–β-cells to islet cAMP in this model is not known.

Assuming that the acute suppression of glucose-induced insulin release proceeds via lowering of intracellular cAMP accumulation, our current and previous (14) data suggest a role for a sufficiently high level of basal cAMP production in the insulin-secretory response of islets to high glucose. Such a conclusion is not supported, however, by a recent study (36) showing that disruption of protein kinase A anchoring in rat islets and RINm5F cells by introduction of anchoring peptides or expression of soluble A-kinase–anchoring proteins blocked GLP-1–stimulated but not glucose-induced insulin release. The experimental designs (the species used, acute versus chronic disruption of islet cAMP signaling) in the study by Lester et al. (36) and the present study are quite different, so that it seems difficult to directly compare results.

Because glucose-induced insulin secretion from GLP-1R–/– islets is well preserved (12 and this study) despite lower cAMP levels, it can be postulated that one or several mechanisms compensate for the disruption of the GLP-1R signaling. As a first possibility, we considered compensation via an increase in the sensitivity to cAMP, e.g., by upregulating catalytic subunits, downregulating regulatory subunits of cAMP-dependent protein kinase or changes in the expression of targets of cAMP-dependent phosphorylation. This possibility was assessed by measuring insulin secretion at 20 mmol/l glucose in the presence of different concentrations of Sp-cAMPS, a potent cAMP analog that is taken up by intact cells (37). The absence of enhanced sensitivity to Sp-cAMPS suggests that increased cAMP sensitivity, particularly at higher levels of intracellular cAMP, is not a generalizable feature of GLP-1R–/– islets. Second, because GLP-1 increases $[Ca^{2+}]_c$ in beta-cells (38) and mobilizes intracellular Ca^{2+} in βTC3-cells (39), we evaluated whether compensating mechanisms in islets of GLP-1R–/– mice could involve changes in $[Ca^{2+}]_c$. A consistent feature of GLP-1R–/– islets was a higher average $[Ca^{2+}]_c$ in the presence of a stimulating glucose concentration. An increased glucose-induced $[Ca^{2+}]$ response is unexpected in the context of a decrease in islet cAMP and the knowledge that

cAMP has a slight positive effect on Ca^{2+}-channel activity in β-cells (21,22). Because the islets isolated from both mouse strains responded identically to high K^+ in the presence of diazoxide, it seems unlikely that the higher $[Ca^{2+}]_c$ response to glucose in GLP-1R–/– islets is the result of an increased number of voltage-dependent Ca^{2+} channels. Furthermore, ACh had similar effects on $[Ca^{2+}]_c$ in both types of islets, indicating that inositol-1,4,5-triphosphate (IP_3)-mediated calcium mobilization from intracellular stores has not been changed substantially in GLP-1R–/– β-cells. It is conceivable that a chronic decrease in cAMP production has effects on Ca^{2+} channel function that are different from those measured in acute in vitro experiments.

So, how might this change in glucose-signaling be achieved? One possibility, to be examined by future experiments, is that higher $[Ca^{2+}]_c$ in GLP-1R–/– β-cells at stimulatory glucose concentrations results from an increase in glucose metabolism. This idea is substantiated by the observation that the threshold glucose concentration for induction of a $[Ca^{2+}]_c$ response was lower in GLP-1R–/– than in GLP-1R+/+ islets. Increased metabolism would not only affect $[Ca^{2+}]_c$ through the K^+-ATP dependent pathway, but also potentiate insulin secretion through the K^+-ATP channel–independent pathway (40). Interestingly, we observed signs of asynchronism of $[Ca^{2+}]_c$ oscillations in response to glucose between subregions in islets of GLP-1R–/– mice. Among several mechanisms that could explain this phenomenon, a difference in metabolic and electric coupling between islet β-cells should be considered. Such coupling has been shown previously to depend on gap junctions between islet cells (41,42). In other tissues it has been shown that gap junctional communication is increased by raising the intracellular cAMP concentration (43,44). Therefore, the low cAMP content in GLP-1R–/– islets may be insufficient to maintain adequate coupling between large numbers of islet cells. Taken together, our data seem to indicate that multiple abnormalities in terms of $[Ca^{2+}]_c$ and cAMP exist in GLP-1R–/– islets and that the larger $[Ca^{2+}]_c$ response to glucose of GLP-1R–/– β-cells compensates, at least in terms of glucose-induced insulin release, for lower cAMP levels.

We have previously reported that GLP-1R–/– mice exhibit upregulation of the GIP system, both in terms of GIP plasma levels and in terms of GIP potentiation of glucose-induced insulin release (13). The present study extends these findings by demonstrating a higher responsiveness of GLP-1R–/– islets to GIP at the level of islet cAMP accumulation (Fig. 2*A*). This hyperresponsiveness could result from upregulation of the number of GIP receptors or increased signaling following activation of the GIP receptor. Consistent with the cAMP data are the significantly larger effects of 10 nmol/l GIP on 20 mmol/l glucose–induced insulin release (Fig. 2*B*) and on 7 mmol/l glucose–induced $[Ca^{2+}]_c$ (Fig. 7) in GLP-1R–/– islets.

In summary, the data presented in this article suggest that, despite the well-preserved insulin secretory response to glucose of GLP-1R–/– mice in vitro, GLP-1R–/– islets exhibit profound changes in basal and stimulated intracellular signaling at the level of both cAMP and $[Ca^{2+}]_c$. It remains to be shown to what extent upregulation of GIP-R–induced cAMP accumulation and the larger $[Ca^{2+}]_c$ response to glucose in GLP-1R–/– β-cells are the pivotal mediators of in vivo compensatory adaptation to the genetic loss of β-cell GLP-1R signaling.

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