Critical Role of cAMP-GEFII-Rim2 Complex in Incretin-potentiated Insulin Secretion*

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Incretins such as glucagon-like peptide-1 and gastric inhibitory polypeptide/glucose-dependent insulinotropic peptide are known to potentiate insulin secretion mainly through a cAMP/protein kinase A (PKA) signaling pathway in pancreatic β-cells, but the mechanism is not clear. We recently found that the cAMP-binding protein cAMP-GEFII (or Epac 2), interacting with Rim2, a target of the small G protein Rab3, mediates cAMP-dependent, PKA-independent exocytosis in a reconstituted system. In the present study, we investigated the role of the cAMP-GEFII-Rim2 pathway in incretin-potentiated insulin secretion in native pancreatic β-cells. Treatment of pancreatic islets with antisense oligodeoxynucleotides (ODNs) against cAMP-GEFII alone or with the PKA inhibitor H-89 alone inhibited incretin-potentiated insulin secretion ~50%, while a combination of antisense ODNs and H-89 inhibited the secretion ~80–90%. The effect of cAMP-GEFII on insulin secretion is mediated by Rim2 and depends on intracellular calcium as well as on cAMP. Treatment of the islets with antisense ODNs attenuated both the first and second phases of insulin secretion potentiated by the cAMP analog 8-bromo-cAMP. These results indicate that the PKA-independent mechanism involving the cAMP-GEFII-Rim2 pathway is critical in the potentiation of insulin secretion by incretins.

Blood glucose levels are precisely controlled by insulin release from pancreatic β-cells. Insulin secretion from the β-cells is regulated positively and negatively by many intracellular signals generated by various factors, including nutrients, hormones, and neurotransmitters (1–4). cAMP is thought to be a most critical intracellular signal in the mechanism of potentiation of insulin secretion (5–8). In fact, insulin secretion varies significantly with treatment by adenylyl cyclase activators and inhibitors and phosphodiesterase inhibitors that alter cAMP significantly with treatment by adenylyl cyclase activators and inhibitors and phosphodiesterase inhibitors that alter cAMP.

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‡‡ The abbreviations used are: PKA, protein kinase A; GLP-1, glucagon-like peptide-1; GIP, gastric inhibitory polypeptide; G-protein, guanine nucleotide-binding protein; 8-Br-cAMP, 8-bromo-cyclic AMP; ODN, oligodeoxynucleotide; HA, hemagglutinin; WT, wild type; NS, not significant; GFP, green fluorescent protein.
GEFII, interacting with Rim2 (26), a target of the small G-protein Rab3, mediates cAMP-dependent, PKA-independent exocytosis in a reconstituted system (26). However, the role of cAMP-GEFII in insulin secretion in native pancreatic β-cells is not known.

In the present study, we have investigated the role of cAMP-GEFII-Rim2 in incretin-potentiated insulin secretion. Our data indicate that a PKA-independent mechanism involving cAMP-GEFII-Rim2 is critical in the potentiation of insulin secretion by both GLP-1 and GIP.

EXPERIMENTAL PROCEDURES

Reagents—Synthetic GLP-1 (7–36) amide and GIP were purchased from Peptide Institute, Inc. (Osaka, Japan). 8-Bromo-cyclic AMP (8-Br-cAMP), carbacarb, and 3-isobutyl-1-methylxanthine were from Sigma. H-89 was from Calbiochem-Novabiochem. MLD 12330A was from RBI (Natick, MA).

Isolation of Mouse Pancreatic Islets and Batch Incubation Experiments—All animal procedures were approved by the Chiba University Animal Care Committee. Mouse pancreatic islets were isolated by collagenase digestion method as described previously (36) and were cultured in RPMI medium 1640 (Invitrogen Corp., Carlsbad, CA) containing 10% (v/v) fetal bovine serum, 60.5 mg/liter penicillin, 100 mg/liter streptomycin, and 11.1 mM glucose under a humidified condition of 95% air and 5% CO₂. The islets then were cultured with the medium containing 4 μM of antisense phosphorothioate-substituted ODNs against mouse cAMP-GEFII (5′-CAACGGCTTCTTACAACGT-3′) or control ODNs (5′-ACCTACGTGACTACGT-3′) (BIOLOGOSTIK, Göttingen, Germany) for 96 h. Batch incubation experiments were performed as described previously (36). After preincubation (30 min) of isolated islets with Hepes-Krebs buffer containing 2.8 mM glucose, five size-matched islets were collected in each tube and incubated in 500 μl of the same buffer containing glucose and test substances at the indicated concentrations for 30 min. GLP-1, GIP, H-89 as a PKA inhibitor, and 3-isobutyl-1-methylxanthine were added in the preincubation period, and 8-Br-cAMP was added in the incubation period. Insulin released into the medium was measured by radioimmunoassay (Eiken Chemical, Tokyo, Japan) (37).

Measurements of cAMP Content in the Islets—The cAMP content of the islets was measured according to the manufacturer’s instructions for the cAMP enzyme immunoassay system (Amersham Pharmacia Biotech) in the presence of 20 mM glucose. 3-Isobutyl-1-methylxanthine (25 μM) was always added to the incubation buffer. Twenty islets were used for cAMP measurements in each batch. The cAMP levels were normalized to the protein concentration.

Glutathione S-Transferase Pull-down Assay—The isolated mouse pancreatic islets, treated with control ODNs or antisense ODNs as described above, were homogenized and incubated at 4 °C for 12 h with 2 μg of glutathione S-transferase-Rim2 (residues 1466–2453) or glutathione S-transferase alone immobilized on glutathione beads. The complexes were washed and then separated by SDS-PAGE and immunoblotted with the IgG-purified anti-cAMP-GEFII antibody (26).

Immunoblot Analysis—Homogenates of mouse pancreatic islets, treated with control ODNs or antisense ODNs, were subjected to SDS-PAGE and immunoblotting with anti-PKA regulatory subunit IIα antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), anti-Rab3A antibody (Transduction Laboratories, Lexington, KY), and anti-VAMP-2 antibody (Calbiochem-Novabiochem).

Co-immunoprecipitation—The wild-type (WT) Rim2 cDNA was subcloned into the modified pCMV containing HA epitope (pCMV-HA). The mutant cAMP-GEFII (G114E,G422D) was subcloned into the pFLAG-CMV-2 (Sigma). The WT cAMP-GEFII cDNA was subcloned into the pCMV containing GFP and Myc epitopes (pCMV-GFP, Myc). COS-1 cells were transfected with plasmid vectors for HA-tagged Rim2, FLAG-tagged mutant cAMP-GEFII (G114E,G422D), or GFP- and Myc-tagged cAMP-GEFII. For co-immunoprecipitation, cellular lysates were incubated with anti-Myc antibody (Santa Cruz Biotechnology), followed by incubation with protein G-Sepharose. The proteins were analyzed by immunoblotting, using anti-HA antibody (Roche Molecular Biochemicals), anti-GFP antibody (Living Colors A.v. Peptide Antibody; CLONTECH Laboratories, Inc., Palo Alto, CA), or anti-FLAG antibody (Sigma).

Measurement of C-peptide Secretion—MIN6 cells were cultured in Dulbecco’s modified Eagle’s medium containing 25 mM glucose, 10% (v/v) fetal bovine serum, 60.5 mg/liter penicillin, and 100 mg/liter streptomycin under a humidified condition of 95% air and 5% CO₂ (37).

RESULTS

GLP-1- and GIP-potentiated Insulin Secretion and cAMP Production in Pancreatic Islets—Both GLP-1 and GIP (100 nM

**Fig. 1. Dose response relationships for the potentiating effects of GLP-1 and GIP on insulin secretion.** A, insulin response to various concentrations of glucose in the presence or absence of GLP-1 (100 nM) was measured. B, insulin response to various concentrations of glucose in the presence or absence of GIP (100 nM) was measured. The data were obtained from three independent experiments (n = 5–6 for each point) in A and B.

MIN6 cells were transfected with human preproinsulin expression vector (pCMV-lrhoins) plus pCMV-luciferase, pCMV-HA-Rim2ΔA, pFLAG-CMV-2-mutant cAMP-GEFII (G114E,G422D), pCMV-HA-Rim2, or pCMV-GFP- and Myc-cAMP-GEFII. The deletion mutant Rim2 (Rim2ΔA) lacks the zinc finger and C₂ domains but retains the cAMP-GEFII binding region and has a dominant negative effect on interaction between WT cAMP-GEFII and WT Rim2 (26). The mutant cAMP-GEFII (G114E,G422D), in which both CAMP binding sites are disrupted, was also used (26). As control, luciferase was used. Three days after transfection, the C-peptide secretory response to 8-Br-cAMP (1 mM) in the presence of glucose (16.7 mM) for 60 min was evaluated by human C-peptide released into medium. Human C-peptide was measured by a human C-peptide radioimmunoassay kit (Linco Research Inc., St. Charles, MO).

Perifusion Experiment—Perfusion of pancreatic islets was performed as described previously (36). Briefly, groups of 100 isolated mouse islets treated for 96 h with 4 μM control or antisense ODNs as described above were loaded onto filters in columns with Bio-Gel P-2 (Bio-Rad) and continuously perifused with Hepes-Krebs buffer at a constant flow rate of 1.0 ml/min. After a 30-min stabilization period with 2.8 mM glucose, the groups of islets were successively stimulated with 16.7 mM glucose with or without 100 μM 8-Br-cAMP. Perifusate solutions were gassed with 95% O₂ and 5% CO₂ and maintained at 37 °C.

Statistical Analysis—Results presented are derived from at least three independent experiments performed on different days. The values are expressed as mean ± S.E. The data were compared using Student’s unpaired t test.
(for each) potentiate insulin secretion in a glucose concentration-dependent manner (Fig. 1, A and B), as has been reported previously (15, 16, 38, 39). We next examined cAMP production by GLP-1 and GIP in pancreatic islets. GLP-1 (100 nM) induced about 2-fold cAMP production in the islets, compared with basal level (basal, 26.5 ± 1.8 ng/μg protein, n = 5; GLP-1 alone, 58.1 ± 7.2 ng/μg protein, n = 6, p < 0.005) (Fig. 2A). The effect of GLP-1 on cAMP production was completely blocked by treatment with MDL12330A (10 μM), an adenylyl cyclase inhibitor (39) (GLP-1 plus MDL12330A, 27.3 ± 1.2 ng/μg protein, n = 6) (Fig. 2A). MDL12330A did not affect the cAMP level at the basal state (Fig. 2A). Similarly, GIP (100 nM) induced about 2-fold cAMP production in the islets, compared with basal level (basal, 27.3 ± 1.0 ng/μg protein, n = 5; GIP alone, 56.4 ± 3.3 ng/μg protein, n = 5, p < 0.0001) (Fig. 2B). The GIP-induced cAMP production was completely abolished by treatment with MDL12330A (GIP plus MDL12330A, 27.2 ± 2.4 ng/μg protein, n = 6) (Fig. 2B).

**Effects of MDL12330A on Incretin-Potentiated Insulin Secretion**—To determine whether cAMP is an essential signal in incretin-potentiated insulin secretion, we examined the effects of MDL12330A (10 μM) on the GLP-1- and GIP-potentiated insulin secretions in the presence of 11.1 mM glucose using the batch incubation method (Fig. 2, C and D). MDL12330A did not alter the insulin secretion at the basal state (basal, 2.53 ± 0.22 ng/islet/30 min; MDL12330A alone, 2.88 ± 0.24 ng/islet/30 min, n = 4) (Fig. 2C). MDL12330A treatment remarkably inhibited GLP-1 (100 nM)-potentiated insulin secretion (GLP-1 alone, 8.50 ± 0.20 ng/islet/30 min; GLP-1 plus MDL12330A, 3.93 ± 0.14 ng/islet/30 min, n = 4, p < 0.0001) (Fig. 2C). Similarly, MDL12330A treatment inhibited GIP-potentiated insulin secretion (basal, 2.50 ± 0.18 ng/islet/30 min; MDL12330A alone, 2.76 ± 0.15 ng/islet/30 min; GIP alone, 7.72 ± 0.50 ng/islet/30 min; GIP plus MDL12330A, 3.08 ± 0.15 ng/islet/30 min, n = 5 for each) (GIP alone versus GIP plus MDL12330A, p < 0.0001) (Fig. 2D). Together, these data indicate that both the GLP-1- and GIP-potentiated insulin secretions depend critically on cAMP production in pancreatic β-cells.

**Effects of the Antisense ODNs against cAMP-GEFII on Incretin-Potentiated Insulin Secretion**—We have shown recently that the cAMP-binding protein cAMP-GEFII is a direct target of cAMP in regulated exocytosis (26). In the present study, we investigated with the purpose of evaluating cAMP-GEFII involvement in the potentiation of insulin secretion by incretins with native pancreatic β-cells. We first ascertained if treatment of the islets with antisense ODNs could suppress the level of endogenous cAMP-GEFII protein. Treatment with antisense ODNs markedly decreased the cAMP-GEFII protein level in the islets (Fig. 3A). To further confirm the specificity of the antisense ODNs, we checked the level of other proteins (i.e. the...
PKA regulatory subunit IIα, small G-protein Rab3A, and VAMP-2 (vesicle-associated membrane protein-2)). There were no differences in expression of protein levels between control ODN-treated and antisense ODN-treated pancreatic islets (Fig. 3A), indicating that the antisense ODNs are specific for cAMP-GEFII. We then examined the effect of antisense ODNs on the insulin secretory responses to GLP-1 and GIP. Glucose-induced insulin secretion was not affected by antisense ODNs treatment (Fig. 3B). The GLP-1 (100 nM)-potentiating insulin secretion in the presence of 11.1 mM glucose in the islets treated with control or antisense ODNs was measured (control ODN-treated, 8.51 ± 0.63 ng/islet/30 min; antisense ODN-treated, 6.26 ± 0.57 ng/islet/30 min, n = 5, p < 0.0001) (Fig. 3C). The GIP (100 nM)-potentiating insulin secretion also decreased significantly (control ODN-treated, 7.01 ± 0.79 ng/islet/30 min; antisense ODN-treated, 5.10 ± 0.44 ng/islet/30 min, n = 6, p < 0.0005) (Fig. 3D). These results indicate that cAMP-GEFII is involved in the potentiation of insulin secretion by both GLP-1 and GIP in pancreatic islets.

**Mechanism of Insulin Secretion by Incretins**

Involvement of cAMP-GEFII in PKA-independent Insulin Secretion Potentiated by GLP-1, GIP, and 8-Br-cAMP—We examined the effect of a combination of H-89 (41) and antisense ODNs on GLP-1-, GIP-, or 8-Br-cAMP-potentiated insulin secretion in mouse pancreatic islets. The insulin secretion potentiated by GLP-1 (100 nM) in the presence of 11.1 mM glucose in the islets treated with control or antisense ODNs was measured (*, p < 0.0001) (Fig. 4A). The GLP-1 (100 nM)-potentiating insulin secretion in the presence of 11.1 mM glucose in the islets treated with control or antisense ODNs was measured (control ODN-treated, 8.49 ± 0.23 ng/islet/30 min; GLP-1 alone, 8.49 ± 0.23 ng/islet/30 min; GLP-1 plus H-89, 5.57 ± 0.20 ng/islet/30 min, n = 5, p < 0.0001). Combination treatment
cAMP in the presence of 16.7 mM glucose. Similar results were obtained in a similar manner as described for A. Using 8-Br-cAMP (1 mM) (**, p < 0.0001; ***. p < 0.0005). C. Effect of H-89 on 8-Br-cAMP-potentiated insulin secretion in the islets. The experiment was done in a similar manner as described for A, using 8-Br-cAMP (1 mM) (**, p < 0.0005; ****, p < 0.0005). 16.7 mM glucose was used to determine the potentiating effect of 8-Br-cAMP. Control ODNs, pancreatic islets treated with control ODNs. Antisense ODNs, pancreatic islets treated with antisense ODNs in A–C. The data were obtained from one of 4–6 similar experiments (n = 5–8 for each) in A–C.

with H-89 and the antisense ODNs caused a further reduction in GLP-1-potentiated insulin secretion (4.27 ± 0.12 ng/islet/30 min, n = 5, p < 0.001). The insulin secretion potentiated by GIP (100 nM) also was measured in the presence of 11.1 mM glucose (Fig. 4B). Similarly, H-89 partially blocked GIP-potentiated insulin secretion (GIP alone, 7.27 ± 0.18 ng/islet/30 min; GIP plus H-89, 4.24 ± 0.13 ng/islet/30 min, n = 5, p < 0.0001), and combination treatment with H-89 and the antisense ODNs caused a further reduction in GIP-potentiated insulin secretion (2.99 ± 0.14 ng/islet/30 min, n = 5, p < 0.0005). To confirm the involvement of cAMP-GEFII in cAMP-dependent, PKA-independent insulin secretion, we used the cAMP analog 8-Br-cAMP in the presence of 16.7 mM glucose. Similar results were obtained with 8-Br-cAMP (Fig. 4C). These results suggest strongly that both GLP-1- and GIP-potentiated insulin secretions are mediated by PKA-independent as well as PKA-dependent mechanisms and that cAMP-GEFII participates in a PKA-independent mechanism.

cAMP-potentiated Insulin Secretion Is Mediated by the cAMP-GEFII-Rim2 Complex—cAMP-GEFII has been shown to interact with Rim2, a target of the small G-protein Rab3 (26). To determine whether the effect of cAMP-GEFII on cAMP-potentiated insulin secretion requires its direct interaction with Rim2, we used two mutants (26) (Fig. 5A). We first examined the effect of Rim2ΔA on 8-Br-cAMP-potentiated exocytosis from MIN6 cells in which endogenous cAMP-GEFII and Rim2ΔA are expressed. For this purpose, we utilized MIN6 cells transfected with human proinsulin cDNA (25, 42). Proinsulin is converted into insulin and C-peptide during the secretory process in pancreatic β-cells (43). Since antibodies against human insulin cross-react with endogenous mouse insulin, we monitored secretion by measuring the human C-peptide release from MIN6 cells transfected with human proinsulin and Rim2ΔA (26). Overexpression of Rim2ΔA in MIN6 cells significantly inhibited the 8-Br-cAMP-induced C-peptide secretion in the presence of 16.7 mM glucose (Fig. 5B). Coexpression of WT cAMP-GEFII with Rim2ΔA in MIN6 cells significantly restored inhibition of the C-peptide secretion by Rim2ΔA, suggesting that the effect of cAMP-GEFII on cAMP-potentiated insulin secretion requires interaction with Rim2. Similarly, we also assessed the effect of the mutant cAMP-GEFII (G114E,G422D). An in vivo binding experiment shows that overexpression of cAMP-GEFII (G114E,G422D) inhibits interaction between the WT cAMP-GEFII and WT Rim2 (Fig. 5C), indicating that the mutant acts as a dominant-negative inhibitor of the interaction. We reasoned that the double mutant (G114E,G422D), when overexpressed in MIN6 cells, might trap endogenous Rim2 to inhibit cAMP-potentiated C-peptide secretion. While overexpression of WT cAMP-GEFII did not alter 8-Br-cAMP-potentiated C-peptide secretion (data not shown), overexpression of the double mutant (G114E,G422D) significantly inhibited it (Fig. 5D). This inhibition of the C-peptide secretion was mostly restored by coexpression of WT Rim2, due probably to its interaction with endogenous WT cAMP-GEFII. These results indicate that cAMP-potentiated insulin secretion is mediated by the cAMP-GEFII-Rim2 complex.

The Effects of cAMP-GEFII Are Dependent on Intracellular Calcium as Well as cAMP—To determine whether the effect of cAMP-GEFII on cAMP-potentiated insulin secretion requires a rise in intracellular calcium concentrations ([Ca2+]i), we examined the effects of 8-Br-cAMP (1 mM), high K+ (60 mM), and their combination, in the presence of 2.8 mM glucose, on insulin secretion in pancreatic islets treated with control ODNs or antisense ODNs. There were no differences in the insulin secretions stimulated by 8-Br-cAMP alone or high K+ alone between control ODN- and antisense ODN-treated islets. In contrast, the insulin secretion stimulated by a combination of 8-Br-cAMP plus high K+ in antisense ODN-treated islets was
significantly lower than that in control ODN-treated islets (control ODNs, 8.81 ± 0.60 ng/islet/30 min; antisense ODNs, 6.24 ± 0.40 ng/islet/30 min, n = 10, p < 0.005) (Fig. 6A). We also examined the effect of carbachol (50 μM) on insulin secretion in the islets treated with control ODNs or antisense ODNs. While there were no differences in insulin secretion stimulated by 8-Br-cAMP alone or carbachol alone, the insulin secretion stimulated by a combination of 8-Br-cAMP plus carbachol in the antisense ODN-treated islets was significantly lower than that in the control ODN-treated islets (control ODNs, 3.48 ± 0.17 ng/islet/30 min; antisense ODNs, 2.45 ± 0.08 ng/islet/30 min, n = 16, p < 0.0001) (Fig. 6B). These results indicate that the effects of cAMP-GEFII on insulin secretion depend on intracellular Ca²⁺ as well as cAMP in pancreatic β-cells.

cAMP-GEFII Is Involved in Both the First and Second Phase of cAMP-potentiated Insulin Secretion—We examined the involvement of cAMP-GEFII in the insulin secretory phase using perifused mouse pancreatic islets. No significant difference was found between control ODN-treated islets and antisense ODN-treated islets in the absence of 8-Br-cAMP (Fig. 7A). When the islets were treated with antisense ODNs, both the first phase and second phase potentions by 8-Br-cAMP were suppressed (Fig. 7B), clearly showing that cAMP-GEFII is involved in both phases of insulin secretion.

**DISCUSSION**

Incretins such as GLP-1 and GIP play an important role in the potentiation of insulin secretion (44–47). It has generally been thought that both GLP-1 and GIP potentiate glucose-induced insulin secretion primarily by cAMP/PKA signaling, which leads to phosphorylation of regulatory proteins associated with the secretory process in pancreatic β-cells (15, 48, 49). A study by capacitance measurements has suggested that cAMP also promotes exocytosis in pancreatic β-cells in a PKA-independent mechanism (32). In the present study, we show that MDL 12330A, an inhibitor of adenylyl cyclase, completely blocks both the GLP-1- and the GIP-stimulated cAMP production in pancreatic islets and that MDL 12330A remarkably...
inhibits both the GLP-1- and the GIP-induced insulin secretions. These results confirm that the effects of both GLP-1 and GIP on insulin secretion depend critically on the intracellular cAMP elevation due to activation of adenylyl cyclase. It is interesting that MDL 12330A did not completely inhibit either GLP-1- or GIP-potentiated insulin secretion under the conditions in which cAMP production was blocked. This suggests that the potentiating effects of the incretins on insulin secretion are mediated at least in part by a cAMP-independent mechanism, although the effects are small.

We recently found that the cAMP-binding protein cAMP-GEFII, by interacting with Rim2, a target of Rab3, participates in cAMP-dependent, PKA-independent exocytosis in a reconstituted system (26). In the present study, we investigated in order to find whether the cAMP-GEFII in native pancreatic β-cells is involved in GLP-1- and GIP-potentiated insulin secretions and if such action is PKA-independent. Treatment of islets with antisense ODNs reduced both GLP-1- and GIP-potentiated insulin secretions, clearly indicating that the effects of the incretins are mediated in part by cAMP-GEFII. Ten μM of H-89, a widely used specific inhibitor of PKA phosphorylation in intact cells (24, 28, 41, 50), was then used to block the phosphorylation of GLUT2, a substrate of PKA in pancreatic β-cells (28), to evaluate incretin-potentiated insulin secretion. Interestingly, although treatment of pancreatic islets with H-89 reduced (about 50%) both GLP-1- and GIP-potentiated insulin secretions, treatment of the islets with H-89 plus antisense ODNs further reduced the insulin secretions (80–90%), suggesting strongly that the potentiation of insulin secretion by both GLP-1 and GIP is mediated by PKA-independent as well as PKA-dependent mechanisms and that cAMP-GEFII is involved in the PKA-independent mechanism.

We then determined whether or not the potentiating effects of cAMP-GEFII on insulin secretion depend on both cAMP and intracellular calcium. A, effects of 8-Br-cAMP (1 mM, 30 min), high K⁺ (60 mM, 30 min), or a combination of 8-Br-cAMP plus high K⁺ (30 min) on insulin secretion in pancreatic islets treated with control or antisense ODNs (*, p < 0.005). 2.8 mM glucose was always added to the incubation buffer. B, effects of 8-Br-cAMP (1 mM, 30 min), carbachol (50 μM, 30 min), or a combination of 8-Br-cAMP plus carbachol (30 min) on insulin secretion in the islets treated with control or antisense ODNs (**, p < 0.0001). 2.8 mM glucose was always added to the incubation buffer. Control ODNs (open columns), pancreatic islets treated with control ODNs. Antisense ODNs (filled columns), pancreatic islets treated with antisense ODNs in A and B. The data were obtained from three or four independent experiments (n = 8–21 for each point) in A and B.
of cAMP on insulin secretion are mediated by Rim2. Overexpression of a dominant negative mutant, Rim2 (Rim2ΔA) or cAMP-GEFII (G114E,G422D double mutant), inhibited the potentiating effect of 8-Br-cAMP on C-peptide secretion from human preproinsulin-transfected MIN6 cells. In addition, the inhibitory effect of Rim2ΔA or the cAMP-GEFII double mutant on C-peptide secretion was mostly restored by coexpression of WT cAMP-GEFII or WT Rim2, respectively, suggesting that the potentiating effects of the incretins are mediated by the cAMP-GEFII-Rim2 complex.

Because intracellular Ca$^{2+}$ is essential in triggering insulin secretion, we investigated to find if the mechanism of potentiation by the cAMP-GEFII-Rim2 complex is also Ca$^{2+}$-dependent. The effects of high K$^+$ and carbachol, which triggers Ca$^{2+}$ influx and mobilizes intracellular Ca$^{2+}$ (51), respectively, on insulin secretion were examined in islets treated with anti-sense DNAs. While there were no differences in the insulin secretions stimulated by 8-Br-cAMP alone, high K$^+$ alone, or carbachol alone between control ODN-treated and anti-sense ODN-treated islets, insulin secretion stimulated by a combination of 8-Br-cAMP plus high K$^+$ or 8-Br-cAMP plus carbachol was significantly reduced in anti-sense ODN-treated islets. These findings indicate that the potentiation of insulin secretion by the cAMP-GEFII-Rim2 pathway depends on intracellular Ca$^{2+}$ as well as cAMP. Since Rim2 has two C$_2$ domains, Ca$^{2+}$ might modulate interaction between cAMP-GEFII and Rim2.

cAMP potentiates both phases of insulin secretion at high glucose concentrations in isolated perfused pancreas (2). Similarly, GLP-1 and GIP are both known to potentiate both the first and second phases of glucose-induced insulin secretion (15, 16). To determine the involvement of cAMP-GEFII in each phase of insulin secretion, we evaluated the potentiating effect of insulin secretion by 8-Br-cAMP in perfused pancreatic islets with antisense or control ODNs treatment. Both the first and second phase enhancement by 8-Br-cAMP was significantly suppressed in islets treated with anti-sense ODNs compared with control, showing that both the first and second phases are potentiated by cAMP in a PKA-independent mechanism.

Considering these findings together, we propose that incretins potentiate glucose-induced insulin secretion primarily by two mechanisms: the pathway involving phosphorylation of regulatory proteins by PKA activation (PKA-dependent) and the pathway involving the cAMP-GEFII-Rim2 complex (PKA-independent). The affinity for cAMP is quite different in PKA and cAMP-GEFII, with $K_d$ of $\approx 100 \, nM$ (52) and $\approx 10 \, \mu M$ (26), respectively. cAMP at basal state in pancreatic islets has been reported in a range of micromolar concentrations (52), suggesting that many substrates for PKA already are maximally phosphorylated in pancreatic islets. This is the case with GLUT2 (28) and the sulfonfurylurea receptor SUR1, a subunit of the $\beta$-cell K$_{ATP}$ channel (29). Accordingly, PKA and cAMP-GEFII have distinct roles in cAMP-potentiated insulin secretion. The mechanism involving the cAMP-GEFII-Rim2 complex might operate upon a rise in local cAMP concentrations by stimulation. The mechanism involving PKA phosphorylation might also be controlled by PKA-anchoring protein (44, 53, 54). Since cAMP-GEFII has guanine exchange factor activity toward the small G-protein Rap1 (33), it is also tempting to speculate that Rap1, which is activated by cAMP-GEFII through incretins, might also be involved in insulin secretion.

Further elucidation of the regulation of the cAMP-GEFII-Rim2 complex by incretins should both clarify the mechanism of the potentiation of insulin secretion and suggest novel anti-diabetic drug therapy.
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