The Novel Imidazoline Compound BL11282 Potentiates Glucose-Induced Insulin Secretion in Pancreatic β-Cells in the Absence of Modulation of K_{ATP} Channel Activity

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The insulinotropic activity of the novel imidazoline compound BL11282 was investigated. Intravenous administration of BL11282 (0.3 mg · kg^{-1} · min^{-1}) to anesthetized rats did not change blood glucose and insulin levels under basal conditions, but produced a higher increase in blood insulin levels and a faster glucose removal from the blood after glucose infusion. Similarly, in isolated Wistar rat pancreatic islets, 0.1–100 μmol/l BL11282 potently stimulated glucose-induced insulin secretion but did not modulate basal insulin secretion. Unlike previously described imidazolines, BL11282 did not block ATP-dependent K^+ channels. Furthermore, the compound stimulated insulin secretion in islets depolarized with high concentrations of KCl or permeabilized with electric shock. Insulinotropic activity of BL11282 was dependent on activity of protein kinases A and C. In pancreatic islets from spontaneously diabetic GK rats, the imidazoline compound restored the impaired insulin response to glucose. In conclusion, the imidazoline BL11282 constitutes a new class of insulinotropic compounds that exerts an exclusive glucose-dependent insulinotropic activity in pancreatic islets by stimulating insulin exocytosis. *Diabetes* 50:797–802, 2001

Compounds with imidazoline structure are potent stimulators of insulin secretion in pancreatic β-cells (1). This effect is thought to be due to the blockade of ATP-dependent K^+ channel (K_{ATP}) activity and the increase in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) (2). Recently, we have shown that the imidazoline compound RX871024, in addition to blocking K_{ATP} channels and increasing $[Ca^{2+}]_i$, exerts its insulinotropic effect by enhancing insulin exocytosis at stages distal to the increase in $[Ca^{2+}]_i$ (3–5). This direct stimulation of exocytosis was only observed at elevated glucose concentrations and required activation of protein kinases A and C.

Sulfonylurea drugs, which are currently used in the treatment of type 2 diabetes, block K_{ATP} channels in β-cells and stimulate insulin secretion at both low and high glucose concentrations (6). The strong insulinotropic effect of sulfonylureas observed at low glucose concentrations often provokes pronounced hypoglycemia in patients treated with these drugs (7). Therefore, agents that do not stimulate basal insulin secretion but augment glucose-induced insulin secretion by modulating Ca^{2+}-stimulated insulin exocytosis could provide a better therapeutic alternative to sulfonylureas.

We now demonstrate that an imidazoline compound, BL11282 (Fig. 1*A*, insert), represents a new class of insulinotropic compounds that do not stimulate basal insulin secretion and lack the effect on K_{ATP} channel activity, but markedly potentiate glucose-induced insulin secretion.

RESEARCH DESIGN AND METHODS

BL11282 and LY333531 were obtained from Eli Lilly (Indianapolis, IN). Rp-cAMPS was from Calbiochem (San Diego, CA). All other reagents were from Sigma (St. Louis, MO).

Intravenous glucose tolerance test. The intravenous glucose tolerance test was performed in overnight-fasted anesthetized male Wistar rats weighing 280–350 g. With the rats under pentobarbitone anesthesia (50 mg/kg i.p.), polyethylene catheters were placed in the left jugular vein and in the left common carotid artery. Glucose was determined by the hexokinase method and insulin by radioimmunoassay.

Isolation of rat pancreatic islets and measurements of insulin secretion. Pancreatic islets were isolated from 2- to 3-month-old male control Wistar and diabetic GK rats by collagenase digestion, as previously described (8). GK rats were obtained from the Karolinska Institutet colony and exhibited major features of type 2 diabetes (9). All experiments were done with islets incubated overnight in RPMI 1640 culture medium (Life Technologies) supplemented with 10% fetal bovine serum, 2 mg/ml glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin, and 11 mmol/l glucose.

Insulin secretion was measured in Krebs-Ringer bicarbonate buffer (KRBB). Islets were incubated in KRBB with 3.3 mmol/l glucose at 37°C for 30–45 min. Groups of three islets were then picked up and incubated in 0.3 ml of the same buffer with the compounds of interest at 37°C for 1 h. After incubation samples were cooled on ice, aliquots were collected and insulin content was measured by radioimmunoassay with rat insulin (Novo Nordisk) as a standard (10).

Insulin secretion in electropermeabilized islets was studied as described previously (3). Briefly, islets were permeabilized in electroporation buffer containing 140 mmol/l potassium glutamate, 1.2 mmol/l $MgCl_2$, 5 mmol/l NaCl, 10 mmol/l EGTA, 25 mmol/l HEPES, pH 7.0, by five electrical pulses of 3 kV/cm. Three islets were then incubated in 0.3 ml modified electroporation medium containing in addition 2 mmol/l MgATP and an ATP-regenerating system (creatine and creatine kinase) at 37°C for 20 min. Insulin content was measured by radioimmunoassay.

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$[Ca^{2+}]_i$, cytosolic free Ca^{2+} concentration; K_{ATP}, ATP-dependent K^+ channel; KRBB, Krebs-Ringer bicarbonate buffer.

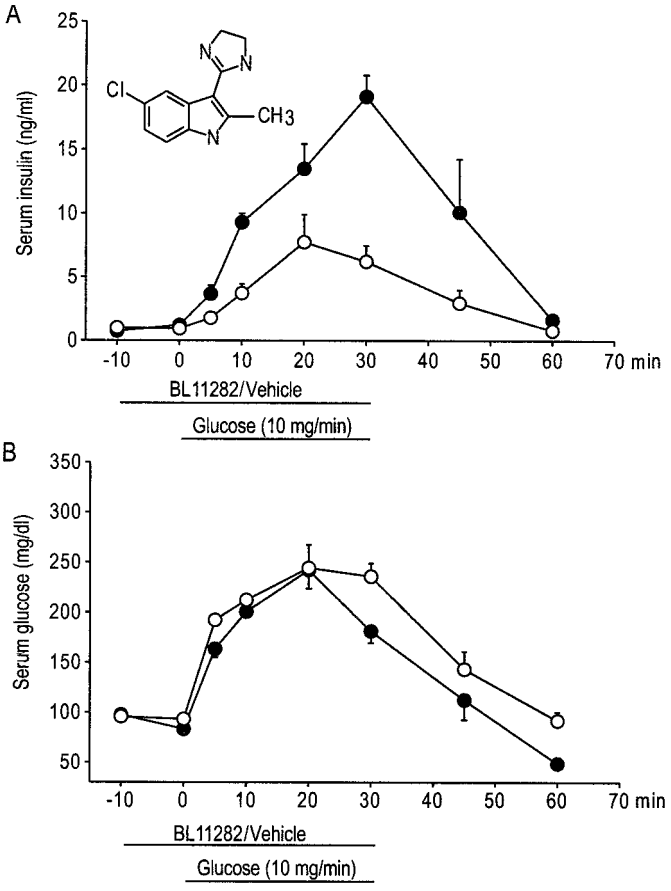

FIG. 1. Effects of the imidazoline compound BL11282 iv infusion (0.3 mg · kg^{-1} · min^{-1}) on serum levels of insulin (*A*) and glucose (*B*) in pentobarbitone-anesthetized rats before and after an intravenous glucose infusion. ●, BL11282 infusion; ○, vehicle. Data are means ± SE from four animals. Insert in *A* depicts chemical structure of the compound.

Measurements of $[Ca^{2+}]_i$ in rat pancreatic islets and β-cells. Single cells were obtained by islet agitation in Ca^{2+}-free medium and placed on the glass cover slip. Measurements of $[Ca^{2+}]_i$ in pancreatic islets and β-cells were performed as previously described (3,10).

Electrophysiology. The membrane potential and activity of K_{ATP} channels were studied in the perforated whole-cell configuration, and the activity of voltage-gated Ca^{2+} channels was studied in the whole-cell configuration of the patch-clamp technique, as previously described (3).

Statistical analysis. Data are expressed as means ± SE for the indicated number of observations. The difference of means was assessed with analysis of variance and Student's *t* test.

RESULTS

Infusion of the novel imidazoline compound BL11282 (0.3 mg · kg^{-1} · min^{-1}) in rats at basal conditions did not produce changes in either insulin or glucose levels (Fig. 1*A* and *B*). However, infusion of BL11282 with glucose (10 mg/min) enhanced both insulin release and glucose removal compared with just glucose infusion (Fig. 1*A* and *B*).

In isolated Wistar rat pancreatic islets, BL11282 did not influence insulin secretion at 3.3 mmol/l glucose (Fig. 2*A* and *F*). An increase in the ambient glucose concentration to 16.7 mmol/l resulted in a potent stimulatory effect of BL11282 on insulin secretion. The compound potentiated glucose-stimulated insulin secretion in the concentration range 0.1–100 μmol/l, and the stimulation at 100 μmol/l was ~500% of glucose-induced insulin secretion in the absence of the compound. In contrast to BL11282, all sulfonylureas tested, i.e., glibenclamide (Fig. 2*B* and *G*), gliclazide (Fig. 2*C* and *H*), tolbutamide (Fig. 2*D* and *I*), and glipizide (Fig. 2*E* and *J*), showed stimulation of insulin secretion at both basal and elevated glucose concentrations. The maximal increases of insulin secretion induced by sulfonylureas were higher at 3.3 mmol/l glucose than at 16.7 mmol/l glucose (Fig. 2*G*–*J*).

The glucose dependency of the effects of the imidazoline compound on insulin secretion was further studied in pancreatic islets from control Wistar and diabetic GK rats. We analyzed the effect of 50 μmol/l BL11282 on insulin secretion at 3, 9, 15, 21, and 27 mmol/l glucose (Fig. 3). In islets from Wistar, rats 50 μmol/l BL11282 stimulated insulin secretion at glucose concentrations of 9 mmol/l and higher (Fig. 3). In pancreatic islets from diabetic GK rats, an elevation of glucose concentration increased insulin secretion only slightly (Fig. 3); 50 μmol/l BL11282 restored glucose-sensitivity of diabetic islets to the levels of secretion observed with glucose alone in islets from control rats.

Because all imidazoline compounds described so far that stimulate insulin secretion also inhibit the activity of K_{ATP} channels (11), and depolarize the membrane, the effects of BL11282 on K_{ATP} channels and membrane potential were studied. BL11282 (50 μmol/l) did not affect either membrane potential or K_{ATP} channel activity at basal (3.3 mmol/l) or stimulated (16.7 mmol/l) glucose levels, as was measured using the perforated-patch configuration of the patch-clamp technique (Fig. 4*A*). BL11282 also did not influence the activity of voltage-dependent Ca^{2+} channels in the whole-cell configuration (Fig. 4*B*).

$[Ca^{2+}]_i$ was measured in single rat pancreatic islets and β-cells using the fura-2 microfluorescence technique. The imidazoline compound did not increase $[Ca^{2+}]_i$ in rat islets (Fig. 5*A* and *B*) at 3.3 mmol/l glucose. At 16.7 mmol/l glucose, 10 μmol/l BL11282 did not significantly influence $[Ca^{2+}]_i$ (Fig. 5*C*) and 50 μmol/l BL11282 increased $[Ca^{2+}]_i$ in seven of nine islets tested (Fig. 5*D*).

Recently, we showed that the imidazoline compound RX871024 stimulated insulin exocytosis under conditions in which Ca^{2+} concentration inside the cell was clamped high (3,4). Whether BL11282 shares similar effects was tested in rat pancreatic islets either depolarized with high concentrations of KCl and glucose or electropermeabilized. BL11282 in the concentration range 1–50 μmol/l increased insulin secretion in islets depolarized with 16.7 mmol/l glucose and 55 mmol/l KCl, but did not modify $[Ca^{2+}]_i$ (Fig. 6*A* and *B*). In electropermeabilized rat pancreatic islets, 50 μmol/l BL11282 induced an increase in insulin secretion at 10^{-5} mol/l Ca^{2+} and had no effect at basal free Ca^{2+} concentration (Fig. 6*C*).

Interaction between the imidazoline compound BL11282 and compounds that stimulate the activities of protein kinase A and phospholipase C—dibutyryl-cAMP and carbamylcholine, respectively—on glucose-induced insulin secretion in rat pancreatic islets was investigated. Both compounds further enhanced the effect of BL11282 on insulin secretion (Fig. 7*A*). Inhibition of protein kinase A with Rp-cAMPS and protein kinase C with LY333531 specifically blocked the potentiation of glucose-induced insulin secretion by 50 μmol/l BL11282 (Fig. 7*B*).

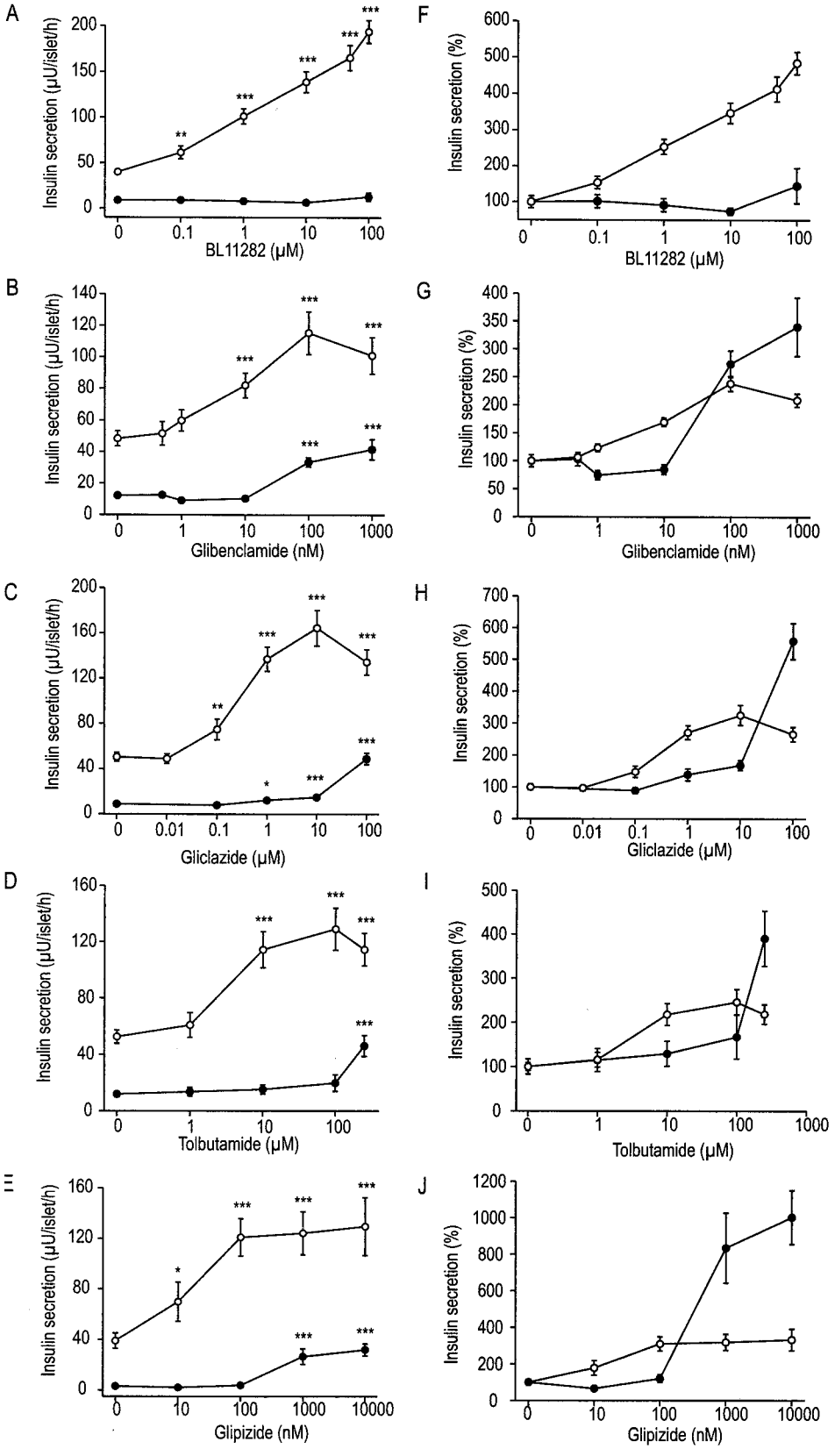

FIG. 2. Effects of the imidazoline BL11282 (*A* and *F*) or the sulfonylureas glibenclamide (*B* and *G*), gliclazide (*C* and *H*), tolbutamide (*D* and *I*), and glipizide (*E* and *J*) on insulin secretion in isolated Wistar rat pancreatic islets at 3.3 (●) and 16.7 (○) mmol/l glucose. *F-J* : Insulin secretion is expressed as percentage of increase over insulin secretion at corresponding glucose concentration without the addition of compounds (100% insulin secretion in the presence of 3.3 or 16.7 mmol/l glucose). Data are means ± SE for 14–20 observations from three or four independent experiments. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ vs. insulin secretion in the absence of the compound.

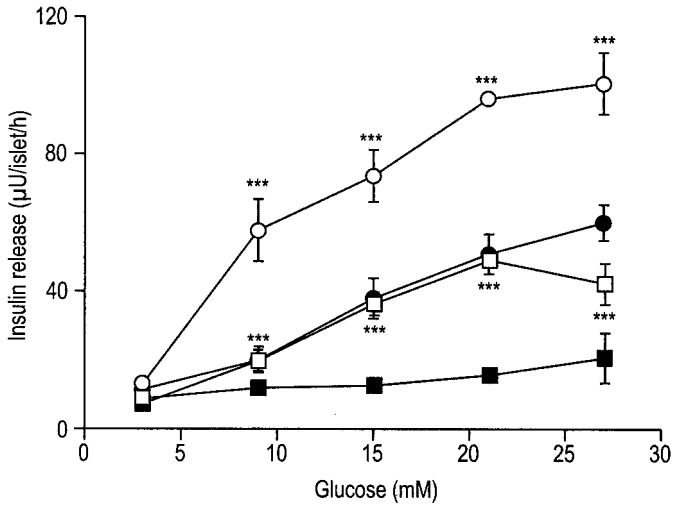

FIG. 3. Dose-response curves for glucose effects on insulin secretion in isolated pancreatic islets from Wistar and GK rats. Dose-response curves were obtained in the presence of 50 μmol/l BL11282 (○, Wistar; □, GK) or without the drug (●, Wistar; ■, GK). Data are means ± SE for 14 observations from four independent experiments. ***$P < 0.001$ vs. insulin secretion at 3 mmol/l glucose.

DISCUSSION

The β-cell K_{ATP} channel has been used as a typical target for many antihyperglycemic agents. Classic blockers of K_{ATP} channel activity, sulfonylureas, have been used extensively in the treatment of type 2 diabetes. Recently, another class of compounds, imidazolines, have been identified as inhibitors of K_{ATP} channels and potential antihyperglycemic agents (1,11). Unfortunately, the effects on insulin secretion produced by sulfonylureas are not critically dependent on ambient glucose concentration (Fig. 2*B–E*), which leads to risk of hypoglycemia in diabetic patients treated with this type of drug (7). Thus, for the rational design of insulinotropic drugs not associated with a hypoglycemic effect, a target distinct from the K_{ATP} channel has to be used. Interaction of therapeutic agents with β-cells should not initiate insulin secretion per se, but rather should work synergistically with an elevated glucose concentration.

We have previously observed that in addition to blocking the activity of K_{ATP} channels, the imidazoline compound RX871024 also markedly potentiates glucose-induced insulin secretion by influencing insulin exocytosis independent of changes in $[Ca^{2+}]_i$ (3–5). Stimulation of insulin exocytosis by RX871024 was dependent on activities of protein kinases A and C. The search for imidazoline compounds possessing only the direct effect on exocytosis resulted in the development of the novel compound BL11282. BL11282 did not affect the activity of K_{ATP} channels, as was assessed in the perforated whole-cell configuration of the patch-clamp technique (Fig. 4).

BL11282 did not initiate insulin secretion at low glucose concentrations. However, the compound markedly potentiated glucose-induced insulin secretion. In contrast, all tested sulfonylureas stimulated insulin secretion at both low and high glucose concentrations. The glucose dependency of BL11282's effects on insulin secretion was observed in pancreatic islets from both normal and diabetic rats. Despite the failure of glucose alone to stimulate insulin secretion properly in islets from diabetic GK rats, the combination of glucose and BL11282 improved the stimulatory effect of glucose to the level observed in control islets.

BL11282 did not influence $[Ca^{2+}]_i$ at low glucose, which confirmed the inability of the compound to block K_{ATP} channels. A high concentration of BL11282 (50 μmol/l) slightly increased $[Ca^{2+}]_i$ at elevated glucose concentrations (Fig. 5*D*). However, 10 μmol/l BL11282 did not increase $[Ca^{2+}]_i$ but produced nearly maximal stimulation of insulin secretion (Fig. 2*A* and 5*C*). Thus, the increase in $[Ca^{2+}]_i$ by BL11282 should make only a minor contribution to the insulinotropic activity of the compound. This notion

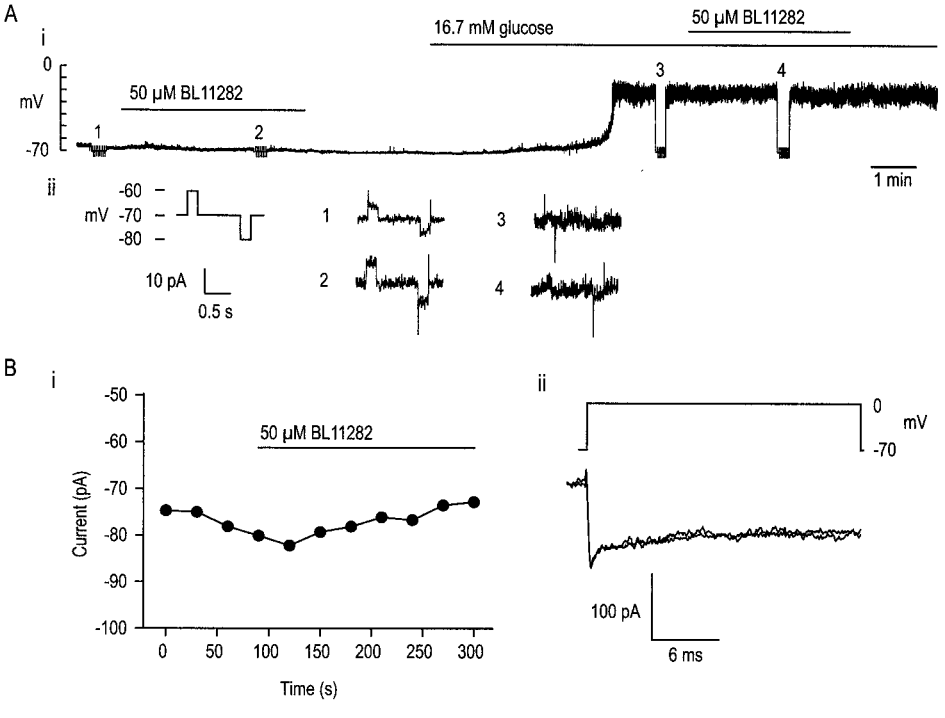

FIG. 4. Modulation of electrical activity by BL11282 in rat pancreatic β-cells. *A*: Effects of 50 μmol/l BL11282 on membrane potential and K_{ATP} channel activity in a perforated patch. Membrane potential was recorded from the single β-cell using the current clamp of the perforated-patch configuration (*i*). To estimate K_{ATP} channel activity, the cell was temporally voltage clamped at –70 mV at various times as indicated by numbers 1–4, and voltage pulses ±10 mV were applied. Resulting currents are shown (*ii*). Traces are representative of four to six experiments. *B*: Effects of BL11282 on voltage-dependent Ca^{2+} channel activity determined in the whole-cell configuration of the rat β-cell by repetitive depolarization pulses from a holding potential of −70 to 0 mV (*i*). Depolarization pulses were applied every 30 s. Recordings of voltage-dependent Ca^{2+} currents are presented (*ii*). Experiment is a representative of four experiments.

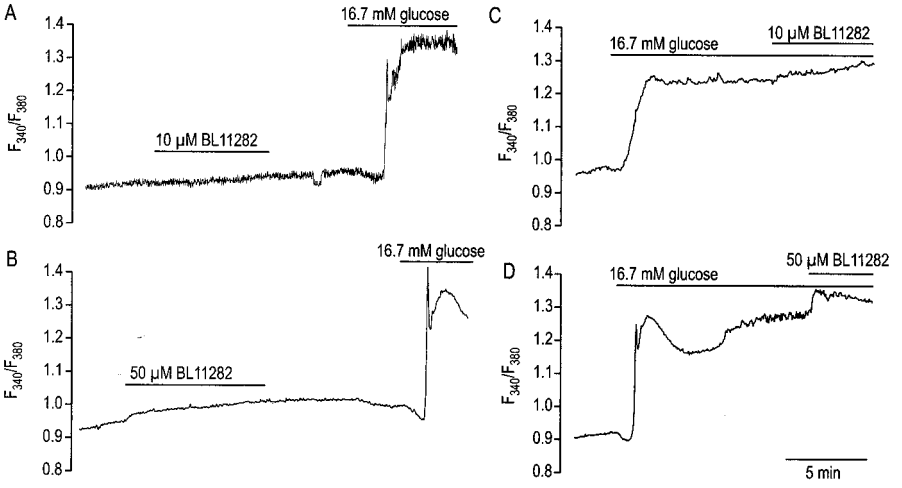

FIG. 5. Effects of BL11282 on fura-2 fluorescence ratio F_{340}/F_{380}, reflecting $[Ca^{2+}]_i$, in the presence of 3.3 (*A* and *B*) and 16.7 (*C* and *D*) mmol/l glucose in rat pancreatic islets. Traces are representative of 8–11 experiments.

is further supported by findings that the compound potentiated insulin secretion without concomitant changes in $[Ca^{2+}]_i$ in islets either depolarized with 55 mmol/l KCl and 16.7 mmol/l glucose or electropermeabilized.

The insulinotropic effect of BL11282 was dependent on the activity of protein kinases A and C. Stimulation of these kinases enhanced, whereas inhibition suppressed, insulin secretion stimulated by the compound and glucose. The important role of protein kinases A and C in regulation of exocytosis is well established (12,13). Therefore, we interpret these findings as well as the results in depolarized and electropermeabilized islets discussed here as indicating that BL11282 stimulates insulin secretion mainly by enhancing the effects of glucose on the exocytotic machinery. This pathway of insulinotropic activity of glucose has recently been recognized, since glucose stimulated insulin secretion in isolated islets and perfused pancreas with clamped intracellular Ca^{2+} (14,15).

Interestingly, sulfonylureas also exert an insulinotropic effect that is independent from the activity of K_{ATP} channels (16). Sulfonylureas and imidazolines share certain similarities in their effects. Both groups of compounds exert a direct effect on insulin exocytosis only in the presence of high glucose concentrations. Stimulation of exocytosis by imidazolines and sulfonylureas is of a similar magnitude and is not additive (4). However, these effects of imidazolines and sulfonylureas are not equivalent. Stimulation of exocytosis by imidazolines depends on activities of protein kinase A and C (3), whereas effects of sulfonylureas were not sensitive to inhibition of protein kinase A (16).

In conclusion, we describe here the new imidazoline compound BL11282, which does not block K_{ATP} channel activity and stimulates insulin secretion only if the glucose concentration is elevated above basal. Because BL11282 did not decrease blood glucose concentration under basal

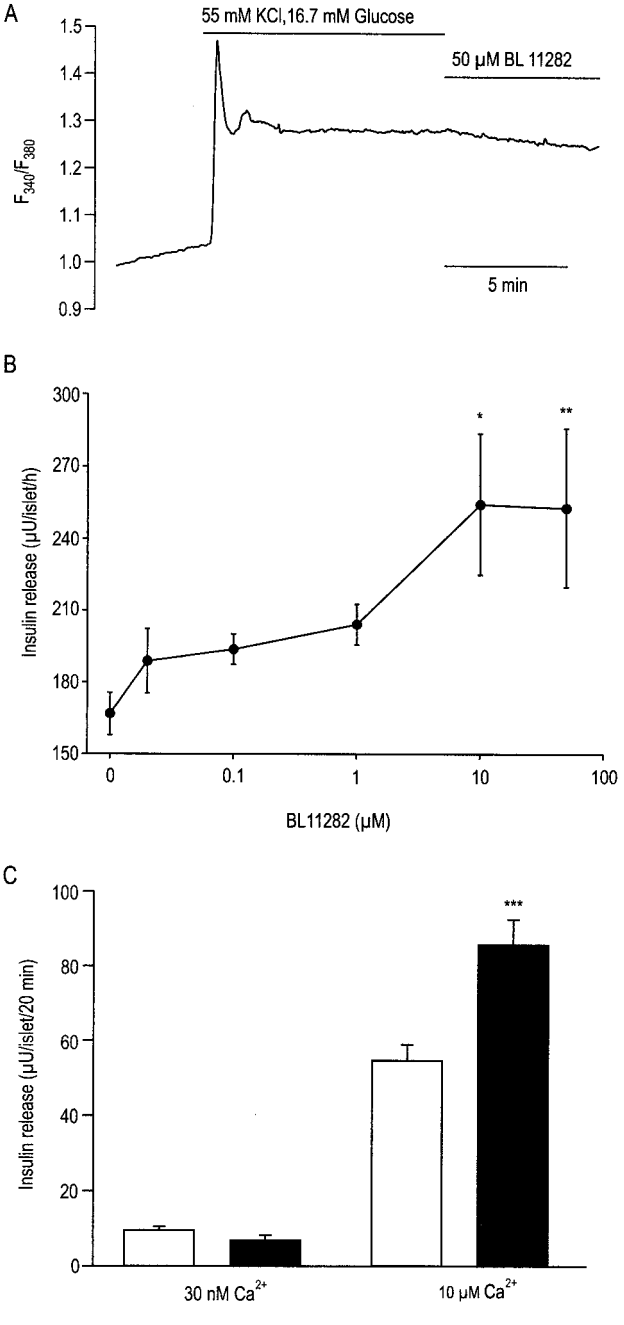

FIG. 6. Stimulation of insulin exocytosis by BL11282. Effects of BL11282 on $[Ca^{2+}]_i$ (*A*) and insulin secretion (*B*) in isolated Wistar rat pancreatic islets depolarized with 55 mmol/l KCl and 16.7 mmol/l glucose. Data are means ± SE from three animals. *C*: Effects of BL11282 on insulin exocytosis in electropermeabilized Wistar rat pancreatic islets. Concentration of free Ca^{2+} in the incubation medium was kept at either 30 nmol/l or 10 μmol/l. Data are means ± SE for 17 observations from four independent experiments. ***$P < 0.001$ vs. insulin secretion in the absence of BL11282.

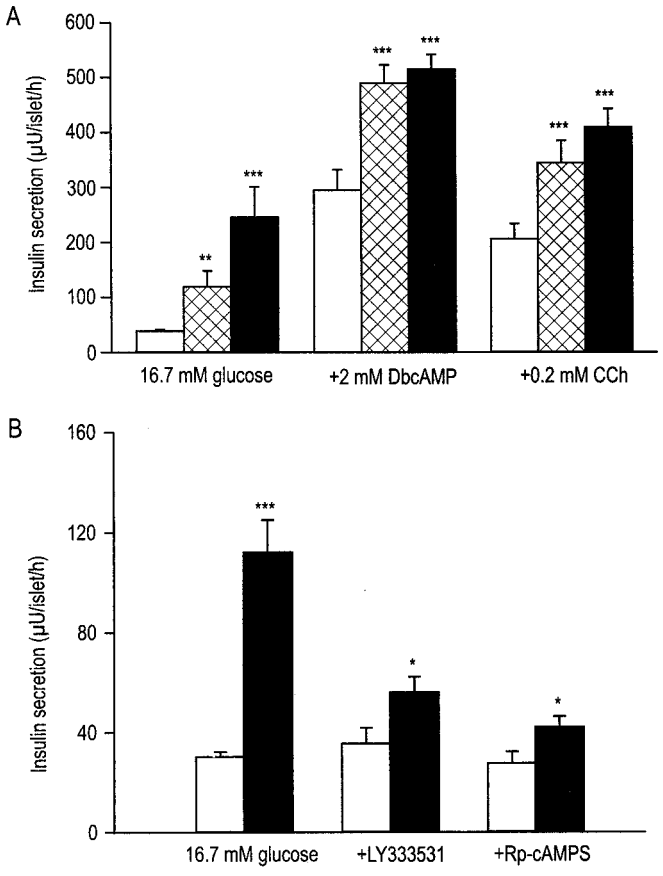

FIG. 7. Interactions between the imidazoline BL11282 and diacylglycerol/cAMP-modulating agents on insulin secretion in isolated Wistar rat pancreatic islets. *A*: Effects of interactions between 10 or 100 µmol/l BL11282 and dibutyryl-cAMP (DbcAMP) or carbamylcholine (CCh) on insulin secretion at 16.7 mmol/l glucose. ▨, insulin secretion with 10 µmol/l BL11282; ■, insulin secretion with 100 µmol/l BL11282; □, insulin secretion without addition of the imidazoline. Data are means ± SE for 12 observations from three independent experiments. *B*: Suppression of 50 µmol/l BL11282–induced insulin secretion (■) by inhibitors of protein kinases C (0.3 µmol/l LY333531) and A (500 µmol/l Rp-cAMPS) at 16.7 mmol/l glucose; effects of inhibitors on insulin secretion stimulated by 16.7 mmol/l glucose alone (□). Data are means ± SE for 14 observations from three independent experiments. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ vs. insulin secretion in the absence of BL11282.

conditions, the risk for hypoglycemia with this type of compound is negligible.

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