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Insulin-like Growth Factor II Inhibits Glucose-Induced Insulin Exocytosis

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We have investigated the effect of IGF-II on glucose-induced insulin release in the pancreatic β-cell. Introduction of IGF-II during perifusion of the cells with 20 mM glucose abolished glucose-induced insulin release. Concomitant addition of IGF-II with 20 mM glucose caused a complete inhibition of insulin release. In addition, IGF-II inhibited Ca^{2+}-induced insulin release from electropermeabilized pancreatic β-cells. IGF-II had no effect on K^+-or tolbutamide-induced insulin release. However, IGF-II could suppress K^+-stimulated insulin release when cells were pretreated with the protein phosphatase inhibitor okadaic acid. The inhibitory effect of IGF-II on insulin release was not associated with significant changes in membrane potential, activity of the voltage-gated L-type Ca^{2+}-channel or cytoplasmic free Ca^{2+} concentration. Pretreatment of the cells with pertussis toxin or the phorbol ester TPA abolished the inhibitory action of IGF-II on insulin release. Hence, the molecular mechanism whereby activation of the IGF-II/M6P receptor by IGF-II inhibits glucose-stimulated insulin exocytosis in the pancreatic β-cell involves pertussis toxin-sensitive G proteins and is dependent on PKC activity.

Increase in cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$), is an integrated part of the stimulus-secretion coupling in a number of secretory cells. The insulin-secreting pancreatic β-cell is an example of such a secretory cell. In this case, glucose is rapidly taken up through the glucose transporter, Glut-2 (1). Glucose metabolism leads to an increase in the ATP/ADP ratio, closure of ATP-regulated K^+-channels, plasma membrane depolarization, opening of voltage-gated L-type Ca^{2+}-channels, increase in $[Ca^{2+}]_i$ and thereby initiation of insulin release (2). Direct activation of exocytosis may involve signaling through a number of plasma membrane receptors coupled to various effector systems, exerting their effects through protein phosphorylation (3). In the pancreatic β cell the latter process reflects a balanced action of protein kinases and protein phosphatases, allowing a sophisticated tuning and orchestration of the molecular mechanisms directly regulating transport and fusion of insulin secretory granules (4). Among the effector systems involved in modulating the insulin secretory process are the phospholipase C system, the cyclic AMP system (5, 3) and the serine/threonine protein phosphatases type 1, type 2A and type 3 (6, 7). Moreover, pertussis toxin-sensitive G proteins have been shown to mediate the inhibitory effects of α_2-adrenergic agonists, galanin and somatostatin on insulin release (8).

Insulin-like growth factor II (IGF-II), like insulin-like growth factor I (IGF-I), is structurally homologous to proinsulin and circulates bound to specific IGF binding proteins (9, 10). The receptor for IGF-II, identical to the cation independent mannose 6-phosphate (M6P) receptor, is responsible for lysosomal enzyme targeting and IGF-II degradation (11, 12). Most biological actions of IGF-II are thought to be mediated through the IGF-I receptor, but not the IGF-II/M6P receptor (11, 13).

IGF-I has been suggested to play a role in insulin secretion, although different results have been observed in different studies (14-17). In contrast, the role of IGF-II on insulin release is less clear. A previous study showed that IGF-II inhibits insulin release, stimulated by high concentration of glucose (18). This effect of IGF-II was thought to be mediated through the IGF-I receptor, as IGF-I was a more potent inhibitor than IGF-II. We recently showed that IGF-II promotes insulin release from pancreatic β-cells isolated from ob/ob mice at non-stimulatory glucose concentrations, an effect that is mediated through the IGF-II/M6P receptor (19). In addition, glucose stimulates both the plasma membrane number and phosphorylation of the IGF-II/M6P receptor in insulin secreting cells (20). These results suggest a potential role for the IGF-II/M6P receptor in insulin exocytosis. In the present study, we have investigated the effect of IGF-II on glucose-induced insulin release.

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MATERIALS AND METHODS

Recombinant IGF-II was a generous gift from Pharmacia & Upjohn, Sweden. Deoxyribonuclease 1 (DNAse 1, from bovine pancreas), ethyleneglycol-bis-(B-aminoethyl ether) N,N′-tetraacetic acid (EGTA), 12-O-tetradecanoylphorbol 13-acetate (TPA), fura-2/acetoxymethylester (Fura-2/AM) and pertussis toxin were purchased from Sigma, USA. Collagenase A (from Clostridium histolyticum) was from Boehringer Mannheim GmbH, Germany. Bio-gel P-4 (fine, 65 ± 20 μm, wet) was obtained from Bio-Rad Laboratories. Tolbutamide was from Hoechst AG, Germany. RPMI-1640 culture medium and fetal calf serum (FCS) were purchased from Life Technologies, UK.

Preparation of cells. Pancreatic islets were isolated from ob/ob mice by collagenase and DNAse digestion. The islets, which contain more than 90% β cells (21), were dispersed into single cells by shaking in medium containing 1 mM EGTA (21). The cells were maintained for 3 h at 37°C in RPMI-1640 tissue culture medium supplemented with 10% (v/v) fetal calf serum (FCS), 100 IU/ml penicillin and 100 μg/ml streptomycin before experiments.

Insulin release. Insulin release was investigated in column-perifusion of pancreatic β-cells, as described previously (22). In the various experimental situations, approximately 2×10^5 cells were carefully mixed with a small volume of pre-wetted Bio-Gel P-4 and placed on top of each of three parallel columns (bed volume 0.5 ml), packed with the same polyacrylamide beads in advance. Perifusion was performed at 37°C with "buffer A" containing (in mM) 125 NaCl, 5.9 KCl, 1.28 $CaCl_2$, 1.2 $MgCl_2$, 25 HEPES and 0.1 % BSA. The flow rate was 0.2 ml/min and fractions were collected every 2 min.

Insulin radioimmunoassay. Fractions collected from perifusion experiments were assayed at 10-to 50-fold dilution. Bound and unbound insulin were separated by activated charcoal (23). The insulin antibodies were raised against porcine insulin in guinea-pigs in our laboratory. Rat insulin was used as standard.

Electropermeabilization and insulin release from permeabilized β-cells. Pancreatic β-cells were permeabilized by exposure to high voltage discharges (24). Isolated cells were washed and resuspended in a buffer containing (in mM) 110 KCl, 10 NaCl, 1 $MgCl_2$, 2 KH_2PO_4 and 25 HEPES (pH 7.0). Cell suspension (0.5 ml, approximately 5×10^5 cells) was placed in a chamber with two platina electrodes. A 2 μF capacitor was charged by high voltage (1.5 kV) and discharged through the chamber. The cells were subjected to the high voltage exposures five times. Insulin release from permeabilized cells was investigated in a 12-well plate at 37°C in a K^+-glutamate buffer containing 140 mM glutamate, 5 mM NaCl, 1 mM $MgCl_2$, 25 mM HEPES, 2 mM creatine phosphate, 4 mM Mg-ATP, 10 mM EGTA, 10 U/ml creatine phosphokinase and 1 mg/ml BSA at different concentrations of Ca^{2+}. The Ca^{2+}-concentration in the buffers was measured by a Ca^{2+}-selective minielectrode and adjusted to 0.1 and 10 μM by the addition of Ca^{2+} and EGTA (24). After 20 min of incubation, the cell suspension was collected from each well and centrifuged. Insulin content in the supernatant was measured by insulin radioimmunoassay.

Measurements of $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ was measured in single pancreatic β-cells using a Zeiss Axiovert 35M inverted epifluorescence microscope connected to a SPEX fluorolog-2 CM1T11I system for dual wavelength excitation fluorimetry (25). The excitation wavelengths were 340 and 380 nm with an emission wavelength of 510 nm. Before measurements, cells were attached to coverslips. In order to load the cells with the Ca^{2+} indicator Fura-2/AM, the coverslips with cells were incubated for 30 min at 37°C in "buffer A", supplemented with 1.5 μM Fura-2/AM. During the experiments, the cells were continuously perifused with "buffer A" at 37°C, at a speed of 0.3 ml/min. Results were expressed as the 340/380 fluorescence ratio, obtained every second (24, 25).

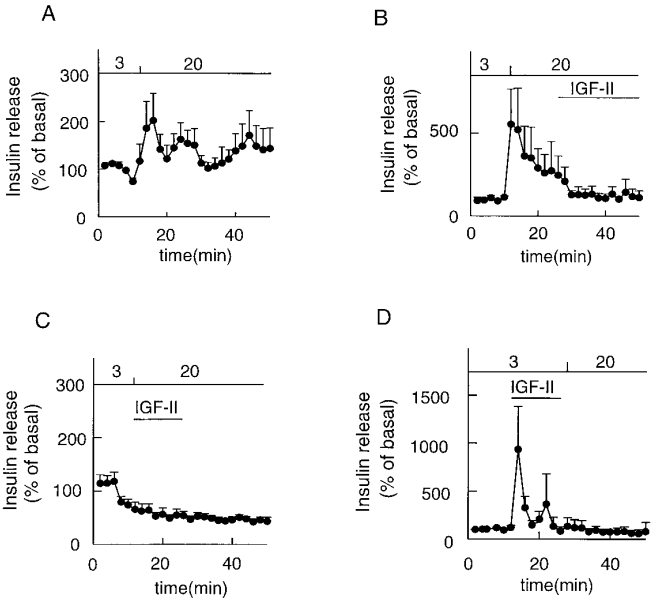

FIG. 1. Effect of IGF-II on glucose-induced insulin release from column-perifused pancreatic β-cells. Cells were perifused in a buffer containing 3 mM glucose for 10 min, followed by stimulation of the cells with 20 mM glucose. Addition of IGF-II (50 ng/ml) is indicated (B, C, D). Insulin release is expressed in percentage of basal secretion, defined as the average insulin release obtained at 3 mM glucose during the first 10 min. Means ± SD derived from three experiments are given.

RESULTS

Stimulation of pancreatic β-cell with 20 mM glucose caused a sustained insulin release (Fig. 1A). Addition of 50 ng/ml IGF-II inhibited glucose-induced insulin release (Fig. 1B). Concomitant addition of IGF-II with 20 mM glucose caused a complete inhibition of insulin secretion (Fig. 1C). The inhibitory effect of IGF-II on glucose-induced insulin release could also be observed when IGF-II was added prior to glucose addition (Fig. 1D). Under these conditions, IGF-II showed a stimulatory effect on insulin release in the presence of 3 mM glucose (19). However, the cells failed to respond to a subsequent addition of 20 mM glucose. In contrast, glucose-induced insulin release from the cells was not affected by IGF-I (data not shown).

The inhibitory effect of IGF-II on insulin release was further evaluated in electropermeabilized β-cells, where membrane barriers are excluded and the exocytotic machinery can be directly affected by changes in the extracellular environment. Under these conditions, high concentration of Ca^{2+} (10^{-5} M) stimulates insulin release in the presence of ATP and an ATP-regenerating system. This stimulatory effect was inhibited by IGF-II (Fig. 2).

Since ATP-regulated K^+ (K_{ATP})-channels and voltage-gated L-type Ca^{2+}-channels are involved in glucose-stimulated insulin release, the actions of IGF-II

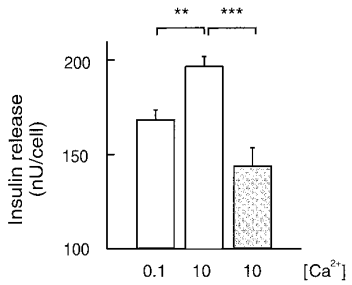

FIG. 2. Effect of IGF-II on Ca^{2+}-induced insulin release from permeabilized pancreatic β-cells. Pancreatic β-cells were permeabilized by high voltage exposures. Insulin release from permeabilized β-cells was investigated in a buffer containing ATP and ATP-regenerating system, at ambient free Ca^{2+} concentrations of 0.1 or 10 μM, in the absence or presence of 50 ng/ml IGF-II (filled bar). Means ± SD (nU/cell) derived from three experiments are shown (** $P < 0.01$, *** $P < 0.001$).

were investigated in the presence of tolbutamide (100 μM) (Fig. 3A) or high concentration of K^+ (25 mM) (Fig. 3B). Tolbutamide and K^+ stimulate insulin release from the pancreatic β-cells by directly closing K_{ATP}-channels and opening voltage-gated L-type Ca^{2+}-channels, respectively. Addition of IGF-II did not interfere with either tolbutamide-or K^+-induced insulin release. However, K^+-stimulated insulin release was significantly suppressed when cells were pre-treated with the protein phosphatase inhibitor okadaic acid (Fig. 3C).

When pancreatic β-cells were perifused with buffer A, containing 3 mM glucose, IGF-II did not cause any changes in $[Ca^{2+}]_i$ and the cells responded to a subsequent addition of high glucose (Fig 4A). Concomitant addition of IGF-II and 20 mM glucose did not reveal significant changes in $[Ca^{2+}]_i$, above the stimulatory effect normally obtained with glucose alone (Fig. 4B). However, IGF-II induced a slight increase in $[Ca^{2+}]_i$ if applied to glucose-stimulated β-cells at a time when $[Ca^{2+}]_i$ was still high, compared with basal level (Fig. 4C). This effect of IGF-II was dependent on Ca^{2+} influx from the extracellular space, as it disappeared when Ca^{2+} was excluded from the perifusion buffer (Fig. 4D). In the absence of extracellular Ca^{2+}, the cells responded to extracellularly applied ATP. Further studies using the patch clamp technique, revealed that IGF-II had no effect on either membrane potential or Ca^{2+} current (data not shown).

DISCUSSION

In the pancreatic β-cell, IGF-II stimulates insulin release at low extracellular glucose concentrations, an effect mediated by the IGF-II/M6P receptor (19). The

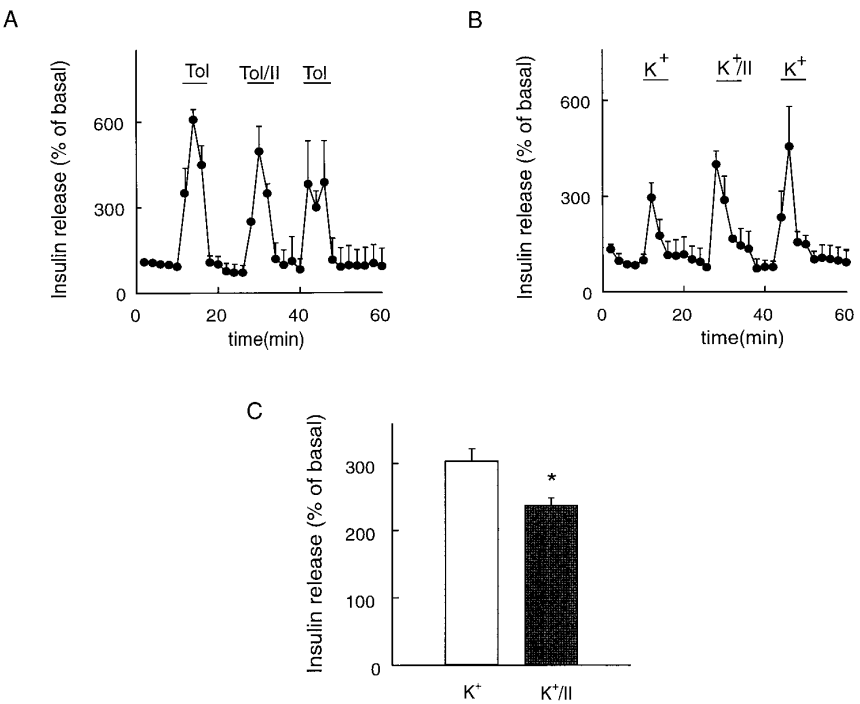

FIG. 3. Effect of IGF-II on tolbutamide-and K^+-induced insulin release. Cells were perifused in a buffer containing 3 mM glucose. Addition of 100 μM tolbutamide (Tol) (A), 25 mM K^+ (B) and 50 ng/ml IGF-II (II) are indicated. Insulin release is expressed as percentage of basal secretion, as described in Fig. 1. Means ± SD derived from three experiments are shown. In C, cells were preincubated with 0.5 μM okadaic acid for 20 min before perifusion with 3 mM glucose. Cells were stimulated with 25 mM K^+ in the presence or absence of 50 ng/ml IGF-II. Insulin release stimulated by K^+ or K^+/IGF-II (II) (expressed as percentage of basal release) for 8 min is shown (Means ± SEM, n = 9, * $P < 0.05$).

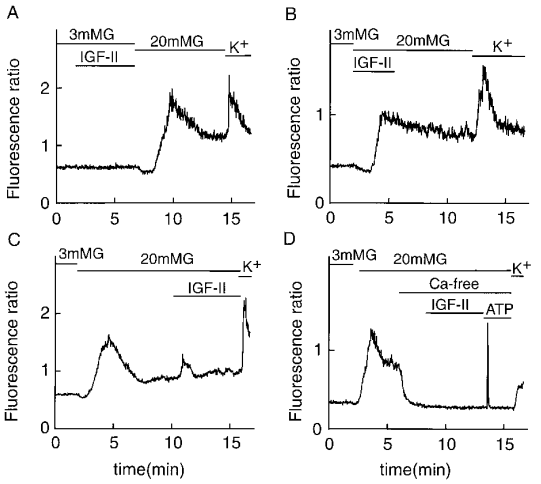

FIG. 4. Effects of IGF-II on $[Ca^{2+}]_i$ in single β-cells. Experiments were performed at 37°C in buffer A containing 1.28 mM Ca^{2+} and 3 mM glucose (3mMG). Cells were subjected to stimulation with 20 mM glucose (20mMG), 25 mM K^+ (K^+), or 200 μM ATP. IGF-II (50 ng/ml) was added as indicated. In D, cells were perifused with a Ca^{2+}-free buffer A (no addition of Ca^{2+}, in the presence of 1 mM EGTA), containing 20 mM glucose. Results are expressed as fluorescence ratios (340 nm/380 nm). Each figure is a representative experiment out of six single cell measurements.

stimulatory effect of IGF-II on insulin release involves pertussis toxin (PTX) sensitive G-proteins and PKC-induced phosphorylation of three major intracellular proteins, but is not accompanied by any changes in $[Ca^{2+}]_i$. The IGF-II/M6P receptor is known to be involved in vesicular trafficking and it is of interest to note that it is localized both in the plasma membrane and in the insulin containing secretory granules in the β-cell (Zhang *et al*, unpublished data). This means that IGF-II-induced insulin exocytosis may involve IGF-II-triggered cycling of the IGF-II/M6P receptor between the plasma membrane and intracellular membranes, such as insulin secretory granules.

The present study shows that IGF-II, at a high extracellular glucose concentration, inhibited insulin release by a similar signalling pathway as that involved in the stimulation of insulin release at low glucose. Thus, the inhibitory effect of IGF-II on glucose-induced insulin release disappeared when the cells were pre-treated with PTX, to ADP ribosylate the α subunit of the G-protein, or were down-regulated with regard to PKC activity (19). This means that IGF-II can either promote or inhibit insulin exocytosis depending on the actual surrounding glucose concentration. Interestingly, the effects of IGF-II on insulin exocytosis are potent, overriding the normal response pattern obtained at low or high concentrations of glucose.

What is then the molecular mechanism behind the inhibitory effect of IGF-II on insulin release at high glucose? Glucose stimulates both the plasma membrane number and phosphorylation of the IGF-II/M6P receptor in insulin secreting cells, an effect dependent on PKC (20). This suggests that phosphorylation of the IGF-II/M6P receptor changes its equilibrium in distribution in favor of the plasma membrane, thereby suppressing translocation to intracellular membranes. It should be noted that, at a low extracellular glucose concentration, sulphonylurea-or K^+-induced insulin release was not suppressed by IGF-II. The protein phosphatase inhibitor okadaic acid is known to increase the phosphorylation of the IGF-II/M6P receptor (26). Interestingly, when we subjected the β-cell to phosphorylation by treatment with okadaic acid prior to stimulation with high concentrations of K^+, the stimulatory effect of the latter on insulin exocytosis was suppressed. Hence, if IGF-II/M6P receptor cycling between the plasma membrane and intracellular membranes is disturbed, as a result of for example glucose-induced and PKC-mediated phosphorylation, the exocytosis process is inhibited by IGF-II. It has been reported that the cytoplasmic tail of the IGF-II/M6P receptor indeed interacts with cytosolic proteins, depending on its state of phosphorylation (27). This may suggest that the IGF-II/M6P receptor, when present in the plasma membrane and activated by IGF-II, serves the purpose of blocking the distal events associated with exocytosis. The interaction of the IGF-II/M6P receptor with the exocytotic machinery, subsequent to IGF-II binding, seems to require a PTX sensitive G-protein. In this context, it is of interest to note that a number of studies have demonstrated that the IGF-II/M6P receptor, when stimulated with IGF-II as a ligand, associates with a G_{i2} protein (28-30) and the intracellular sequence of the receptor responsible for this interaction has been identified to amino acids 2410-2423 (30).

The present data together with our previous study (19), suggest that IGF-II binding to the IGF-II/M6P receptor confers ability of the receptor to interact with the exocytotic machinery in the pancreatic β-cell. Two different processes can then happen depending on the phosphorylation state of the receptor. Exocytosis is stimulated when the IGF-II-IGF-II/M6P receptor complex is internalized, a scenario that occurs at a low phosphorylation state, i.e. a low extracellular glucose concentration. On the contrary, exocytosis is inhibited when the IGF-II/M6P receptor complex is preferentially localized to the plasma membrane, a scenario that occurs at a high phosphorylation state, i.e. a high extracellular glucose concentration.

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