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Regulation of glucose-dependent insulin secretion by insulin: Possible role of AMP-activated protein kinase

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ABSTRACT

Aims: Extracellular insulin affects insulin secretion from pancreatic β-cells in an autocrine fashion, but the role of glucose in this signaling pathway remains unclear. This study was conducted to evaluate the glucose dependency of extracellular insulin-mediated regulation of insulin secretion and the potential underlying mechanism.
Main methods: Pancreatic β-cells from male Sprague–Dawley rats and INS-1, a rat insulinoma cell line, were used. The mechanism of extracellular insulin-mediated, glucose-dependent insulin secretion was explored by analyzing the activity of ATP-sensitive K^+ (K_{ATP}) channels, changes in cell membrane potential, and cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_c$), as well as phosphorylation of the insulin signaling pathway and the metabolic sensor AMP-activated protein kinase (AMPK).
Key findings: Treatment of native β-cells with 100 nM insulin under basal glucose conditions (≤5 mM) reduced subsequent high glucose-induced insulin secretory responses, demonstrating less inhibition of K_{ATP} channels and decreased elevation of $[Ca^{2+}]_c$. In contrast, insulin treatment under high glucose conditions potentiated the insulin secretory responses of β-cells. While insulin treatment attenuated phosphorylation on the Thr172 of AMPK and the Ser789 of insulin receptor substrate (IRS)-1, which was increased by lowering glucose concentration, it enhanced phosphorylation of AMPK and IRS-1, which was decreased by elevating glucose concentration. This glucose-dependent regulation of insulin even occurred in the presence of LY294002, a phosphoinositide-3 kinase inhibitor.
Significance: Considering that the phosphorylated AMPK could inhibit K_{ATP} currents in β-cells, which triggers glucose-stimulated insulin secretion, extracellular insulin may regulate the phosphorylation status of AMPK through IRS-1 to modulate insulin secretion in a glucose-dependent way.

Introduction

In pancreatic β-cells, insulin feedback signaling has been known to be involved in insulin secretion, insulin biosynthesis, and cell growth (Kulkarni et al. 2004; Xu and Rothenberg 1998). Secreted insulin appeared to enhance insulin biosynthesis and cell survival via insulin receptors (IR) and the insulin receptor substrates (IRS) (Leibiger et al. 2001). Regarding insulin secretion, it has been previously addressed that a negative insulin feedback signaling reduces insulin secretion and this is primarily mediated by phosphatidylinositol triphosphate (PIP3) generated by phosphoinositide (PI)-3 kinase (Khan et al. 2001). PIP3 has been shown to activate ATP-sensitive K^+ (K_{ATP}) channels and hyperpolarize the cell membrane, resulting in a decrease in the concentration of cytosolic free Ca^{2+} ($[Ca^{2+}]_c$) and, therefore, a reduction of insulin secretion. In contrast, it has been rather shown that an insulin feedback signaling increases $[Ca^{2+}]_c$ and thus enhances insulin secretion (Aspinwall et al. 1999, 2000; Roper et al. 2002; Xu et al. 1999, 2000). Interestingly, gene disruption of β-cell IR or IRS-1 was linked to impairment of glucose-dependent insulin secretion (Kulkarni et al. 1999a), suggesting that β-cell insulin feedback signaling may be influenced by changes in glucose concentration for the regulation of insulin secretion. The mechanism underlying the glucose sensitivity of the insulin feedback signaling, however, is still under investigation.

The 5′-AMP-activated protein kinase (AMPK) is a cellular glucose sensor found in various tissues. AMPK was shown to be activated through phosphorylation on the Thr172 of α-subunit residues during conditions of energy deprivation, such as hypoglycemia (Long and Zierath 2006). Physiologically, while this cellular sensor is activated at low concentrations of glucose, AMPK is inhibited at high glucose concentrations (Eto et al. 2002a). An association between AMPK and insulin feedback signaling for cell function and survival has been addressed in intact mouse C2C12 myotubes, in which the activated

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form of AMPK leads to phosphorylation of the Ser789 of IRS-1, thereby facilitating insulin-stimulated, IRS-1-associated PI-3 kinase activity (Jakobsen et al. 2001). In fact, the phosphorylation of Ser789 of IRS-1 may be really limited to that of a reporter site for AMPK activity, given that IRS-1 has many other serine/threonine and tyrosine phosphorylation sites and the phosphorylated tyrosine residues of IRS-1 are more critical in linking cellular insulin signaling to various vital cellular functions and in recruiting PI-3 kinase and protein kinase B (Eto et al. 2002b). However, the linkage between AMPK and IRS-1 could offer a possibility that AMPK might participate in a short-term regulatory role of insulin feedback signaling in glucose-dependent insulin secretion. Hence, in this study, we examined whether regulation by extracellular insulin of β-cell insulin secretion was glucose-dependent and sought to elucidate the potential cellular mechanism.

Materials and methods

Molecular biological reagents

Anti-AMPK, anti-phospho-AMPK (Thr172), anti-IRS-1, anti-phospho-IRS-1 (Ser789), anti-protein kinase B (Ser473; Akt), and anti-phospho-Akt antibodies were obtained from Cell Signaling Technology (Danvers, MA, USA). Fetal bovine serum (FBS) and bovine serum albumin (BSA) were purchased from Gibco Invitrogen (Carlsbad, CA, USA). Other chemicals were from Sigma (St. Louis, MO, USA).

Preparation of β-cells from the rat pancreas

Islets of Langerhans were isolated from the pancreas of male Sprague–Dawley rats by the collagenase digestion technique, as previously described (Han et al. 2004). Briefly, following anaesthetization with Nembutal, the rats were exsanguinated by puncture of the abdominal aorta. After confirming that the heart had stopped beating, 1 mg/ml collagenase in Hank's Balanced Salts solution was transfused at the common bile duct retrogradely into the pancreatic ducts. The pancreas was dissected and incubated for 15 min at 37 °C in a shaking water bath. The islets were placed into Krebs Ringer bicarbonate buffer containing 10% BSA, 100 U/ml penicillin, and 0.1 mg/ml streptomycin. To collect single islet cells, the islets were further triturated and incubated in RPMI-1640 media with 11.1 mM glucose, 10% FBS, and antibiotics in a humidified incubator at 37 °C in 5% CO_2/95% air. All protocols were approved by the institutional animal care and use committee at the Dongsan Medical Institute for Life Sciences in Daegu, Korea.

Patch-clamp analysis of whole-cell K_{ATP} channel currents and membrane potential in β-cells

The perforated whole-cell configuration of conventional patch-clamp technique was used for measuring the K_{ATP} channel current and membrane potential of β-cells. Nystatin was prepared as a stock solution (50 mg/ml DMSO), diluted (10 μl/5 ml of pipette solution), and back-filled to measuring pipettes. To measure the membrane potential, a current-clamp mode was used, and the holding potential was −70 mV to obtain whole-cell K_{ATP} channel currents. A 3-s step pulse toward −50 and then −90 mV was sequentially applied. The time gap between pulses was 30 s. The PI-3 kinase inhibitor LY294002 (10 μM) was added to the pipette solution before the whole-cell experiments. Patch pipettes had a resistance of 3–5 MΩ. The potential and currents were recorded using an Axopatch 200B patch-clamp amplifier (Axon Instruments, Forster, CA, USA) and later analyzed with the Pclamp 9.1 software (Axon Instruments). Data were filtered at 5 kHz and sampled at 1 kHz. The cells were perfused with bath solution containing 138 mM NaCl, 5.6 mM KCl, 1.2 mM $MgCl_2$, 2.6 mM $CaCl_2$, and 5 mM hydroxyethyl piperazine ethane sulfonate (HEPES) (pH adjusted to 7.4 with NaOH). Insulin or glucose was then added to the bath solution, and the solution exchange was completed within 20 s. The pipette solution contained 76 mM K_2SO_4, 1 mM $MgCl_2$, 10 mM KCl, 10 mM NaCl, and 10 mM HEPES (pH adjusted to 7.4 with KOH). All experiments were performed at room temperature (22–25 °C).

Measurement of $[Ca^{2+}]_c$ in β-cells

Cultured cells were bathed in a solution containing 126 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 10 mM HEPES (pH 7.4). The calcium indicator dye Fura-2/AM was added to a concentration of 3 μM, and the cells were incubated for 30 min at room temperature. Next the cells were further incubated for 30 min in dye-free solution to allow esterase cleavage of Fura-2/AM to liberate Fura-2. Imaging was achieved with an InCa dual-wavelength system (Intracellular Imaging, Cincinnati, OH, USA). $[Ca^{2+}]_c$ was calculated from the ratio of emissions at 510 nm at excitation wavelengths of 340 and 380 nm. The ratio images were processed every 5 s and converted to $[Ca^{2+}]_c$ by comparison to a standard curve generated using a Calcium Calibration Buffer Kit (Molecular Probe, Eugene, OR, USA). All imaging experiments were performed at room temperature.

Measurement of C-peptide secretion by islets

C-peptide secretion from islets was measured by enzyme immunoassay using batch incubation. The islets were incubated for 1 h at 37 °C in a modified Krebs Ringer bicarbonate buffer (114 mM NaCl, 4.4 mM KCl, 1 mM $MgSO_4$, 29.5 mM $NaHCO_3$, 1.28 mM $CaCl_2$, and 10 mM HEPES; pH 7.4) containing 0.1% BSA and 3 mM glucose. Islets were then treated in groups as follows: 1) incubation with 3 mM glucose for 40 min followed by stimulation with 3 or 20 mM glucose for an additional 40 min in the absence of 100 nM insulin; 2) incubation with 3 mM glucose for 40 min in the presence of insulin followed by stimulation with 3 or 20 mM glucose in the presence of 100 nM insulin; and 3) incubation with 3 mM glucose for 40 min followed by stimulation with 20 mM glucose, plus 100 nM insulin or 2 mM AICAR for an additional 40 min. After the incubation period, an aliquot of the medium was collected from each well and centrifuged for 5 min at 700 ×*g*. The supernatant (200 μl) was collected and stored at −20 °C for later analysis. To determine the protein concentration, the remaining pellet was washed in 400 μl of RPMI-1640 medium and lysed in 150 μl of ice-cold lysis buffer (50 mM HEPES, 0.1% Triton X-100, 1 μM PMSF, 10 μM E-64, 10 μM TLCK, 10 μM pepstatin A and 100 μM leupeptin; pH 7.4). After sonication and centrifugation for 2 min at 10,000 ×*g*, the resulting supernatant was assayed for islet protein concentration. The level of C-peptide secretion was measured with an enzyme immunoassay kit purchased from Yanihara Institute Inc. (Shizuoka, Japan).

Western blotting

INS-1 cells were seeded in 6-well plates at 8×10^5 cells/well and kept in the culture medium containing 11.1 mM glucose for 3 days. Cells were then washed with phosphate-buffered saline and incubated in a low glucose (3 mM)-containing serum-free buffer (114 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.16 mM $MgSO_4$, 20 mM HEPES, 2.5 mM $CaCl_2$, and 25.5 mM $NaHCO_3$; pH 7.4) for 2 h. Next, the cells were exposed to the serum-free buffer containing various concentrations of glucose in the absence of 100 nM insulin. To elucidate PI-3 kinase dependency, another set of experiments was performed in the presence of 10 μM LY294002. In both sets of experiments, 2 mM AICAR in the absence of LY294002 was employed at high glucose concentrations as a verifying control. After 2 h of incubation, some batches of cells were treated with 100 nM insulin for 30 min. Then cells were lysed in lysis buffer [20 mM Tris–HCl, 137 mM NaCl, 10% glycerol, 1% Triton X-100, 2 mM EDTA, 1 mM PMSF, 1× Phosphatase Inhibitor Cocktail Set II (Calbiochem, Darmstadt, Germany), and 1× protease inhibitor (Roche Applied Science, Mannheim, Germany)] for 20 min at 4 °C. Lysates were separated by SDS-PAGE and