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Angiopoietin-like peptide 4 regulates insulin secretion and islet morphology

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ABSTRACT

Insulin secretion from pancreatic islet β-cells is primarily regulated by the blood glucose level, and also modulated by a number of biological factors produced inside the islets or released from remote organs. Previous studies have shown that angiopoietin-like protein 4 (Angptl4) controls glucose and lipid metabolism through its actions in the liver, adipose tissue, and skeletal muscles. In this present study, we investigated the possible role of Angptl4 in the regulation of insulin secretion from pancreatic islets. Angptl4 was found to be highly expressed in the α-cells but not β-cells of rodent islets. Moreover, treatment of rodent islets with Angptl4 peptide potentiated glucose-stimulated insulin secretion through a protein kinase A-dependent mechanism. Consistently, Angptl4 knockout mice showed impaired glucose tolerance. In the cultured islets from Angptl4 knockout mice, glucose-stimulated insulin secretion was significantly lower than in islets from wild type mice. Angptl4 peptide replacement partially reversed this reduction. Moreover, Angptl4 knockout mice had dysmorphic islets with abnormally distributed α-cells. In contrast, the β-cell mass and distribution were not significantly altered in these knockout mice. Our current data collectively suggest that Angptl4 may play a critical role in the regulation of insulin secretion and islet morphogenesis.

1. Introduction

Elevated blood glucose during the postprandial period triggers insulin secretion from pancreatic β-cells and suppresses glucagon secretion from α-cells. These responses of α- and β-cells to glucose are blunted by type 2 diabetes in human, leading to inappropriate insulin and glucagon secretion and hyperglycemia [1]. Interactions between the α (glucagon secreting)-, β (insulin secreting)-, and δ (somatostatin secreting)-endocrine cells in the pancreatic islets modulate insulin secretory responses to both nutrient and non-nutrient stimuli [2]. These communication pathways may arise through direct cell-to-cell contact via cell adhesion molecules, by the exchange of small molecules and/or ions via gap junctions, and by paracrine interactions through various secreted products [2]. In rodent islets, the β-cells are located in the core of the islet whilst α-cells are mainly distributed in the mantle zone of islets. These differential distributions may reduce the direct contact between these two cell populations and thus interactions via paracrine mechanisms may be more important in rodents [3].

Angiopoietin-like proteins (Angptls) are a family of secreted proteins that share common structures with angiopoietins i.e. an *N*-terminal coiled-coil domain (CCD) and a *C*-terminal fibrinogen-like domain (FLD). Whereas angiopoietins critically regulate angiogenesis and vascular stability, the Angptls have been implicated in

Abbreviations: Angptls, angiopoietin-like proteins; Angptl4, angiopoietin-like protein 4; cAMP, cyclic adenosine monophosphate; CCD, coiled-coil domain; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; Fiaf, fasting-induced adipose factor; FLD, fibrinogen-like domain; GLP1, glucagon-like peptide-1; GSIS, glucose-stimulated insulin secretion; GTT, glucose tolerance test; ITT, insulin tolerance test; KRBH, Krebs-Ringer bicarbonate HEPES buffer; PKA, protein kinase A; PPAR, peroxisome proliferator-activated receptor.

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both angiogenesis and metabolic control [4]. Angiopoietin-like protein 4 (Angptl4), also known as the fasting-induced adipose factor (Fiaf), was originally identified in the liver and adipose tissue as a target gene of peroxisome proliferator-activated receptor (PPAR)-α and PPAR-γ [5,6]. Fat-specific Angptl4 transgenic mice display reduced adiposity due to enhanced lipolysis and fatty acid oxidation [7]. Moreover, the adenovirus-mediated overexpression of Angptl4 potently decreases the blood glucose concentration and improves glucose tolerance in C57 mice and *db/db* diabetic mice, which is partly mediated through the suppression of hepatic gluconeogenesis [8]. In the intestine, Angptl4 regulates lymphangiogenesis [9] and mediates the anti-obesity effects of germ-free conditions [10]. Angptl4 has also been found in the systemic circulation where it strongly inhibits the activity of lipoprotein lipase [11]. The genetic and viral-mediated overexpression of Angplt4 thus leads to hyperlipidemia [8,11]. The plasma Angplt4 concentrations increase with fasting and exercise [12] but are suppressed by chronic high fat feeding and in type 2 diabetes [5,8]. These findings collectively support a critical role for Angptl4 in the maintenance of normal glucose and lipid metabolism in multiple metabolic organs. However, the expression and function of Angptl4 in the endocrine pancreas has not been evaluated previously. In our current study, we therefore investigated a potential role for Angptl4 in the regulation of insulin secretion using rodent pancreatic islets.

2. Materials and methods

2.1. Peptides and chemicals

Mouse Angptl4 peptide was synthesized as described previously [13]. In brief, mouse Angptl4 cDNA was amplified from a liver library. Full length, CCD and FLD fragments of Angptl4 were cloned into expression vectors and transfected into Cos7 cells. Culture supernatant fractions were collected and concentrated through ultrafiltration. FLAG-tagged mouse Angptl4 was purified using anti-FLAG affinity column and endotoxin was removed using the Detoxigel (Pierce, Rockford, IL). Forskolin and H89 were purchased from Sigma (St. Louis, MO).

2.2. Animals

Adult male C57BL/6 mice and Sprague-Dawley rats of 8–10 weeks old were purchased from Orient Bio (Seoul, Korea). Angptl4 knockout mice in a C57BL/6J genetic background were generated as described previously [10], and kindly donated by Dr. Andras Nagy, Mount Sinai Hospital, Canada. All animals were allowed free access to a standard chow diet (Agripurina Inc., Seoul, Korea) and water unless otherwise indicated and were housed at a controlled temperature (21 ± 1 °C) and under a 12 h light-dark cycle, with light on from 08:00 to 20:00 h. All procedures followed the Principles of Laboratory Animal Care (NIH, Washington, DC) and were approved by the Institutional Animal Care and Use Committee at the Asan Institute for Life Sciences (Seoul, Korea).

2.3. Cell culture

MIN6 cells were maintained in Dulbecco's modified Eagle's medium (DMEM; 25 mmol/L glucose; Invitrogen, Carlsbad, CA) supplemented with 15% fetal bovine serum (FBS) and penicillin/streptomycin (100 units/mL and 0.1 mg/mL, respectively). The cells were incubated at 37 °C in a 5% CO_2 humidified incubator. The culture medium was changed every 48–72 h.

2.4. Immunostaining

Pancreas tissue was isolated from the mice and fixed in 4% formaldehyde for 24 h. The fixed tissues were then dehydrated and embedded in paraffin, cut into 4 μm-thick slices, permeabilized with 0.1% (*v/v*) Tween 20, and blocked with 3% (*v/v*) donkey serum (Sigma) for 1 h. For double immunofluorescence staining, tissue slices were co-incubated with anti-Angptl4 antibody (1:200, rabbit, Adipogen, Incheon, Korea) and either anti-insulin antibody (1:5000, mouse, Sigma) or anti-glucagon antibody (1:10000, mouse, Sigma) at 4 °C overnight. After washing with PBS, the slices were subsequently immunoreacted with Alexa Fluor 488- or 555-conjugated secondary antibodies (1:500; Invitrogen) for 1 h at room temperature. Stained tissues were examined using a confocal microscope (Leica, Wetzlar, Germany). For immunohistochemical staining, tissue slices were incubated with either anti-insulin antibody (1:20000, mouse, Sigma) or anti-glucagon antibody (1:20000, mouse, Sigma) at 4 °C overnight. After washing with PBS, the slices were incubated with ImmPRESS™ HRP anti-mouse IgG peroxidase polymer detection reagent (MP-7402; Vector Laboratories, Burlingame, CA) for 30 min and followed by 5 min-treatment of ImmPACT™ DAB peroxidase substrate (SK-4105; Vector Laboratories). Stained tissues were examined and photographed using an Olympus BX50 microscope (Olympus, Tokyo, Japan).

2.5. Islet culture and insulin secretion test

Islets were isolated from rats or mice and cultured as described previously [14]. For insulin secretion analysis, islets were incubated overnight in RPMI1640 (Invitrogen) supplemented with 10% FBS and penicillin/streptomycin. On the following day, the islets were washed twice with Krebs-Ringer bicarbonate HEPES buffer (KRBH; 115 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 1 mM KH_2PO_4, 24 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 10 mM HEPES, pH 7.4), counted and seeded into 48-well plates (about 10 islets per well), and preincubated in KRBH for 1 h. After a further 1 h incubation with KRBH containing 2.5 mM glucose, supernatants were collected to measure basal insulin secretion. Subsequently, the islets were incubated with KRBH containing 12.5 mM or 25 mM glucose for 1 h, and supernatants were collected to determine the glucose-stimulated insulin secretion (GSIS) level. Insulin concentrations in the supernatant were measured using commercial radioimmunoassay kits for rat insulin (LINCO Research, Charles, MO) or mouse insulin (Shibayagi, Gunma, Japan). Insulin secretion was corrected by the islet DNA amounts. Experiments were repeated at least three times.

2.6. Cyclic AMP (cAMP) assay

MIN6 cells were treated with Angptl4 peptides (10 or 100 nM) or forskolin (5 μM), as a positive control, for 1 h. Cellular cAMP contents were measured using a cAMP enzyme immunoassay kit (Sigma) in accordance with the manufacturer's protocol.

2.7. Glucose and insulin tolerance tests

Angptl4 knockout mice and their wild type littermates were subjected to a glucose tolerance test (GTT) and insulin tolerance test (ITT) at 11–12 weeks of age. For GTT, mice were injected with glucose (2 g/kg) intraperitoneally following a 5 h fasting. For ITT, mice received regular insulin (0.35 units/kg) (Humulin-R®; Eli Lilly, Indianapolis, IN) intraperitoneally under 5 h-fasting conditions. Blood was collected from the tail vein at the indicated time points. Blood glucose concentrations were measured using a glucometer (Accu-Check®; Roche, Basel, Switzerland).