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Globular Adiponectin Augments Insulin Secretion from Pancreatic Islet β Cells at High Glucose Concentrations

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Adiponectin plays an important role in improving insulin resistance and preventing atherosclerosis. However it has been rarely reported that adiponectin influences insulin secretion because its receptor was identified in human islet β cells. In order to investigate the direct effect of adiponectin on pancreatic islet β cells, we performed an insulin secretion test in purified rat islets, which were incubated with adiponectin (100 ng/mL) at low (3.3 m*M*) and high (16.7 m*M*) glucose concentrations. Furthermore, cell lysates were extracted from the adiponectin-treated islets for p-AMPKα assay. RT-PCR and immunohistochemical examination showed both adiponectin receptor 1 (AdipoR1) and receptor 2 (AdipoR2) were expressed in islet cells and AdipoR1 was predominantly expressed. Insulin secretion was significantly increased in the presence of adiponectin for 6 h at high glucose concentration. Meanwhile, the levels of phosphorylated AMPK increased with adiponectin treatment at high glucose concentrations. It is concluded that adiponectin augments insulin secretion from pancreatic islet β cells at high glucose concentration through AMPK activation.

Key Words: Adiponectin; islet beta cells; insulin secretion; AMPK.

Introduction

Adiponectin, a 30 kDa protein predominantly produced by adipose tissues, also termed as apM1, Acrp30, GBP28, or AdipoQ , is well known to have antidiabetic and antiatherogenic properties *(1–3)*. Plasma adiponectin concentrations are decreased in patients with obesity, type 2 diabetes, and cardiovascular diseases in South-Asians and Caucasians, which is usually associated with insulin resistance and hyperinsulinemia *(4–7)*. Added to this, with the identification of its receptors expressed in brain, heart, kidney, liver, lung, etc. *(8)*, the biological functions of adiponectin appear much more than what is already known. In 2003, Kharroubi et al. *(9)* reported the marked mRNA expression of the adiponectin receptors AdipoR1 and AdipoR2 in human and rat pancreatic β cells, at levels similar to liver and greater than muscle. And the adiponectin receptor expression in β cell was upregulated by the unsaturated free fatty acid (FFA) oleate. Then, a group from the other research lab *(10)* confirmed the adiponectin receptor AdipoR1 existed in mouse islets, and discovered a dual action of adiponectin on insulin secretion in insulin-resistant mice. In that study, the acute effects of adiponectin on islets isolated from normal or diet-induced insulin-resistant mice were examined. In islets from mice rendered insulin resistant by high-fat feeding, adiponectin inhibited insulin secretion at 2.8 m*M* but augmented insulin secretion at 16.7 m*M* glucose, while in normal islets, adiponectin had no significant effect on insulin secretion. In our previous clinical studies, we not only found hypo-adiponectinemia in Chinese obese and type 2 diabetic patients, but also identified the correlation of adiponectin with islet β cell function evaluated by acute insulin response to glucose (AIRg), which was output from FSIVGTT combined with minimal model *(11,12)*.

On the other hand, it is also known that adiponectin promotes glucose utilization and fatty-acid oxidation by upregulating AMP-activated protein kinase (AMPK) *(13,14)* and PPAR-α *(15)*. Adenosine-5'-monophosphate activated protein kinase (AMPK) acts as a metabolic sensor of the cellular energy status. Once activated by allosteric binding of AMP in conditions of ATP depletion, AMPK phosphorylates and inhibits the rate-limiting enzymes in cholesterol and fatty-acid synthesis, 3-hydroxy-3-methylglutaryl-CoA (HMGCoA) reductase and acetyl CoA carboxylase (ACC). Thus, AMPK activation promotes fatty-acid oxidation and glucose uptake in myocytes, reduces gluconeogenesis in hepatocytes, and inhibits lipogenesis in adipocytes. Since pancreatic β cells were functionally affected by AMPK activation in previous studies *(16,17)*, it is indispensable to

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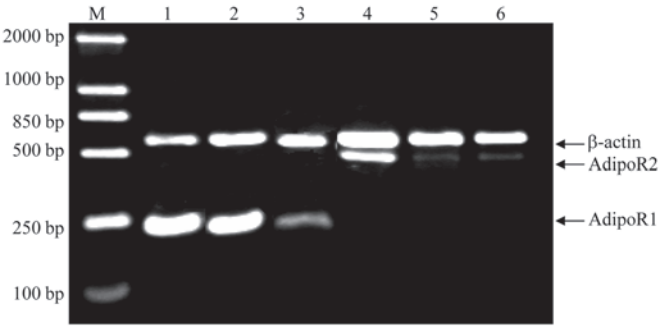

Fig. 1. RT-PCR analysis of AdipoR1 and AdipoR2 mRNA in rat liver tissue, Min6 cells and rat islet. (Lanes: M, marker; 1,4, liver tissue; 2,5, Min6 cells; 3,6, islets; β-actin, 572 bp; AdipoR1, 226bp; AdipoR2, 466 bp).

identify AMPK activation in β cells treated with adiponectin. In the present study, we explored the possibility that adiponectin acts on pancreatic β cells as an insulin secretagogue and whether adiponectin played the role through AMPK activation.

Results

Adiponectin Receptors Identified in Pancreatic Islet β Cells

The adiponectin receptor 1 (AdipoR1) and receptor 2 (AdipoR2) expressions were detected in rat islets and Min6 cells by RT-PCR (shown in Fig. 1). AdipoR1 was predominantly expressed in the liver and Min6 cells. Furthermore, immunohistochemistry showed AdipoR1 expression was located on cell membranes and cytoplasm of rat pancreatic islets (shown in Fig. 2).

Insulin Secretion Augmented by Adiponectin at High Glucose Concentration

Insulin secretion suppression was observed in isolated rat islets incubated with adiponectin for 2 h at low glucose concentration (3.3 m*M*) ($p < 0.05$). The suppression could not be observed with an elongation of incubation with adiponectin till 6 and 24 h. Insulin secretion was not altered by adiponectin at 5.6 m*M* glucose concentration for 2–24 h.

Insulin secretion was significantly augmented by adiponectin at high glucose concentration (16.7 m*M*) for 24 h. The maximum insulin secretion was reached at 6 h (shown in Fig. 3). The dynamic tests showed that insulin secretion and the stimulating index were significantly enhanced following 6 h incubation with adiponectin compared with the control (shown in Fig. 4).

*AMPK Phosphorylation Increased by Adiponectin at 16.7 m*M *Glucose Concentration*

p-AMPK-α declined in intact isolated islets at high glucose concentration (shown in Fig. 5A). Total AMPK was not changed. p-AMPK-α increased at 5.6 and 16.7 mmol/L glucose compared to 3.3 mmol/L in the islets treated with adiponectin. Interestingly, p-AMPK-α was also increased at 5.6 and 16.7 mmol/L glucose in adiponectin-treated islets compared with the islets without adiponectin treatment (shown in Fig. 5B).

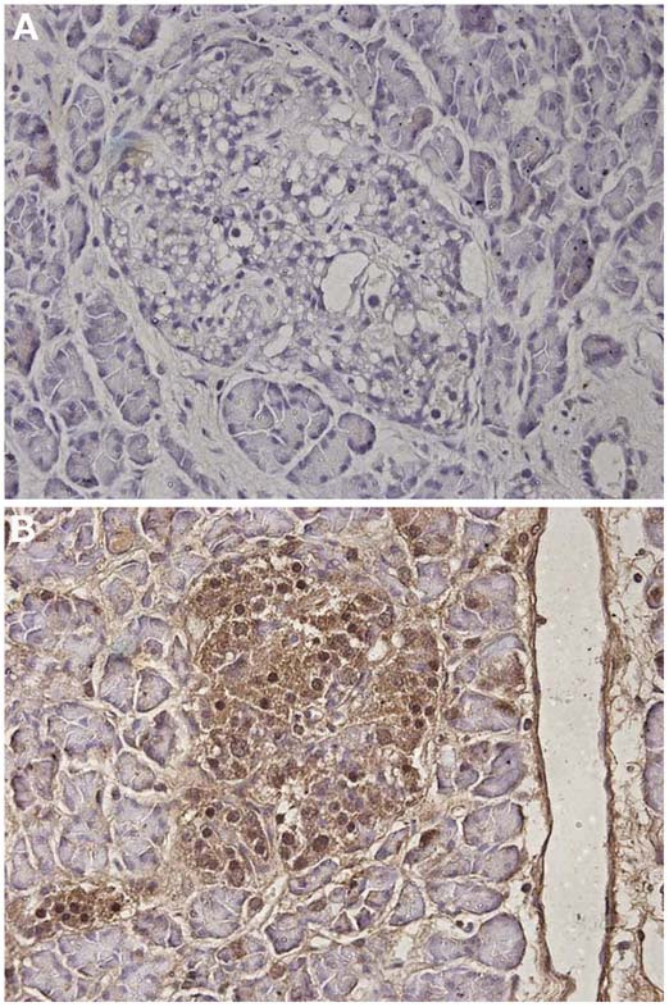

Fig. 2. Immunohistochemistry for adiponectin receptor1 was performed on rat pancreas slice (see Materials and Methods). Compared to the background control (**A**), positive DAB staining for AdipoR1 was demonstrated in the islets (**B**) (400×).

Discussion

It has been known that adipose tissue is not only an energy warehouse, but also an active endocrine organ *(18,19)*. Substantial researches have explored the notion that adipose tissue secretes a variety of biologically active molecules, including cytokines, growth factors, and complement factors, into the circulation. One of these molecules, adiponectin, is produced predominantly by white adipose tissues and plays an important role in improving insulin resistance and antiatherogenesis. It has been reported that adiponectin, like leptin, could regulate food intake and energy expenditure through hypothalamic centers *(20,21)*. More than that, from leptin, the evidence for the existence of an adipoinsular axis was found *(22)*. While insulin increases body fat

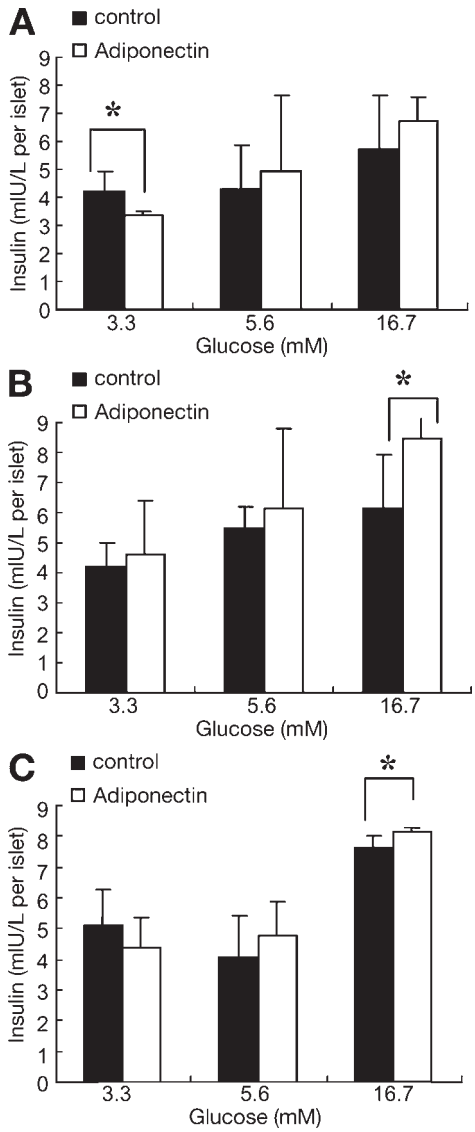

Fig. 3. Insulin secretion after incubation with adiponectin (100 ng/mL) of 2 h, 6 h, and 24 h (**A**, **B**, and **C**, respectively). *$p < 0.05$.

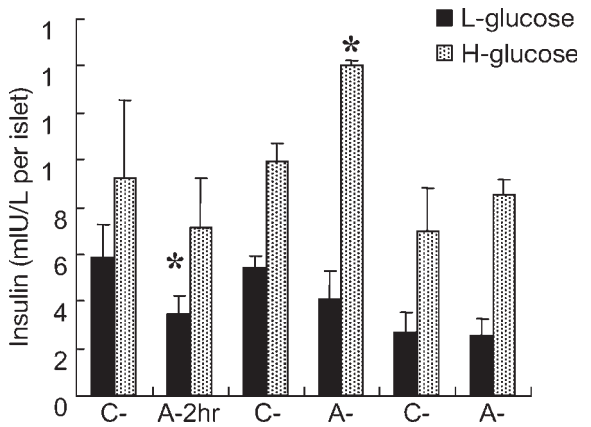

Fig. 4. Glucose stimulated insulin secretion in dynamic tests after incubation with adiponectin at 5.6 m*M* glucose of 2 h, 6 h, and 24 h, respectively. Note: compared to the control group, *$p < 0.05$.

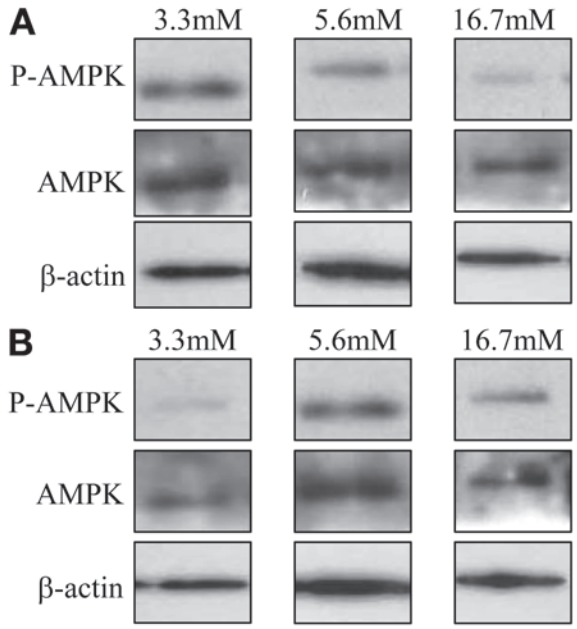

Fig. 5. Effects of adiponectin on phosphorylation of AMPK in rat islets. Islets were untreated (**A**) and treated with adiponectin at100 ng/mL for 6 h (**B**). The AMPK-phosphorylation was detected by Western blot.

mass, and stimulates the production and secretion of leptin, leptin in turn suppresses insulin secretion by both central actions and direct actions on β cells. Therefore, a hypothesis that the dysregulation of this adipoinsular axis may contribute to obesity and the development of hyperinsulinemia associated with diabetes was proposed. In regards to adiponectin, a novel protein contributing to the development of insulin resistance and atherosclerotic complications in diabetes mellitus, it almost has an opposing tendency in plasma level and a different manifestation with leptin either in obese or in diabetic people. However, the possibility still exists that, like leptin, adiponectin may directly influence the function of β cells too. In the present study we detected adiponectin receptors (AdipoR1 and AdipoR2) on islet β cells, which supported a potential role of adiponectin on pancreatic islets. Adiponectin receptors were primarily isolated from Ba/F3 cells infected with a library of retrovirally expressed cDNA, which was derived from human skeletal muscles. AdipoR1 is abundantly expressed in skeletal muscle, whereas AdipoR2 is predominantly expressed in the liver. Our study showed, compared to AdipoR2, AdipoR1 was predominantly expressed in islet β cells.

Because globular adiponectin was bound to AdipoR1 with higher affinity than full-length form in C2C12 myocytes *(23)*, we adopted globular forms of adiponectin for insulin-secretion tests in this study. The results showed, compared to control, that the insulin secretion became higher with the increasing concentration of the glucose in the culture medium. Adiponectin promoted insulin secretion by 37.3% in the islets at 16.7 m*M* glucose for 6–24 h as well

as in dynamic tests by high glucose concentration flow (16.7 m*M*). Thus, it was encouraging that adiponectin might have a positive role in the prevention of the development of diabetic mellitus. Under the physiological condition, adiponectin may not enhance insulin secretion from islet β cells, while under a pathological condition as in diabetes, hypoadiponectinemia may exacerbate the impairment of islet β cell function. However, adiponectin systematically administered to rats did not change the circulatory insulin levels *(24–26)*, which was possibly due to its short half-life, insufficient dose, and improper blood glucose.

Furthermore, to explore the mechanism of adiponectin acting on islet β cells, as it does in peripheral tissues, we examined AMPK activation. AMPK is a crucial molecule participating in many metabolic processes, such as ketogenesis, cholesterol synthesis, lipogenesis, lipolysis, triglyceride synthesis, and fatty-acid oxidation in the livers, adipose tissues, muscles, and even pancreatic islets *(27–30)*. Adiponectin can activate AMPK by phosphorylation of its α subunits *(31)*. Our results showed pAMPK-α was increased in isolated rat islets at high glucose concentration (16.7 m*M*) with the treatment of adiponectin, which correlated with insulin secretion. Marchetti et al. *(32)* demonstrated that metformin, as an AMPK activator too, restored pancreatic beta cell function and prevented diabetic human islet apoptosis. Even a study about AICAR showed its beneficial properties in clonal beta cells under lipotoxic conditions *(33)*. And the latest study on the antioxidant compound α-lipoic acid (α-LA) discovered that α-LA regulating AMP-activated protein kinase and inhibiting insulin secretion from beta cells may result from an effect probably involving the ROS-induced impairment of mitochondrial function besides *(34)*. Therefore, more studies are needed to prove the mechanism of adiponectin to promote the insulin secretion.

In conclusion, adiponectin can augment insulin secretion from pancreatic islet β cells at high glucose concentration. It is possibly promising that adiponectin acts as an insulin secretagogue, especially in high blood glucose environment.

Materials and Methods

Rat Islet Isolation

Adult male SD rats (250–270g) were provided by the Animal Center, Chinese Academy of Science. The animal experiments were performed in the Animal Center of Shanghai Jiao Tong University School of Medicine. The pancreatic islets were isolated by perfusion of the pancreatic duct and subsequently digested with collagenase dissolved in HBSS at 0.5 mg/mL (Sigma, St. Louis, MO, USA). The digested pancreas was filtered through a 500 μm mesh and rinsed with cold 0.1% BSA HBSS three times. After being purified by Ficoll density gradients, the islets were handpicked for the insulin-secretion test. Aliquots of islets were stained with DTZ for purity assay.

RT-PCR for Identification of Adiponectin Receptors

Total RNA was extracted from rat livers, islets, and Min6 cells (kindly provided by Dr. J. Miyamoto, University of Kyoto, Japan) using TRIzol® Reagent (Invitrogen, USA). Reverse transcription reaction was performed in 20 μL containing 1 μg RNA templates, 5 unit Super Script TM (Invitogen, USA), 10 m*M* dNTPs, 25 pg/μL oligo(dT) primer, and 20 U RNase inhibitor (Promega, SA). The PCR amplification reaction was performed in 50 μL containing 2 μL cDNA templates, 1 unit Ampli Taq Gold (PE Applied Biosystems), and the primers (AdipoR1, forward 5'-GTTT CTCCTGGCTCTTCCACACT-3' and reverse 5'-AGTGG CAAACCGGTCCCACT-3'; AdipoR2, forward 5'-GGAG CCATTCTCTGCCTTTC-3' and reverse 5'-ACCAGATG TCACATTTGCCA-3'). Denaturation was performed at 95°C for 3 min, followed by 30 s at 95°C, 45 s at 58°C, and 45 s at 72°C for 30 cycles. The PCR products were loaded onto 1.2% agarose gels and visualized by ethidium bromide under the UV light. The DNA sequences were identified by direct sequencing and subclone sequencing.

Immunohistochemistry

The rat pancreas was examined for AdipoR1 expression by immunohistochemistry. The sections were fixed in 4% formalin and embedded in paraffin. For the staining, the sections were deparaffinized in xylene and rehydrated through descending ethanol to distill water. Endogenous peroxidase was quenched by incubating the slides in 3% H_2O_2 for 10 min at room temperature. The primary antibodies used were rabbit anti-AdipoR1 at 1:500 dilutions (Phoenix Biotech. Co, USA) and the secondary antibodies (universal) were at 1:2000 dilution (Vector Laboratories, USA). The immunoreactive adipoR1 was visualized using Peroxidase Substrate Kit (Vector Laboratories). The section staining without primary antibody was used as the background control.

Insulin-Secretion Assay

The handpicked islets were seeded at 10 islets/well and four wells for each treatment in a 24-well plate and cultured in DMEM medium supplemented with 10% heat-inactivated fetal calf serum (FBS), 100 U/mL penicillin, 100 μg/mL streptomycin, and varying concentration of glucose (3.3 m*M*, 5.6 m*M*, and 16.7 m*M*). The islets were incubated with recombinant globular adiponectin (Wako Pure Chemical Industries, Osaka, Japan) (100 ng/mL) for 2 h, 6 h, and 24 h. The medium was collected and stored at –20°C after incubation until insulin assay.

The dynamic insulin secretion tests were also performed. The islets were incubated with globular adiponectin (100 ng/mL) at 5.6 m*M* glucose concentration for 2 h, 6 h, or 24 h. The medium was replaced with Krebs–Ringer bicarbonate solution (KRBS) after incubation. Thereafter, the islets were incubated with 3.3 m*M* and 16.7 m*M* glucose concentration, respectively, for 45 min at 37°C and KRBS were

harvested for insulin assay. The insulin level was measured by Rat Insulin RIA Kit (Linco, USA). The data were statistically analyzed with Student's *t* test.

Western Blot

The cell lysates were extracted from the adiponectin treated islets in lysis buffer (RIPA, 1X PBS, 1% NP40, 5 m*M* EDTA, 0.5% SDS, 1 m*M* sodium orthovanadate, 1% PMSF, complete protease inhibitor cocktail, and complete phosphatase inhibitors). The protein concentrations were determined with DC Protein Assay Reagent (Bio-Rad Laboratories, USA). Fifty micrograms of cell lysates were loaded onto SDS/PAGE. The protein bands were stained with Ponceaus' solution and transferred to Hybond-ECL Nitrocellulose membrane (Amersham Biosciences, USA). The membrane was blocked with 5% nonfat milk at room temperature for 1 h and incubated with anti β-actin, anti AMPK-α or anti-phospho-AMPK-α (Thr172) (Cell Signaling Technology, USA) antibodies at 4°C overnight. p-AMPK-α was visualized with ECL plus kit (Amersham Biosciences, USA).

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