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Attenuation of insulin secretion by insulin-like growth factor binding protein-1 in pancreatic β-cells

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Abstract

IGFBP-1 is involved in glucohomeostasis, but the direct action of IGFBP-1 on the β-cell remains unclear. Incubation of dispersed mouse β-cells with IGFBP-1 for 30 min inhibited insulin secretion stimulated by glucose, glucagon-like peptide 1 (GLP-1) or tolbutamide without changes in basal release of insulin and in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) and NAD(P)H evoked by glucose. In contrast, IGFBP-1 augmented glucose-stimulated insulin secretion in intact islets, associated with a reduced somatostatin secretion. These results suggest a suppressive action of IGFBP-1 on insulin secretion in isolated β-cells through a mechanism distal to energy generating steps and not involving regulation of $[Ca^{2+}]_i$. In contrast, IGFBP-1 amplifies glucose-stimulated insulin secretion in intact islets, possibly by suppressing somatostatin secretion. These direct modulatory influences of IGFBP-1 on insulin secretion may imply an important regulatory role of IGFBP-1 *in vivo* and in the pathogenesis of type 2 diabetes, in which loss of insulin release is an early pathogenetic event.

Keywords: Insulin-like growth factor; Insulin-like growth factor binding protein-1; Insulin secretion; Exocytosis; Cytosolic free Ca^{2+} concentration; Islet; Somatostatin

Insulin-like growth factors (IGFs) play important roles in regulating glucose metabolism, β-cell function and regeneration [1,2]. The effects of IGFs are mediated through cell surface IGF receptors and are modulated by IGF binding proteins (IGFBPs) through sequestration of IGFs [3,4]. The unbound, free form of IGFs takes only small portion among the total IGFs and determines IGF actions [5]. One of the important functions of IGFBPs is to limit the hypoglycemic effect of IGFs. Among IGFBPs, IGFBP-1 has been shown to be involved in glucose homeostasis. Serum levels of IGFBP-1 vary considerably depending on the metabolic conditions [5,6], correlate inversely with both body mass and serum levels of insulin [7,8]. Down-regulation of IGFBP-1 levels by insulin contributes to the metabolic response to food intake because a decrease in IGFBP-1 would increase the bioavailability of IGFs, which exert insulin-like metabolic functions [9]. The role of IGFBP-1 in the regulation of insulin remains poorly understood. IGFBP-1 is elevated in type 1 diabetes [10,11] and is also found to be associated with development of complications [10,12]. Overexpression of IGFBP-1 in mice has provided additional insights into the physiological role of IGFBP-1 in glucose metabolism. Transgenic mice overexpressing the IGFBP-1 gene under the control of different promoters showed impaired glucose tolerance and abnormalities of insulin action [13–16], suggesting that IGFBP-1 may participate in disruption of the physiological control of glucose homeostasis. In spite of these studies, a direct action of IGFBP-1 on pancreatic β-cell function has not been reported. In the present study, we have investigated the role of IGFBP-1 in insulin secretion from isolated mouse pancreatic β-cells and intact islets *in vitro*.

Materials and methods

Recombinant IGFBP-1 was from GroPep Limited (Adelaide, Australia). Fura-2/acetoxymethylester (Fura-2/AM) was from Sigma (St

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Louis, MO). Bio-gel P-4 (fine, 65 ± 20 μm, wet) was from Bio-Rad Laboratories (Hercules, CA) and collagenase A was from Roche Diagnostics (Mannheim, Germany). Mouse insulin ELISA kits were from Mercodia (Uppsala, Sweden) and somatostatin ELISA kits were from Phoenix Pharmaceuticals Inc (Belmont, CA). RPMI-1640 culture medium and fetal calf serum (FCS) were from Life Technologies Invitrogen (Paisley, UK).

Preparation of pancreatic islets and cells. Pancreatic islets containing >90% β-cells were isolated from 12-month-old obese (*ob/ob*) mice, by collagenase and DNAse digestion, bred at the KISÖS Stockholm colony. The Stockholm *ob/ob* colony was established at KISÖS in 2004 from breeding pairs kindly provided by Professor Janove Sehlin, Umeå University. Spherical islets, free of connective tissue were collected. For single cell preparation, islets were dispersed into single cells as described [17]. Islets or cells were kept in RPMI-1640 culture medium supplemented with 10% FCS, 2 mM L-glutamine and antibiotics overnight in an incubator with gentle shaking. Islets or cells were pre-incubated in the culture medium in the presence of IGFBP-1 (20 nM) or equimolar concentrations of BSA for 30 min at 37 °C before experiments.

Insulin secretion. Insulin secretion from dispersed pancreatic β-cells was monitored by perifusion using two micro-columns, packed with Bio-gel P-4 and performed in parallel [17]. At the end of pre-incubation, about 1×10^5 cells were carefully mixed with a small volume of pre-wetted Bio-gel P-4 and placed on the top of each of the columns. Perifusion was performed at 37 °C at a flow rate of 0.25 ml/min with buffer A containing (in mM) 125 NaCl, 5.9 KCl, 1.28 $CaCl_2$, 1.2 $MgCl_2$, 25 Hepes, 3 glucose and 0.1% BSA in the presence of IGFBP-1 (20 nM) or equimolar concentration of BSA. Fractions were collected every 2 min and the insulin content in each fraction was measured using ELISA kits.

Insulin secretion from intact pancreatic islets was performed in 24-well plates. After pre-incubation of the islets in culture medium in the presence of IGFBP-1 (20 nM) or equimolar concentrations of BSA for 30 min, islets were washed three times with buffer A, followed by incubation of the islets in the same buffer containing 3 or 20 mM glucose in the presence of IGFBP-1 (20 nM) or equimolar concentrations of BSA for 30 min. At the end of the incubation, islets were collected and lysed for subsequent analyses of protein and insulin content in the supernatants.

Measurements of $[Ca^{2+}]_i$. Dispersed β-cells prepared from *ob/ob* mice were placed on glass cover slips and were incubated in culture medium overnight. After pretreatment, cells attached on cover slips were loaded with Fura-2/AM (1 μM) in buffer A for 30 min at 37 °C in the presence of IGFBP-1 (20 nM) or equimolar concentrations of BSA. The cover slips were subsequently rinsed once in the same buffer without the Ca^{2+} indicator and were mounted as the exchangeable bottom of an open perifusion chamber on the stage of an inverted epifluorescence microscope (Olympus CK40). The superfusion chamber was designed to allow rapid exchange of fluids and was thermostatically controlled to maintain a temperature of 37 °C in the perifusate. Measurements of dynamic changes in $[Ca^{2+}]_i$ were performed as previously described [18,19] using a time-sharing spectrofluorometer (RM-5 system, PhotoMed, Denmark) providing light flashes of 1 ms duration at 340 and 380 nm every 10 ms. Fluorescence was recorded at 510 nm from single cells.

Measurement of NAD(P)H. Temporal fluctuations in cellular [NAD(P)H] were measured using the fluorescence system above as described [20]. After pretreatment, cells attached on cover slips were incubated in buffer A at 3 mM glucose for 30 min during which IGFBP-1 (20 nM) or equimolar concentrations of BSA were continuously present. During the experiments, cells were perifused as in the $[Ca^{2+}]_i$ measurements described above. NAD(P)H fluorescence was monitored at an excitation wavelength of 366 nm, a dichroic mirror at 400 nm, and an emission bandpass filter at 450–470 nm [20].

Somatostatin secretion from pancreatic islets. After pretreatment, islets were washed and divided into four groups, each containing 10 islets, and transferred into 24-well plates preloaded with 1 ml of buffer A per well in the presence of IGFBP-1 (20 nM) or equimolar concentrations of BSA with the indicated concentrations of glucose and incubated for 40 min at 37 °C. The same volume of buffer was placed in the parallel-performed wells without islets as background control. At the end of the incubation, the buffer was collected and briefly centrifuged; the supernatants were concentrated by lyophilization. Somatostatin content was analyzed using ELISA kits. The islets were then collected, washed in PBS and lysed for protein determination.

Results

The effect of IGFBP-1 on insulin secretion was investigated in column-perifused dispersed β-cells after incubation in IGFBP-1 (20 nM) or equimolar concentrations of BSA. Insulin secretion in response to stimulation with glucose, GLP-1 or tolbutamide was significantly attenuated by 30-min incubation with IGFBP-1 (Fig. 1A) without any change in basal release (Fig. 1A inset). Glucose- and GLP-1-induced secretion of insulin was suppressed by approximately 50%, while tolbutamide-induced secretion was inhibited up to 70% by IGFBP-1.

Insulin secretion from intact islets was also examined during batch incubation. Treatment of the islets with IGFBP-1 (20 nM) did not alter insulin secretion at low glucose. However, insulin secretion stimulated by high glucose was almost doubled in the presence of IGFBP-1 (Fig. 1B).

Since an increase in $[Ca^{2+}]_i$ is a crucial step in insulin secretion stimulated by glucose and many other insulin secretogogues, we next investigated if the suppressive effect of IGFBP-1 on insulin secretion noted in dispersed β-cells was associated with a change in $[Ca^{2+}]_i$. However, treatment of the dispersed β-cells with IGFBP-1 did not result in any changes in $[Ca^{2+}]_i$ in response to either glucose or tolbutamide (Fig. 2). In addition, IGFBP-1 did not interfere with glucose-stimulated NAD(P)H production in the dispersed β-cells (Fig. 3).

In order to investigate whether treatment of pancreatic islets interfered with the function of delta cells, we examined somatostatin release from the intact islets after IGFBP-1 treatment. As expected, somatostatin secretion was increased approximately 70% by 20 mM glucose compared to 3 mM glucose. IGFBP-1 treatment resulted in an approximately 55% reduction in somatostatin secretion induced by high glucose, but did not influence basal somatostatin secretion (Fig. 4).

Discussion

IGFBP-1 plays important role in maintaining normal glucose homeostasis and its circulating levels are elevated in diabetes. The direct action of IGFBP-1 on pancreatic β-cells has remained incompletely understood. Here we report that exposure of dispersed β-cells to IGFBP-1 attenuates insulin secretion stimulated by insulin secretagogues, whereas in intact pancreatic islets the opposite occurs.

Studies on transgenic mice overexpressing IGFBP-1 revealed impaired glucose tolerance associated with hyperglycemia [13,21]. In the transgenic models, glucose-stimulated insulin secretion was potentiated in the pancreatic islets. In concordance with the latter *in vivo* finding, the present study shows that exposure of intact pancreatic islets to IGFBP-1 *in vitro* augmented glucose-stimulated