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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

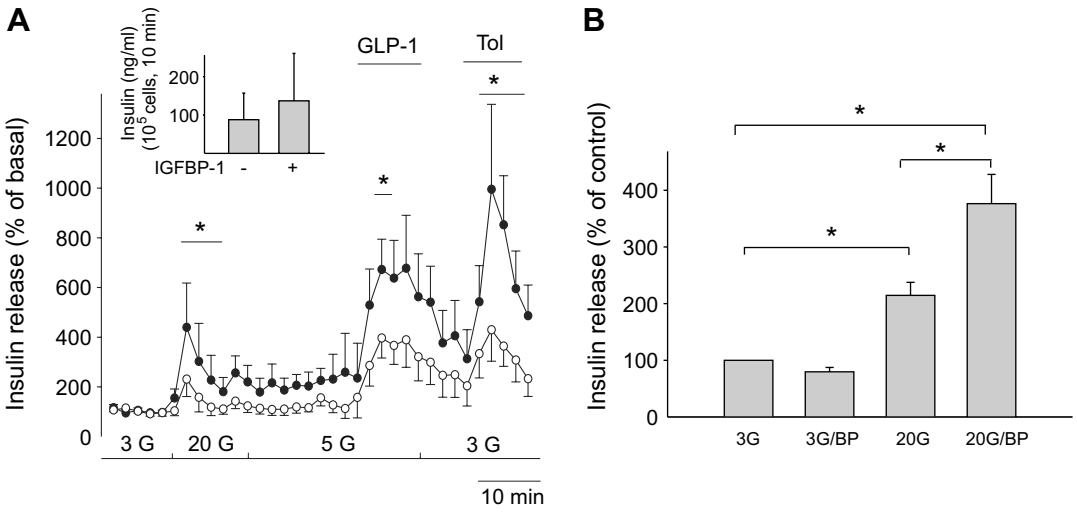

Fig. 1. Effect of IGFBP-1 on insulin secretion from isolated β-cells and intact islets. Insulin secretion was evaluated in column-perifused isolated β-cells from *ob/ob* mice, as described in Materials and methods. Cells were perifused with 3 mM glucose before glucose stimulation (G, 20 mM). Cells were further perifused in 5 mM glucose before and during GLP-1 (100 nM) stimulation, followed by 3 mM glucose perifusion before and during tolbutamide (Tol, 100 μM) addition. Values represent means ± SEM for seven independent experiments and are expressed as percentage of insulin release obtained in 3 mM glucose during the first 10 min of perifusion. *Denotes $P < 0.05$ for a chance difference vs control using ANOVA. Filled circles: control; empty circles: IGFBP-1 treated (A). The inset shows insulin secretion at 3 mM glucose in the presence or absence of IGFBP-1 during 10 min. Insulin secretion from pancreatic islets from *ob/ob* mice was examined during 30 min of batch incubation (B). Bars represent means ± SEM for four independent experiments and are expressed as percentage of insulin released in 3 mM glucose. *Denotes $P < 0.05$ for a chance difference using ANOVA.

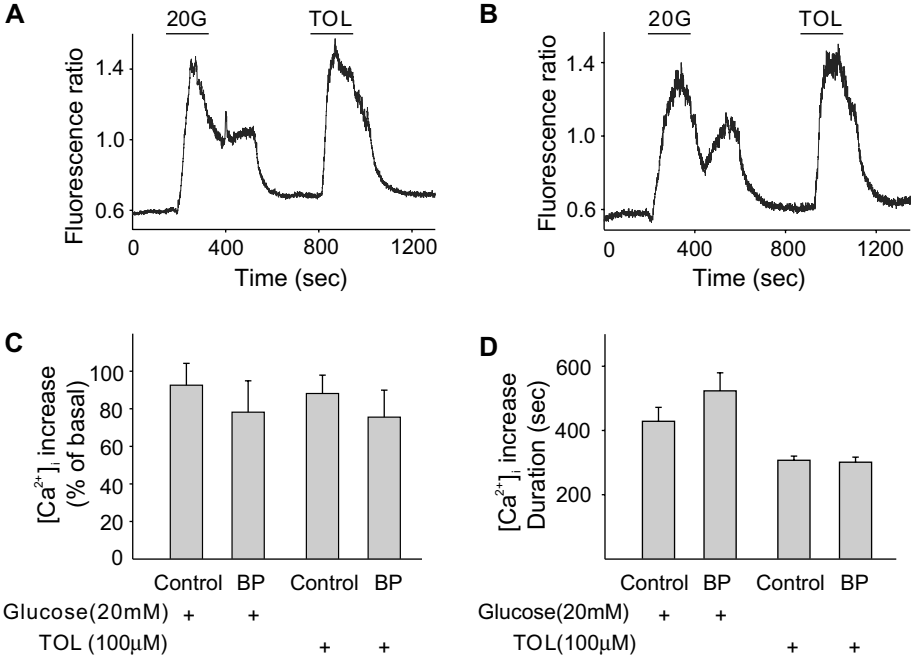

Fig. 2. IGFBP-1 does not influence $[Ca^{2+}]_i$ in isolated β-cells. Measurement of $[Ca^{2+}]_i$ was done in single cells loaded with the fluorescent Ca^{2+} probe Fura-2 as described in Materials and methods. Results are expressed as changes in fluorescence ratio (*F340/F380*) obtained every second. All experiments were performed on different cell preparations. Representative experiments out of seven (control) or nine (IGFBP-1 treated) are shown in (A) and (B), respectively. Statistical analysis of the peaks stimulated by 20 mM glucose (20G) or tolbutamide (TOL) in amplitude (C, percentage increase compared to 3 mM glucose) or duration (D) is shown. BP, IGFBP-1. Bars represent means ± SEM for 7–9 independent experiments.

insulin secretion. Our results from isolated pancreatic islets suggest that the enhanced glucose-stimulated insulin secretion observed in the transgenic mice may be due to a direct stimulatory effect of IGFBP-1 on the islet cells.

In contrast to intact pancreatic islets, treatment of isolated β-cells from *ob/ob* mice suppressed glucose-stimulated insulin secretion. The inhibition of insulin secretion evoked by IGFBP-1 is not likely to reflect cytotoxicity as incubation

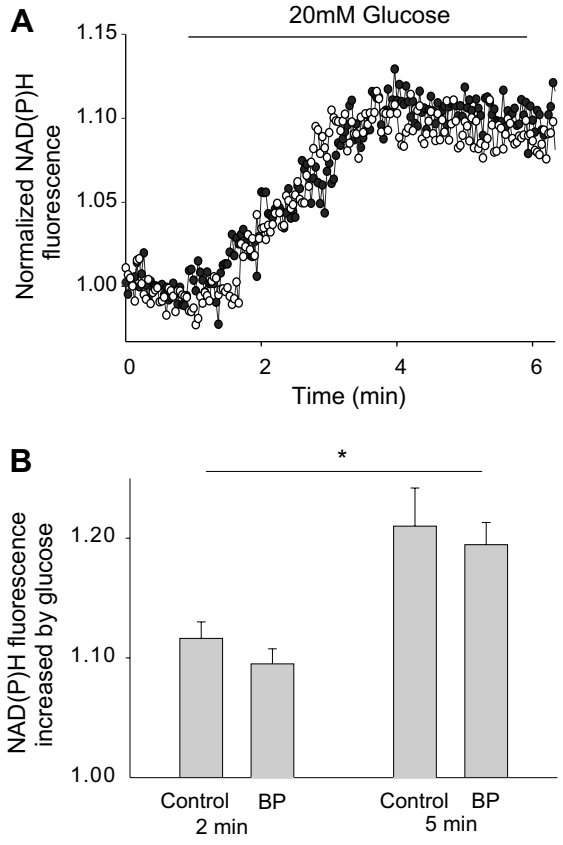

Fig. 3. IGFBP-1 does not influence NAD(P)H levels in isolated β-cells. NAD(P)H fluorescence was monitored in single cells during perifusion with 3 mM and stimulation with 20 mM glucose. All of the experiments were performed on different cell preparations. Fluorescence was normalized by setting basal fluorescence at 100%. A representative trace out of six (control, filled circles) and seven (IGFBP-1 treated, empty circles) experiments is shown (A). Summary data of the increase in NAD(P)H fluorescence stimulated by glucose after 2 min or 5 min are shown in (B). Bars represent means ± SEM for 6–7 independent experiments. BP, IGFBP-1. *Denotes $P < 0.05$ for a chance difference between 5 and 2 min in both groups using ANOVA.

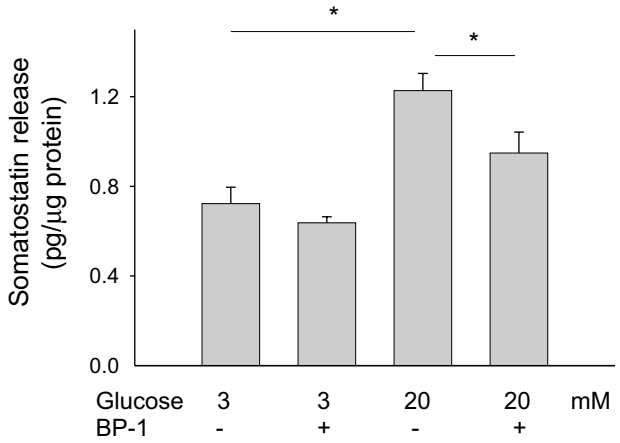

Fig. 4. IGFBP-1 attenuates glucose-stimulated somatostatin secretion from intact islets. Somatostatin release from *ob/ob* mouse intact pancreatic islets was evaluated by ELISA after 40 min incubation. Amounts of released hormone were normalized by protein content in lysed islets. Bars represent means ± SEM for six independent experiments. BP-1, IGFBP-1. *Denotes $P < 0.05$ for a chance difference between groups using ANOVA.

of the β-cells with IGFBP-1 for up to 6 h did not affect cell viability as assessed by Trypan blue exclusion (data not shown). In addition, the cells responded normally to glucose in terms of NAD(P)H production and $[Ca^{2+}]_i$ after IGFBP-1 treatment. Our data suggest that IGFBP-1 inhibits, rather than potentiates, glucose-induced insulin secretion in the isolated β-cell. Studies in mice lacking IGF-1R specifically in β-cells [2,22] suggest that deficiency in IGF-1 signaling does not affect the β-cell proliferation. The glucose/insulin ratio even falls in transgenic mice using a cosmid clone encompassing the entire human IGFBP-1 gene. In addition, the mechanism behind the enhanced insulin secretion in IGFBP-1 overexpressing mice has not been elucidated. The presence of excess IGFBP-1 reduces the bioavailability of IGF-1, which is normally stimulating insulin secretion. The important role of the IGF-1 system in maintaining normal insulin secretory responses has been documented at both receptor and post-receptor levels [2,23]. In agreement with the inhibitory effect of IGFBP-1 on function of isolated β-cells, disruption of IGF-1 signaling in β-cells results in impaired glucose tolerance and a decrease in glucose-dependent insulin release [2,22].

Studies on intact islets may yield results different from those obtained in dispersed β-cells due to the intercellular communication between different cell types in the islets and paracrine actions of hormones on adjacent cells [24,25]. Deletion of IGF-1 signaling impairs glucose tolerance in mice lacking IGF-1R specifically in the β-cell, but not in the pancreatic specific IGF-I inactive mice [26]. Accordingly, our results show that glucose-stimulated insulin secretion was suppressed by excess IGFBP-1 in isolated β-cells, whereas it was increased in intact islets. In perifused dispersed islet cells, any paracrine actions of islet hormones are disrupted. Exposure of the pancreatic islets to high glucose increased somatostatin release, consistent with the report from rat islets [27]. Notably, IGFBP-1 attenuated glucose-stimulated somatostatin secretion. This may be a consequence of reduced bioavailability of IGF-1, a peptide that has been demonstrated to stimulate somatostatin release [28]. That the suppressive effect of IGFBP-1 on somatostatin release became evident at high glucose might be due to an increased secretion and sensitivity of IGF-1 under the conditions [29,30]. The IGFBP-1-induced decrease in somatostatin release at high glucose may explain the enhanced glucose-induced insulin secretion by the binding protein in intact islets, since (paracrine) somatostatin is a potent inhibitor of glucose-stimulated insulin secretion.

The molecular mechanisms behind the inhibitory effect of IGFBP-1 on insulin secretion remain elusive. Exposure of isolated β-cells to IGFBP-1 caused impaired insulin secretion in response to GLP-1 and tolbutamide, in addition to glucose, indicating that IGFBP-1 treatment impaired a common pathway in insulin secretion. Neither glucose-stimulated NAD(P)H, nor $[Ca^{2+}]_i$ was altered by

the binding protein, suggesting that IGFBP-1 may be targeting pathways distal to energy-generating steps and cytosolic Ca^{2+} increase. Although the precise nature of the mechanisms by which IGFBP-1 negatively impact insulin secretion in isolated β-cells remains to be elucidated, the present study nonetheless suggests a direct action of IGFBP-1 on the insulin exocytotic machinery. Since serum levels of IGFBP-1 are elevated in diabetes, the direct effects of IGFBP-1 on β-cell and islet function noted here may also be relevant in the pathogenesis of diabetes.

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References

[1] M. George, E. Ayuso, A. Casellas, C. Costa, J.C. Devedjian, F. Bosch, Beta cell expression of IGF-I leads to recovery from type 1 diabetes, J. Clin. Invest. 109 (2002) 1153–1163.
[2] S. Xuan, T. Kitamura, J. Nakae, K. Politi, Y. Kido, P.E. Fisher, M. Morroni, S. Cinti, M.F. White, P.L. Herrera, D. Accili, A. Efstratiadis, Defective insulin secretion in pancreatic β cells lacking type 1 IGF receptor, J. Clin. Invest. 110 (2002) 1011–1019.
[3] S.M. Firth, R.C. Baxter, Cellular actions of the insulin-like growth factor binding proteins, Endocr. Rev. 23 (2002) 824–854.
[4] A. Denley, L.J. Cosgrove, G.W. Booker, J.C. Wallace, B.E. Forbes, Molecular interactions of the IGF system, Cytokine Growth Factor Rev. 16 (2005) 421–439.
[5] V. Hwa, Y. Oh, R.G. Rosenfeld, The insulin-like growth factor-binding protein (IGFBP) superfamily, Endocr. Rev. 20 (1999) 761–787.
[6] L.E. Underwood, J.P. Thissen, S. Lemozy, J.M. Ketelslegers, D.R. Clemmons, Hormonal and nutritional regulation of IGF-I and its binding proteins, Horm. Res. 42 (1994) 145–151.
[7] T. Kamoda, H. Saitoh, M. Inudoh, K. Miyazaki, A. Matsui, The serum levels of proinsulin and their relationship with IGFBP-1 in obese children, Diabetes Obes. Metab. 8 (2006) 192–196.
[8] C.A. Conover, P.D. Lee, J.A. Kanaley, J.T. Clarkson, M.D. Jensen, Insulin regulation of insulin-like growth factor binding protein-1 in obese and nonobese humans, J. Clin. Endocrinol. Metab. 74 (1992) 1355–1360.
[9] L.J. Murphy, The role of the insulin-like growth factors and their binding proteins in glucose homeostasis, Exp. Diabesity Res. 4 (2003) 213–224.
[10] A. Wedrychowicz, H. Dziatkowiak, J. Nazim, K. Sztefko, Insulin-like growth factor-1 and its binding proteins, IGFBP-1 and IGFBP-3, in adolescents with type-1 diabetes mellitus and microalbuminuria, Horm. Res. 63 (2005) 245–251.
[11] C.A. Hedman, J. Frystyk, T. Lindstrom, J.W. Chen, A. Flyvbjerg, H. Orskov, H.J. Arnqvist, Residual β-cell function more than glycemic control determines abnormalities of the insulin-like growth factor system in type 1 diabetes, J. Clin. Endocrinol. Metab. 89 (2004) 6305–6309.
[12] H.R. Mogul, M. Marshall, M. Frey, H.B. Burke, P.S. Wynn, S. Wilker, A.L. Southern, S.R. Gambert, Insulin like growth factor-binding protein-1 as a marker for hyperinsulinemia in obese menopausal women, J. Clin. Endocrinol. Metab. 81 (1996) 4492–4495.
[13] P.A. Crossey, J.S. Jones, J.P. Miell, Dysregulation of the insulin/IGF binding protein-1 axis in transgenic mice is associated with hyperinsulinemia and glucose intolerance, Diabetes 49 (2000) 457–465.
[14] E. Gay, D. Seurin, S. Babajko, S. Doublier, M. Cazillis, M. Binoux, Liver-specific expression of human insulin-like growth factor binding protein-1 in transgenic mice: repercussions on reproduction, ante- and perinatal mortality and postnatal growth, Endocrinology 138 (1997) 2937–2947.
[15] K. Rajkumar, S.T. Dheen, L.J. Murphy, Hyperglycemia and impaired glucose tolerance in IGF binding protein-1 transgenic mice, Am. J. Physiol. 270 (1996) E565–E571.
[16] M.R. Schneider, H. Lahm, M. Wu, A. Hoeflich, E. Wolf, Transgenic mouse models for studying the functions of insulin-like growth factor-binding proteins, FASEB. J. 14 (2000) 629–640.
[17] Q. Zhang, P.O. Berggren, O. Larsson, K. Hall, M. Tally, Insulin-like growth factor II inhibits glucose-induced insulin exocytosis, Biochem. Biophys. Res. Commun. 243 (1998) 117–121.
[18] F. Zhang, Q. Zhang, A. Tengholm, A. Sjoholm, Involvement of JAK2 and Src kinase tyrosine phosphorylation in human growth hormone-stimulated increases in cytosolic free Ca^{2+} and insulin secretion, Am. J. Physiol. Cell Physiol. 291 (2006) C466–C475.
[19] Q. Zhang, M. Kohler, S.N. Yang, F. Zhang, O. Larsson, P.O. Berggren, Growth hormone promotes $Ca^{(2+)}$-induced Ca^{2+} release in insulin-secreting cells by ryanodine receptor tyrosine phosphorylation, Mol. Endocrinol. 18 (2004) 1658–1669.
[20] L. Juntti-Berggren, D.L. Webb, P.O. Arkhammar, V. Schultz, E.K. Schweda, K. Tornheim, P.O. Berggren, Dihydroxyacetone-induced oscillations in cytoplasmic free Ca^{2+} and the ATP/ADP ratio in pancreatic β-cells at substimulatory glucose, J. Biol. Chem. 278 (2003) 40710–40716.
[21] S.T. Dheen, K. Rajkumar, L.J. Murphy, Effects of insulin-like growth factors (IGF) on pancreatic islet function in IGF binding protein-1 transgenic mice, Diabetologia 39 (1996) 1249–1254.
[22] R.N. Kulkarni, M. Holzenberger, D.Q. Shih, U. Ozcan, M. Stoffel, M.A. Magnuson, C.R. Kahn, β-cell-specific deletion of the Igf1 receptor leads to hyperinsulinemia and glucose intolerance but does not alter β-cell mass, Nat. Genet. 31 (2002) 111–115.
[23] R.N. Kulkarni, Receptors for insulin and insulin-like growth factor-1 and insulin receptor substrate-1 mediate pathways that regulate islet function, Biochem. Soc. Trans. 30 (2002) 317–322.
[24] H. Ishihara, P. Maechler, A. Gjinovci, P.L. Herrera, C.B. Wollheim, Islet β-cell secretion determines glucagon release from neighbouring α-cells, Nat. Cell Biol. 5 (2003) 330–335.
[25] A. Wendt, B. Birnir, K. Buschard, J. Gromada, A. Salehi, S. Sewing, P. Rorsman, M. Braun, Glucose inhibition of glucagon secretion from rat α-cells is mediated by GABA released from neighboring β-cells, Diabetes 53 (2004) 1038–1045.
[26] Y. Lu, P.L. Herrera, Y. Guo, D. Sun, Z. Tang, D. LeRoith, J.L. Liu, Pancreatic-specific inactivation of IGF-I gene causes enlarged pancreatic islets and significant resistance to diabetes, Diabetes 53 (2004) 3131–3141.
[27] E. Dumonteil, C. Magnan, B. Ritz-Laser, A. Ktorza, P. Meda, J. Philippe, Glucose regulates proinsulin and prosomatostatin but not proglucagon messenger ribonucleic acid levels in rat pancreatic islets, Endocrinology 141 (2000) 174–180.

[28] M. Berelowitz, M. Szabo, L.A. Frohman, S. Firestone, L. Chu, R.L. Hintz, Somatomedin-C mediates growth hormone negative feedback by effects on both the hypothalamus and the pituitary, Science 212 (1981) 1279–1281.

[29] L. Goya, A. de la Puente, S. Ramos, M.A. Martin, F. Escriva, A.M. Pascual-Leone, Regulation of insulin-like growth factor-I and -II by glucose in primary cultures of fetal rat hepatocytes, J. Biol. Chem. 274 (1999) 24633–24640.

[30] M.J. Horney, D.W. Shirley, D.T. Kurtz, S.A. Rosenzweig, Elevated glucose increases mesangial cell sensitivity to insulin-like growth factor I, Am. J. Physiol. 274 (1998) F1045–F1053.