IGF-1 Protects Against Dexamethasone-Induced Cell Death in Insulin Secreting INS-1 Cells Independent of AKT/PKB Phosphorylation

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Key Words

Apoptosis • Proliferation • Insulin secreting cells • Dexamethasone • IRS-2 • PI3K • PKB/Akt • ERK • Phosphorylation

Abstract

Appropriate insulin secretion depends on β -cell mass that is determined by the balance between cell proliferation and death. IGF-1 stimulates proliferation and protects against apoptosis. In contrast, glucocorticoids promote cell death. In this study we examined molecular interactions of the glucocorticoid dexamethasone (dexa) with IGF-1 signalling pathways in insulin secreting INS-1 cells. IGF-1 (50 ng/ml) increased the growth rate and stimulated BrdU incorporation, while dexa (100 nmol/l) inhibited cell growth, BrdU incorporation and induced apoptosis. Dexa-induced cell death was partially antagonized by IGF-1. This protection was further increased by LY294002 (10 μ mol/l), an inhibitor of PI3 kinase. In contrast, MAP kinase inhibitor PD98059 (10 μ mol/l) significantly reduced the protective effect of IGF-1. The analysis of signalling pathways by Western blotting revealed that dexa increased IRS-2 protein abundance while the expression of PI3K, PKB and ERK remained unchanged. Despite increased IRS-2

protein, IRS-2 tyrosine phosphorylation stimulated by IGF-1 was inhibited by dexa. Dexa treatment reduced basal PKB phosphorylation. However, IGF-1-mediated stimulation of PKB phosphorylation was not affected by dexa, but ERK phosphorylation was reduced. LY294002 restored IGF-1-induced ERK phosphorylation. These data suggest that dexa induces apoptosis in INS-1 cells by inhibiting phosphorylation of IRS-2, PKB and ERK. IGF-1 counteracts dexa-mediated apoptosis in the presence of reduced PKB but increased ERK phosphorylation.

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Introduction

There is increasing evidence that insulin and IGF-1 receptor signalling pathways are involved in the regulation of β -cell mass and that alterations in these pathways increase the incidence of diabetes mellitus [1-6]. The pivotal role of IRS-dependent signalling in β -cells was convincingly demonstrated by targeted gene disruption. The IRS-2 knockout mouse developed lethal hyperglycaemia that was rescued by β -cell specific overexpression of IRS-2 [7]. The IRS-1 knockout directed to insulin

secreting β -cells leads to impaired glucose homeostasis [8, 9]. Thereby, both, impaired glucose-induced insulin secretion and the reduction of β -cell mass contribute to these phenotypes [10].

Insulin and IGF-1 trigger autophosphorylation of their respective receptors and activates several divergent signalling cascades. One pathway involves tyrosine phosphorylation of specific insulin receptor substrates (IRS-1 to IRS-4) and subsequent activation of phosphatidylinositol-3 kinase (PI3K), PDK-1 and protein kinase B (PKB/Akt), that regulates gene expression, glucose transport, glycogen synthesis and has anti-apoptotic effects [11-15]. Another pathway leads to the stimulation of the mitogen-activated protein kinase (MAPK) cascade through Grb/SOS and ras inducing gene expression and cell proliferation [16]. Reduced tyrosine kinase signalling, inappropriately high cytosolic Ca^{2+} activity during sustained hyperglycaemia and oxidative stress have been found to induce apoptotic β -cell death [17-19]. In contrast, PKB stimulation through insulin and IGF-1 receptor activation or overexpression of PKB effectively counteracts cell death [13]. The anti-apoptotic effects of PKB signalling depend on the rate and duration of activation, i.e. phosphorylation [20]. Beside PKB activation, a contribution of MAP kinase pathways, especially ERK activation, to proliferation and protection against cell death has been observed but its significance is not completely understood [20-22].

Glucocorticoids are diabetogenic agents due to the stimulation of hepatic glucose production and induction of peripheral insulin resistance. In healthy subjects, glucocorticoid treatment results in stimulation of insulin secretion [23]. However, long term treatment with immunosuppressive synthetic glucocorticoid dexamethasone induces steroid diabetes mellitus as a result of impaired insulin secretion [24-26]. Glucocorticoids have been described to inhibit insulin secretion by increasing α_2 -adrenoceptor signalling [27, 28] and K_v channel activity [29] as well as by impairing insulin biosynthesis and glucose metabolism [30-32]. Previously, we showed that dexamethasone (dexa) induces apoptotic cell death in insulin secreting cells, an effect that may contribute to impaired β -cell function [33]. The dexa-stimulated cell death was accompanied by reduced protein levels of the anti-apoptotic protein Bcl-2 and reduced phosphorylation of Bad, changes that are known to trigger mitochondrial dependent apoptosis [33, 34]. Both proteins of the Bcl-2 family are regulated by PI3K and MAPK signalling pathways [34-37].

In the present study, we examined whether dexa

affects β -cell survival via interference with PKB or MAPK, or both signalling pathways. We explored the role of these pathways in the insulin secreting cell line INS-1 by manipulating the expression and activation of endogenously expressed proteins by long term treatment with glucose, the synthetic glucocorticoid dexamethasone and IGF-1 in the presence and absence of PKB and MAP kinase inhibitors.

Materials and Methods

Reagents

The antibody for PKB (# 06-558) was purchased from Upstate (Lake Placid, NY, USA), the antibodies for P-PKB (Ser 473, # 9271), P-ERK (Thr202/Tyr204, # 9101) and ERK (# 9102) were from Cell Signaling Technology (Beverly, MA, USA). Antibodies against PY-IRS-2 and β -tubulin-HRP (# sc-5274HRP) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), peroxidase-linked donkey anti-rabbit IgG was from Amersham Biosciences (Buckinghamshire, UK). The IRS-2 antibody was a kind gift from M. White (Harvard Medical School, Boston, USA). Cell culture media were purchased from GIBCO (Invitrogen GmbH, Karlsruhe, Germany), fetal calf serum from Biochrom (Berlin, Germany). The protein assay was purchased from Biorad Laboratories GmbH (München, Germany), the protein ladder (#10748-010) from Invitrogen (Karlsruhe, Germany). LY294002 and PD98059 were purchased from Calbiochem (Merck Biosciences, Bad Soden, Germany). The TUNEL assay and proliferation kits were purchased from Roche Diagnostic GmbH (Mannheim, Germany). All other chemicals were from Sigma (Deisenhofen, Germany) and of analytical grade unless otherwise stated.

Cell culture and cell counting

INS-1 cells (kindly provided by C. B. Wollheim, University of Geneva, Switzerland) derived from a rat insulinoma were cultured in HEPES-buffered RPMI 1640 supplemented with 10 % foetal calf serum (FCS, Biochrom, Berlin, Germany), 10 mmol/l Hepes, 2 mmol/l L-glutamine, 1 mmol/l Na pyruvate, 10 $\mu\text{mol/l}$ β -mercaptoethanol (Sigma, München, Germany) and antibiotics as described previously [29]. Unless otherwise stated the culture medium contained 10 % FCS and 11 mmol/l glucose (standard condition). For proliferation analysis, cells were seeded at a concentration of 0.2×10^6 cells/ 11 cm^2 culture dish, cultured for 2 d and thereafter test substances were added with fresh medium as indicated. At the respective time points cells were detached by trypsin and counted using a hemocytometer.

Cell proliferation assay

Cells were seeded into 96 well plates in RPMI 1640 medium. They were cultured for 24 h in serum-free medium containing 5 mmol/l glucose. IGF-1 (50 ng/ml) and LY294002 (10 $\mu\text{mol/l}$) were added 1 d before dexa (100 nmol/l). One day later cells were labelled for 8 h with bromodeoxyuridine (100 $\mu\text{mol/l}$). Incorporated BrdU was bound by a peroxidase-coupled anti-

BrdU antibody and the immune complexes were detected and quantified in an ELISA reader.

Cell death detection assays

INS-1 cells were seeded onto glass cover slips coated with poly-L-ornithine (0.001%) at subconfluent densities and cultured for 2 d. Thereafter, test substances were added with fresh medium as indicated. After incubation for the appropriate time period, cells were fixed with ethanol (100 %) for 5 min at room temperature and then incubated with 4'-diamidino-2-phenylindole (DAPI, Sigma) at a concentration of 0.1 µg/ml in ethanol for 15 min at 37°C. After washing the cells with cold PBS, the morphology of stained nuclei was analysed under an inverted fluorescence microscope (10 x objective; Nikon, Japan) using an excitation wavelength of 350 nm. Apoptotic nuclei were identified by fractionated and condensed chromatin. More than 200 cells/condition/experiment were counted and the percentage of apoptotic nuclei determined. For TUNEL staining, cells were processed as described by the provider of the kit.

Western blotting

Cells were cultured for 3 d, treated with test substances as indicated for each experiment and processed as described previously [33]. Briefly, cells were lysed in buffer containing 125 mmol/l NaCl, 1 % (v/v) Triton X-100, 0.5 % sodiumdeoxycholate, 0.1 % SDS, 10 mmol/l EDTA, 25 mmol/l HEPES pH 7.3, 10 mmol/l NaPP, 10 mmol/l NaF, 1 mmol/l Na-vanadate, 10 µg/ml pepstatin A, 10 µg/ml aprotinin and 0.1 mmol/l PMSF. Proteins of a 10000 g supernatant were subjected to a SDS-PAGE (8-12 %) and blotted onto nitrocellulose membranes (Schleicher and Schuell, Dassel, Germany). All antibodies were diluted 1:1000. Quantification of Western blots was performed by measuring the O.D. of the respective protein dot on the film using a Molecular Imager Gel Doc System (Bio-Rad Laboratories).

Statistical analysis

Data are provided as means ± S.E., n represents the number of independent experiments. All data were tested for significance using Student's t-test, ANOVA and Dunnett's test as post-test. Results with $p < 0.05$ were considered statistically significant.

Results

IGF-1 inhibits while dexamethasone promotes apoptotic cell death of insulin secreting INS-1 cells

INS-1 cells cultured under standard conditions had a proliferation rate with a doubling time of 2 d (Fig. 1A). IGF-1, 50 ng/ml for 1 d, significantly increased proliferation and BrdU incorporation (Fig. 1A and 1B). Dexamethasone, 100 nmol/l, inhibited cell proliferation by 82 % after 2 - 4 d of treatment. Dexamethasone exerted its effect through the activation of the glucocorticoid receptor as 1 µmol/l RU486, a glu-

Fig. 1. Dexamethasone inhibits proliferation and induces apoptotic cell death. (A) INS-1 cell population doubling time was measured as described under Materials and Methods. Cells were incubated with 100 nmol/l dexamethasone, 1 µmol/l RU486 and 50 ng/ml IGF-1 as indicated. (B) BrdU incorporation was measured as described under Material and Methods after 1 d treatment. (C) Cell death is expressed as % of condensed and fractionated nuclei visualized by DAPI staining after 1 d treatment. Shown are means ± S.E. for the given number (n) of independent experiments. * denotes significant effect of dexamethasone to the respective control of untreated cells; \$ denotes significance to IGF-1 in control cells, # denotes significant different to dexamethasone.

cocorticoid receptor inhibitor, antagonized its effect. In the presence of IGF-1, dexamethasone inhibited cell proliferation by 50 % and 78 % after 2 d and 4 d, respectively (Fig. 1A). Dexamethasone inhibited IGF-1 mediated BrdU staining (Fig. 1B). In addition, dexamethasone stimulated cell death 3-fold (Fig. 1C). IGF-1 reduced the amount of condensed apoptotic nuclei by 71 % (Fig. 1C). These observations indicate that IGF-1 and dexamethasone compete for opposing effects on signalling pathways involved in proliferation and cell death.

IGF-1 protects against dexamethasone-induced cell death in the presence of PI3K inhibition

To further analyse the signalling pathways mediating dexamethasone-induced cell death and IGF-1 dependent protection, their effects in the presence of selective inhibitors for PI3K and MAPK were examined (Fig. 2). Surprisingly, the inhibition of PI3K by 10 µmol/l LY294002 did not antagonize IGF-1-dependent protection against dexamethasone-induced cell death but supported the anti-apoptotic effect of IGF-1 (Fig. 2A). The increased amount of TUNEL positive cells and increased caspase 3 activity (not shown) corroborate that apoptotic rather than necrotic cell death was induced by dexamethasone (Fig. 2B and D).

Fig. 2. Dexa-induced apoptosis is counteracted by IGF-1 in the presence of LY294002. INS-1 cells were treated for 4 d with 100 nmol/l dexa, 50 ng/ml IGF-1, 10 μmol/l LY294002 (LY) and 10 μmol/l PD98059 (PD) as indicated. (A and C) Cell death is expressed as % of condensed DAPI-stained nuclei. Shown are means ± S.E. for the number (n) of independent experiments (B and D) INS-1 cell apoptosis estimated by TUNEL assay. Shown are representative pictures of INS-1 cells and the correspondent fluorescent stained TUNEL positive cells. * denotes significance to control; # to dexa, & to IGF-1.

The protection against dexa-induced apoptotic cell death was mediated by IGF-1 and not by LY294002, since LY294002 augmented cell death (Fig. 2A). These results indicate that signalling pathways distinct from PI3K are effective in counteracting dexa-induced cell death when PI3K is inhibited. Indeed, the inhibition of the MAPK pathway by PD98059, 10 μmol/l, reversed the protective effect of IGF-1 on dexa-induced apoptosis, regardless whether LY294002 was present or not (Fig. 2C and 2D). These observations indicate that MAPK pathways are able to exert anti-apoptotic effects in INS-1 cells, especially when the PI3K pathway is concomitantly inhibited.

Dexa increases IRS-2 protein but inhibits phosphorylation of IRS-2

Next we examined whether long term treatment with dexa changed the expression of components of the IGF-1/insulin signalling cascades. The quantification of cytosolic proteins on Western blots revealed that dexa

significantly increased IRS-2 protein by 44 ± 15 (n = 5, Fig. 2A). PI3K, PKB and ERK protein concentrations remained unchanged. To analyse whether dexa affects IRS-2 stimulation, tyrosine phosphorylation of IRS-2 was examined after immunoprecipitation of IRS-2. IGF-1 mediated stimulation of IRS-2 phosphorylation was significantly reduced in dexa treated INS-1 cells. This observation indicates that dexa blunts the stimulation of the IGF-1 signalling pathways by inhibition of IRS-2 phosphorylation despite increasing IRS-2 protein.

PI3K inhibition leads to increased ERK phosphorylation in dexa-treated INS-1 cells

Since IRS-2 stimulates PI3K and the phosphorylation of PKB/Akt further experiments were performed to examine the effects of dexa and kinase inhibitors, LY294002 (10 μmol/l) and PD98059 (10 μmol/l), on PKB and ERK phosphorylation (Fig. 4 and Fig. 5). IGF-1 (50 ng/ml) transiently stimulated PKB and ERK

Fig. 3. Dexamethasone increased IRS-2 protein and reduced IRS-2 tyrosine phosphorylation stimulated by IGF-1. INS-1 cells were treated with dexamethasone for 1 d and stimulated with IGF-1 for 5 min as indicated. Shown is a representative Western blot for IRS-2, PI3K, PKB and ERK (A) and PY-IRS-2 and reblot for IRS-2 after immunoprecipitation with IRS-2 (B). Data are presented as means $\pm$ S.E. The number of independent experiments is given in each column. * denotes significance to control, # to dexamethasone.

Fig. 4. Effect of LY294002 and PD98059 on IGF-1 stimulated PKB phosphorylation. Cells were treated with dexamethasone (100 nmol/l) for 1 d, with kinase inhibitors (each 10 μ mol/l) for 1 h before the addition of IGF-1 (50 ng/ml) for 5 min as indicated (A-D). Shown are representative Western blots and means $\pm$ S.E. for the indicated number (n) of independent experiments. (A and B) P-PKB of control INS-1 cells; (C and D) P-PKB and PKB of dexamethasone-treated cells; (E and F) P-PKB and PKB after 1 d treatment with dexamethasone, inhibitors and IGF-1 as indicated. * denotes significance to control, # to dexamethasone, § to dexamethasone + IGF-1.

phosphorylations in control cells (Fig. 4A and B, Fig. 5A and B). As expected, PI3K inhibition by LY294002 (10 μ mol/l) decreased PKB but not ERK phosphorylation, while MAPK inhibition by PD98059 (10 μ mol/l) reduced ERK but not PKB phosphorylation. Dexamethasone treatment for 1 d reduced PKB phosphorylation, but did not affect the stimulation with IGF-1, 50 ng/ml for 5 min (Fig. 4C and D) and 1 d (Fig. 4E and F). The effect of IGF-1 on PKB phosphorylation in dexamethasone-treated cells was inhibited by LY294002 but not by PD98059. In contrast, ERK

phosphorylation was not changed by dexamethasone treatment of the cells for 1 d (Fig. 5C and D). Furthermore, IGF-1, applied for 5 min, was unable to stimulate ERK phosphorylation (Fig. 5C and D). The addition of LY294002 restored stimulation of ERK phosphorylation by IGF-1. This effect was transient and not observed after 1 d treatment with IGF-1 (Fig. 5E and F). These observations support the idea that increased ERK phosphorylation exerts anti-apoptotic effects in dexamethasone-treated cells when PI3K is inhibited.

Fig. 5. Effect of LY294002 and PD98059 on IGF-1 stimulated ERK phosphorylation. Cells were treated with dexamethasone (100 nmol/l) for 1 d, with kinase inhibitors (each 10 μ mol/l) for 1 h before the addition of IGF-1 (50 ng/ml) for 5 min as indicated (A-D). Shown are representative Western blots and means $\pm$ S.E. for the indicated number (n) of independent experiments. (A and B) P-ERK of control INS-1 cells; (C and D) P-ERK and ERK of dexamethasone-treated cells; (E and F) P-ERK and ERK after 1 d treatment with dexamethasone, inhibitors and IGF-1 as indicated. * denotes significance to control, § to dexamethasone + IGF-1, & to dexamethasone + IGF-1 + LY294002.

Discussion

This study demonstrates that dexamethasone-induced apoptotic cell death is effectively inhibited by activation of MAPK pathways in insulin-secreting INS-1 cells when PI3K is inhibited. Dexamethasone itself interferes with IGF-1/insulin receptor signalling pathways. Dexamethasone increased IRS-2 protein but inhibited the phosphorylation of IRS-2 and PKB/Akt (Fig. 6). Similar observations have been made in other systems. In adipocytes, dexamethasone also increased IRS-2 protein but decreased IRS-1 [38]. The reduction of IRS-1 and insulin-stimulated phosphorylation of PKB was associated with development of insulin resistance during glucocorticoid excess in adipocytes [38, 39]. Similarly, in rat liver, dexamethasone treatment *in vivo* decreased insulin-dependent IRS-1 and IRS-2 phosphorylation, reduced PI3K signalling and PKB/Akt phosphorylation [40]. In insulin-secreting cells, we were unable to detect an effect of dexamethasone treatment on IRS-1 protein and IRS-1 tyrosine phosphorylation (data not shown). The reduced phosphorylation of PKB may account for the reduced phosphorylation of the pro-apoptotic protein BAD that we observed previously and the activation of apoptotic cell death by the glucocorticoid [33].

Fig. 6. Effects of glucocorticoids on IGF-1 dependent signalling pathways. Glucocorticoids inhibit phosphorylation of IRS-2, PKB, Bad, induce apoptosis and reduce proliferation. IGF-1 counteracts glucocorticoid-dependent apoptosis most efficiently through activation of MAPK pathways.

IGF-1 antagonized dexamethasone-induced inhibition of PKB phosphorylation, an observation that may explain the protective effect of IGF-1 on dexamethasone-induced apoptosis. This conclusion confirms studies from other groups showing that PKB exerts anti-apoptotic effects in insulin secreting cells [13, 15, 41, 42]. Paradoxically, IGF-1 dependent protection against apoptosis was not antagonized but enforced by PI3K inhibition. Thus, although dephosphorylation of PKB/Akt accompanies dexamethasone-mediated apoptosis, increased activation of MAPK pathways by IGF-1 is able to prevent cell death despite PKB inhibition. The anti-apoptotic effect of IGF-1 in the presence of PI3K inhibition suggests that PKB activity is not essential for the survival of insulin secreting cells during glucocorticoid excess.

Interestingly, it has been shown that glucocorticoids inhibit the development of the endocrine pancreas and suppress insulin gene expression through reduced PDX-1 activation [43-47]. Furthermore, dexamethasone increases apoptotic death in rat islets in which proliferation was stimulated by prolactin [48]. This discloses the possibility of *in vivo* regulation of β -cell mass by glucocorticoids during embryonic development and late pregnancy. Thus, these mechanisms might not only be important for the prevention of steroid diabetes mellitus during treatment with immunosuppressive drugs but also for physiological regulation of insulin secretion in pregnancy and other metabolic situations of increased demand of insulin.

In conclusion, this study demonstrates that phosphorylation of PKB is not essential for the protection against

glucocorticoid-induced cell death in insulin secreting cells.

Abbreviations

Ac-DEVD-pNA (p-nitroanilide); PKB/Akt (protein kinase B); dexamethasone; CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate); DAPI (4',6-diamidino-2-phenylindole); DTT (dithiothreitol); EDTA (Ethylenediamine tetraacetic acid); FCS (foetal calf serum); HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid); HRP (horse radish peroxidase); IGF-1 (insulin like growth factor 1); IRS-1, IRS-2 (insulin receptor substrate-1 (-2)); LY294002 (PI3K inhibitor); MAPK (mitogen activated protein kinase); NaPP (sodium pyrophosphate) PD98059 (MAPK inhibitor); PI3K (phosphatidylinositol 3 kinase); PMSF (phenylmethylsulfonylfluoride); SDS (sodium dodecyl sulfate); TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP nick end labelling).

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References

- Okada T, Liew CW, Hu J, Hinault C, Michael MD, Krtzfeldt J, Yin C, Holzenberger M, Stoffel M, Kulkarni RN: Insulin receptors in beta-cells are critical for islet compensatory growth response to insulin resistance. *Proc Natl Acad Sci U S A* 2007;104:8977-82.
- Burks DJ, White MF: IRS proteins and beta-cell function. *Diabetes* 2001;50 Suppl 1:S140-S145.
- Ueki K, Okada T, Hu J, Liew CW, Assmann A, Dahlgren GM, Peters JL, Shackman JG, Zhang M, Artner I, Satin LS, Stein R, Holzenberger M, Kennedy RT, Kahn CR, Kulkarni RN: Total insulin and IGF-I resistance in pancreatic beta cells causes overt diabetes. *Nat Genet* 2006;38:583-8.
- Lingohr MK, Buettner R, Rhodes CJ: Pancreatic beta-cell growth and survival: a role in obesity-linked type 2 diabetes? *Trends Mol Med* 2002;8:375-84.
- van Haeften TW, Twickler TB: Insulin-like growth factors and pancreas beta cells. *Eur J Clin Invest* 2004;34:249-55.
- Cantley J, Choudhury AI, Asare-Anane H, Selman C, Lingard S, Heffron H, Herrera P, Persaud SJ, Withers DJ: Pancreatic deletion of insulin receptor substrate 2 reduces beta and alpha cell mass and impairs glucose homeostasis in mice. *Diabetologia* 2007;50:1248-56.
- Hennige AM, Burks DJ, Ozcan U, Kulkarni RN, Ye J, Park S, Schubert M, Fisher TL, Dow MA, Leshan R, Zakaria M, Mossa-Basha M, White MF: Upregulation of insulin receptor substrate-2 in pancreatic beta cells prevents diabetes. *J Clin Invest* 2003;112:1521-32.
- Kulkarni RN, Winnay JN, Daniels M, Bruning JC, Flier SN, Hanahan D, Kahn CR: Altered function of insulin receptor substrate-1-deficient mouse islets and cultured beta-cell lines. *J Clin Invest* 1999;104:R69-R75.
- Hennige AM, Ozcan U, Okada T, Jhala US, Schubert M, White MF, Kulkarni RN: Alterations in growth and apoptosis of IRS-1 deficient beta-cells. *Am J Physiol Endocrinol Metab* 2005;289:E337-E346.
- Rhodes CJ, White MF: Molecular insights into insulin action and secretion. *Eur J Clin Invest* 2002;32 Suppl 3:3-13.
- Yoshida K, Murao K, Imachi H, Cao WM, Yu X, Li J, Ahmed RA, Kitanaka N, Wong NC, Unterman TG, Magnuson MA, Ishida: Pancreatic glucokinase is activated by insulin-like growth factor-I. *Endocrinol* 2007;148:2904-13.

- 12 Farese RV: Insulin-sensitive phospholipid signaling systems and glucose transport. Update II. *Exp Biol Med* (Maywood) 2001;226:283-95.
- 13 Tuttle RL, Gill NS, Pugh W, Lee JP, Koerberlein B, Furth EE, Polonsky KS, Naji A, Birnbaum MJ: Regulation of pancreatic beta-cell growth and survival by the serine/threonine protein kinase Akt1/PKBalpha. *Nat Med* 2001;7:1133-7.
- 14 Avruch J: Insulin signal transduction through protein kinase cascades. *Mol Cell Biochem* 1998;182:31-48.
- 15 Dickson LM, Rhodes CJ: Pancreatic beta-cell growth and survival in the onset of type 2 diabetes: a role for protein kinase B in the Akt? *Am J Physiol Endocrinol Metab* 2004;287:E192-E198.
- 16 Meloche S, Pouyssegur J: The ERK1/2 mitogen-activated protein kinase pathway as a master regulator of the G1- to S-phase transition. *Oncogene* 2007;26:3227-39.
- 17 Kajimoto Y, Kaneto: Role of oxidative stress in pancreatic beta-cell dysfunction. *Ann N Y Acad Sci* 2004;1011:168-76.
- 18 Kaiser N, Leibowitz G, Neshet R: Glucotoxicity and beta-cell failure in type 2 diabetes mellitus. *J Pediatr Endocrinol Metab* 2003;16:5-22.
- 19 Rhodes CJ: Type 2 diabetes-a matter of beta-cell life and death? *Science* 2005;307:380-4.
- 20 Lingohr MK, Dickson LM, McCuaig JF, Hugl SR, Twardzik DR, Rhodes CJ: Activation of IRS-2-mediated signal transduction by IGF-1, but not TGF-alpha or EGF, augments pancreatic beta-cell proliferation. *Diabetes* 2002;51:966-76.
- 21 Briaud I, Lingohr MK, Dickson LM, Wrede CE, Rhodes CJ: Differential activation mechanisms of Erk-1/2 and p70(S6K) by glucose in pancreatic beta-cells. *Diabetes* 2003;52:974-83.
- 22 Lawrence MC, McGlynn K, Park BH, Cobb MH: ERK1/2-dependent activation of transcription factors required for acute and chronic effects of glucose on the insulin gene promoter. *J Biol Chem* 2005;280:26751-9.
- 23 Kautzky-Willer A, Thomaseth K, Clodi M, Ludvik B, Waldhausl W, Prager R, Pacini G: Beta-cell activity and hepatic insulin extraction following dexamethasone administration in healthy subjects. *Metabolism* 1996;45:486-91.
- 24 McMahon M, Gerich J, Rizza R: Effects of glucocorticoids on carbohydrate metabolism. *Diabetes Metab Rev* 1988;4:17-30.
- 25 Wajngot A, Giacca A, Grill V, Vranic M, Efendic S: The diabetogenic effects of glucocorticoids are more pronounced in low- than in high-insulin responders. *Proc Natl Acad Sci U S A* 1992;89:6035-9.
- 26 Henriksen JE, Alford F, Ward GM, Beck-Nielsen H: Risk and mechanism of dexamethasone-induced deterioration of glucose tolerance in non-diabetic first-degree relatives of NIDDM patients. *Diabetologia* 1997;40:1439-48.
- 27 Hamamdžić D, Džuric E, Sherlock JD, Lanier S: Regulation of alpha 2-adrenergic receptor expression and signaling in pancreatic beta-cells. *Am J Physiol* 1995;269:E162-E171.
- 28 Lambilliotte C, Gilon P, Henquin JC: Direct glucocorticoid inhibition of insulin secretion. An in vitro study of dexamethasone effects in mouse islets. *J Clin Invest* 1997;99:414-23.
- 29 Ullrich S, Berchtold S, Ranta F, Seeböhm G, Henke G, Lupescu A, Mack AF, Chao CM, Su J, Nitschke R, Alexander D, Friedrich B, Wulff P, Kuhl D, Lang F: Serum- and Glucocorticoid-Inducible Kinase 1 (SGK1) Mediates Glucocorticoid-Induced Inhibition of Insulin Secretion. *Diabetes* 2005;54:1090-9.
- 30 Jeong IK, Oh SH, Kim BJ, Chung JH, Min YK, Lee MS, Lee MK, Kim K: The effects of dexamethasone on insulin release and biosynthesis are dependent on the dose and duration of treatment. *Diabetes Res Clin Pract* 2001;51:163-71.
- 31 Gremlich S, Roduit R, Thorens B: Dexamethasone induces posttranslational degradation of GLUT2 and inhibition of insulin secretion in isolated pancreatic beta cells. Comparison with the effects of fatty acids. *J Biol Chem* 1997;272:3216-22.
- 32 Ling ZC, Khan A, Delaunay F, Davani B, Ostenson CG, Gustafsson JA, Okret S, Landau BR, Efendic S: Increased glucocorticoid sensitivity in islet beta-cells: effects on glucose 6-phosphatase, glucose cycling and insulin release. *Diabetologia* 1998;41:634-9.
- 33 Ranta F, Avram D, Berchtold S, Düfer M, Drews G, Lang F, Ullrich S: Dexamethasone induces cell death in insulin secreting cells, an effect reversed by Exendin-4. *Diabetes* 2006;55:1380-90.
- 34 Lizcano JM, Morrice N, Cohen P: Regulation of BAD by cAMP-dependent protein kinase is mediated via phosphorylation of a novel site, Ser155. *Biochem J* 2000;349:547-57.
- 35 Datta SR, Brunet A, Greenberg ME: Cellular survival: a play in three Akts. *Genes Dev* 1999;13:2905-27.
- 36 Alessi DR, Cohen P: Mechanism of activation and function of protein kinase B. *Curr Opin Genet Dev* 1998;8:55-62.
- 37 Chang F, Steelman LS, Shelton JG, Lee JT, Navolanic PM, Blalock WL, Franklin R, McCubrey JA: Regulation of cell cycle progression and apoptosis by the Ras/Raf/MEK/ERK pathway (Review). *Int J Oncol* 2003;22:469-80.
- 38 Buren J, Liu HX, Jensen J, Eriksson JW: Dexamethasone impairs insulin signaling and glucose transport by depletion of insulin receptor substrate-1, phosphatidylinositol 3-kinase and protein kinase B in primary cultured rat adipocytes. *Eur J Endocrinol* 2002;146:419-29.
- 39 Sakoda H, Ogihara T, Anai M, Funaki M, Inukai K, Katagiri H, Fukushima Y, Onishi Y, Ono H, Fujishiro M, Kikuchi M, Oka Y, Asano T: Dexamethasone-induced insulin resistance in 3T3-L1 adipocytes is due to inhibition of glucose transport rather than insulin signal transduction. *Diabetes* 2000;49:1700-8.
- 40 Rojas FA, Hirata AE, Saad MJ: Regulation of insulin receptor substrate-2 tyrosine phosphorylation in animal models of insulin resistance. *Endocrine* 2003;21:115-22.
- 41 Trumper K, Trumper A, Trusheim H, Arnold R, Goke B, Horsch D: Integrative mitogenic role of protein kinase B/Akt in beta-cells. *Ann N Y Acad Sci* 2000;921:242-50.
- 42 Wrede CE, Dickson LM, Lingohr MK, Briaud I, Rhodes CJ: Protein kinase B/Akt prevents fatty acid-induced apoptosis in pancreatic beta-cells (INS-1). *J Biol Chem* 2002;277:49676-84.
- 43 Shinozuka Y, Okada M, Oki T, Sagane K, Mizui Y, Tanaka I, Katayama K, Murakami-Murofushi K: Altered expression of HES-1, BETA2/NeuroD, and PDX-1 is involved in impaired insulin synthesis induced by glucocorticoids in HIT-T15 cells. *Biochem Biophys Res Commun* 2001;287:229-35.
- 44 Shen CN, Seckl JR, Slack JM, Tosh D: Glucocorticoids suppress beta-cell development and induce hepatic metaplasia in embryonic pancreas. *Biochem J* 2003;375:41-50.
- 45 Gesina E, Tronche F, Herrera P, Duchene B, Tales W, Czernichow P, Breant B: Dissecting the role of glucocorticoids on pancreas development. *Diabetes* 2004;53:2322-9.
- 46 Breant B, Gesina E, Blondeau B: Nutrition, glucocorticoids and pancreas development. *Horm Res* 2006;65 Suppl 3:98-104.
- 47 Gesina E, Blondeau B, Milet A, Le N, I, Duchene B, Czernichow P, Scharfmann R, Tronche F, Breant B: Glucocorticoid signalling affects pancreatic development through both direct and indirect effects. *Diabetologia* 2006;49:2939-47.
- 48 Weinhaus AJ, Bhargava NV, Brelje TC, Sorenson RL: Dexamethasone counteracts the effect of prolactin on islet function: implications for islet regulation in late pregnancy. *Endocrinol* 2000;141:1384-93.