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Glucose and leptin induce apoptosis in human β-cells and impair glucose-stimulated insulin secretion through activation of c-Jun N-terminal kinases

Kathrin Maedler,*,[1] Fabienne T. Schulthess,* Christelle Bielman,[†] Thierry Berney,[‡] Christophe Bonny,[†] Marc Prentki,[§] Marc Y. Donath,[‖] and Raphael Roduit[†,¶]

*Larry L. Hillblom Islet Research Center, University of California, Los Angeles, California, USA; [†]Medical Genetic Service, Centre Hospitalier Universitarie Vaudois Lausanne, Lausanne, France; [‡]Department of Surgery, University Medical Center, Geneva, Switzerland; [§]Molecular Nutrition Unit and the Montreal Diabetes Research Center, Centre de Recherche du CHUM, and Department of Nutrition and Biochemistry, Université de Montréal, Montréal, Quebec, Canada; [‖]Division of Endocrinology and Diabetes, University Hospital Zurich, University of Zurich, Switzerland; and [¶]Institut de Recherche en Ophtalmologie, Sion, Switzerland

ABSTRACT **c-Jun N-terminal kinases (SAPK/JNKs) are activated by inflammatory cytokines, and JNK signaling is involved in insulin resistance and β-cell secretory function and survival. Chronic high glucose concentrations and leptin induce interleukin-1β (IL-1β) secretion from pancreatic islets, an event that is possibly causal in promoting β-cell dysfunction and death. The present study provides evidence that chronically elevated concentrations of leptin and glucose induce β-cell apoptosis through activation of the JNK pathway in human islets and in insulinoma (INS 832/13) cells. JNK inhibition by the dominant inhibitor JNK-binding domain of IB1/JIP-1 (JNKi) reduced JNK activity and apoptosis induced by leptin and glucose. Exposure of human islets to leptin and high glucose concentrations leads to a decrease of glucose-induced insulin secretion, which was partly restored by JNKi. We detected an interplay between the JNK cascade and the caspase 1/IL-1β-converting enzyme in human islets. The caspase 1 gene, which contains a potential activating protein-1 binding site, was up-regulated in pancreatic sections and in isolated islets from type 2 diabetic patients. Similarly, cultured human islets exposed to high glucose- and leptin-induced caspase 1 and JNK inhibition prevented this up-regulation. Therefore, JNK inhibition may protect β-cells from the deleterious effects of high glucose and leptin in diabetes.—Maedler, K., Schulthess, F. T., Bielman, C., Berney, T., Bonny, C., Prentki, M., Donath, M. Y., Roduit, R. Glucose and leptin induce apoptosis in human β-cells and impair glucose-stimulated insulin secretion through activation of c-Jun N-terminal kinases. *FASEB J.* 22, 1905–1913 (2008)**

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TYPE 2 DIABETES MELLITUS (T2DM) occurs in insulin-resistant individuals because of β-cell insulin secretory dysfunction and a decrease in β-cell mass (1), a major underlying mechanism thereof being β-cell apoptosis (2, 3). For many years now, evidence has shown that chronic hyperglycemia and hyperlipidemia, endoplasmic reticulum and oxidative stress, cytokines, and autoimmunity may trigger the increase in β-cell apoptosis that occurs during the pathogenesis of type 2 diabetes (4, 5).

In T2DM, hyperglycemic episodes lead to reduction of insulin secretion as well as insulin stores and to further β-cell destruction in the so-called glucotoxicity process (6). Various mechanisms of glucose-induced β-cell dysfunction have been studied, including formation of advanced glycation end products (7), endoplasmic reticulum stress (8), reactive oxygen species (6), direct impairment of insulin gene transcription and proinsulin biosynthesis (9, 10), and reduced binding activity of pancreatic duodenal homeobox 1 (11). Recently, we showed up-regulation of the Fas receptor by elevated glucose *per se*, leading to cleavage of downstream caspases and β-cell apoptosis in isolated human islets (12). We found that interleukin-1β (IL-1β) was expressed and secreted by the β-cell itself after chronic glucose exposure, initiating its self-destruction (13). Also, in three animal models of T2DM, the *Psammomys obesus* (13), the GK rat (14), and the human islet amyloid polypeptide transgenic rat (15, P. C. Butler, personnel communication, May 1, 2005), pancreatic β-cell expression of IL-1β in direct association with the onset of hyperglycemia has been observed.

Leptin, mainly produced and secreted by the adipose tissue, is an important factor regulating body weight and glucose homeostasis to the amount of body fat (16). In "leptin resistance," high levels of leptin may

[1] Correspondence: Centre for Biomolecular Interactions Bremen, University of Bremen, NW2, Box 33 04 40, D-28334 Bremen, Germany. E-mail: kmaedler@uni-bremen.de

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contribute to the dysregulation of the adipo-insular axis that leads to hyperinsulinemia and promotes T2DM (16). *In vitro*, chronic exposure of human islets to leptin decreases β-cell production of interleukin-1 receptor antagonist (IL-1Ra) and induces IL-1β release from islet preparation, leading to impaired β-cell function and apoptosis (17). Long-term treatment of β-cells with leptin also decreases insulin biosynthesis (18) and secretion (19–21).

Activation of members of the mitogen-activated protein kinase family has been described in several apoptotic processes including those induced by reactive oxygen species and IL-1β *via* Fas (22–24). Although leptin- and glucose-induced activation of p38 and phosphorylated extracellular signal-regulated kinase have been studied extensively in different cellular systems, little is known about the role of the c-Jun N-terminal kinase (JNK) pathway in glucose and leptin signaling. It has been shown that the JNK pathway is activated by inflammatory cytokines and free fatty acids in several tissues under conditions of diabetes and obesity (25). Inhibition of the JNK pathway in the *db/db* mouse model leads to improved insulin resistance and glucose tolerance (26). Absence of JNK1 in the obese *ob/ob* mouse enhances the signaling capacity of the insulin receptor (27). Also, JNK activation by loss-of function mutations in the JNK scaffold protein (JIP1/IB1) is a mediator of cytokine-induced β-cell apoptosis (28). Finally, *IB1* has been proposed as a candidate gene implicated in T2DM (29).

In view of this knowledge, the aim of the present study was to test the hypothesis that the JNK pathway is implicated in the action of chronically elevated glucose and leptin to promote β-cell dysfunction and apoptosis.

MATERIALS AND METHODS

Islet isolation and cell culture

Islets were isolated from pancreases of five organ donors at the Department of Surgery, University of Geneva Medical Center, as described (30–32). The islet purity was >95%, as judged by dithizone staining. In some isolations, when this degree of purity was not primarily achieved by routine isolation, islets were handpicked. The donors, aged 50–70 yr, were heart-beating cadaver organ donors, and none had a previous history of diabetes or metabolic disorders. For long-term *in vitro* studies, the islets were cultured on extracellular matrix-coated plates derived from bovine corneal endothelial cells (Novamed Ltd., Jerusalem, Israel), allowing the cells to attach to the dishes and spread, to preserve their functional integrity. Islets were cultured in CMRL 1066 medium containing 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal calf serum (Invitrogen, Paisley, UK), hereafter referred to as culture medium. Two days after plating, when most islets were attached and began to flatten, the medium was changed to culture medium containing 5.5, 11.1, or 33.3 mM glucose. In some experiments, islets were additionally cultured with 10 nM recombinant human leptin (PeproTech, London, UK) or 1 μM dominant inhibitor JNK-binding domain of IB1/JIP-1 (JNKi) and 1 μM Tat-peptide (control), prepared as described before (33). INS 832/13 cells (34) were cultured in complete RPMI-Glutamax medium containing 11 mM glucose supplemented with 10% fetal calf serum, 10 mM Hepes (pH 7.4), 1 mM sodium pyruvate, and 50 μM β-mercaptoethanol. INS 832/13 cells were incubated for 18 h in complete culture medium at 5.5 or 20 mM glucose in the absence or presence of 10 nM recombinant human leptin, 1 μM JNKi, and 1 μM Tat-peptide (control).

Western blot analysis and protein kinase assays

Islets were cultured in culture medium in nonadherent plastic dishes. One day after isolation, medium was changed, and groups of 200 islets were incubated for 18 h in culture medium conditions as described above.

At the end of the incubations, islets were washed in PBS, suspended in 100 μl of lysis buffer containing 20 mM Tris acetate, 0.27 M sucrose, 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 1% Triton X-100, 5 mM sodium pyrophosphate, 10 mM β-glycerophosphate, 1 mM dithiothreitol (DTT), 1 mM Na_3VO_4, 1 mM 4-nitrophenol phosphate, 1 mM benzamidine, and 4 μg/ml leupeptin and were lysed for 30 min on ice. The detergent-insoluble material was pelleted by centrifugation at 15,000 rpm for 5 min at 4°C. The supernatants containing whole cell lysates were used for Western blotting or whole cell lysate kinase assays.

For Western blot analysis, equivalent amounts of protein from each treatment group were run on 15% sodium dodecyl sulfate (SDS) -polyacrylamide gels. Proteins were electrically transferred to nitrocellulose filters and incubated with rabbit anti-caspase 1 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) or rabbit anti-actin (Cell Signaling Technology Inc., Beverly, MA, USA) antibodies followed by incubation with horseradish-peroxidase-linked anti-rabbit IgG peroxidase-conjugated antibodies (Santa Cruz Biotechnology Inc.). Immune complexes were detected by chemiluminescence using LumiGLO (Cell Signaling Technology Inc.).

For protein kinase assays, 1 μg of glutathione *S*-transferase (GST) -cJun (amino acids 1–89) coupled to glutathione-agarose beads was added to cellular extracts and incubated for 3 h at 4°C. The beads were washed 3× with washing buffer (lysis buffer with 0.1% Triton X-100 instead of 1% Triton X-100) and 2× with kinase buffer (20 mM HEPES, pH 7.5; 20 mM glycerophosphate; 10 mM $MgCl_2$; 1 mM DTT; and 50 μM Na_3VO_4). Finally, they were resuspended in kinase buffer supplemented with 5 μCi of [γ-^{33}P]ATP. After incubation at 30°C for 30 min, reaction products were separated by SDS-polyacrylamide gel electrophoresis on a denaturing 10% polyacrylamide gel. The gels were stained with Coomassie Blue to check for equal loading of the samples, dried, and subsequently exposed to X-ray films. Density of the bands was analyzed using Labworks 4.5 software (BioImaging Systems, Upland, CA, USA).

β-Cell apoptosis

Free 3-hydroxy strand breaks resulting from DNA degradation were detected by the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) technique according to the manufacturer's instructions (In Situ Cell Death Detection Kit, AP; Boehringer Mannheim, Mannheim, Germany) as described before (13). To detect the β-cells, islets were incubated with guinea pig anti-insulin antibody (DakoCytomation A/S, Copenhagen, Denmark), followed by detection using the streptavidin-biotin-peroxidase complex (Zymed Laboratories, Burlingame, CA, USA). The samples were immediately evaluated by fluorescence microscopy for positively stained apoptotic nuclei. To determine apoptosis in

INS 832/13 cells, cells were double-stained with the fluorescent DNA-staining dyes Hoechst 33342 and propidium iodide. Stained nuclei were immediately visualized by fluorescence microscopy (Axiovert 25; Zeiss, Oberkochen, Germany). For each experiment a minimum of 1000 cells were counted using an inverted fluorescence microscope. Cells were defined as apoptotic when they exhibited a condensed nuclear chromatin or a fragmented nuclear membrane when visualized with Hoechst 33342.

Caspase 1 immunostaining of islets and pancreases

Islets were incubated for 18 h in culture medium containing 5.5 or 33.3 mM glucose without or with 10 nM recombinant human leptin, 1 μM JNKi, or 1 μM Tat-peptide. At the end of the incubations, islets were washed in PBS, fixed in Bouin solution for 15 min, and resuspended in 40 μl of 2% melted agarose in PBS (40°C), followed by rapid centrifugation and paraffin embedding. Pancreases from routine necropsies were immersion-fixed in formalin, followed by paraffin embedding. Sections were deparaffinized and rehydrated, endogenous peroxidase was blocked by submersion in 0.3% H_2O_2 for 15 min, and sections were incubated in methanol for 4 min. Sections were incubated with a 1:50 dilution of anti-caspase 1 (Santa Cruz Biotechnology Inc.) antibody, detected by donkey anti-rabbit Cy3-conjugated antibody (Jackson ImmunoResearch Laboratories, West Grove, PA, USA), and double-stained for insulin with pig anti-insulin antibody (DakoCytomation A/S) and fluorescein isothiocyanate-conjugated donkey anti-guinea pig antibody (Jackson ImmunoResearch).

NF-κB activation

Human islets were cultured as described above and washed with PBS. Activation of the NF-κB complex was quantified with an ELISA-based kit (Trans-AM NF-κB; Active Motif LLC, Carlsbad, CA, USA) using attached oligonucleotides corresponding to an NF-κB consensus binding site and detected by an anti-p65 subunit antibody according to the manufacturer's instructions. INS 832/13 cells were cultured in RPMI-Glutamax medium before transfection with Lipofectamine 2000, with a pNFkB-Luc reporter vector encoding the firefly luciferase gene as the reporter gene and with an internal control pRL-SV40 vector encoding the *Renilla* luciferase 24 h after the transfection. A dual luciferase assay (Promega Corp., Madison, WI, USA) was then performed to measure NF-κB activity accordingly to the manufacturer's instructions.

RNA extraction and quantitative reverse transcription (RT) -polymerase chain reaction (PCR)

Total RNA was extracted from the cultured islets by using an RNeasy Mini Kit (Qiagen, Basel, Switzerland), and RT-PCR was performed using the SuperScript Double-Stranded cDNA Synthesis Kit according to the manufacturer's instructions (Life Technologies, Inc., Gaithersburg, MD, USA). A quantitative PCR system was used to perform RT-PCR with a commercial kit (Light Cycler DNA Master SYBR Green I; Roche Diagnostics, Basel, Switzerland). Primers used were 5′-AGAGTCGCGCTGTAAGAAGC-3′ and 5′-TGGTCTTGTCACTTGGCATC-3′ (α-tubulin), 5′-TACGGGTCCTGGCATCTTGT-3′ and 5′-CCATTTGTGTTGGGTCCAGC-3′ (cyclophilin), 5′-TTCTGTGGAAAAGAGGCAGGC-3′ and 5′-GCTCCGTTTTAGCTCGTTCCT-3′ (c-Myc), 5′-CCCAGTCTGCATAGAAGG-3′ and 5′TGATACACTCCAAGCGGAGAC3′ (c-Fos), and 5′-AAATCTCACTGCTTCGGACAT-3′ and 5′-GGGCAGTTCTTGGTATTCAAC-3′ (caspase 1).

Insulin release and content

To determine acute insulin release in response to glucose stimulation, islets were washed in Krebs-Ringer bicarbonate (KRB) buffer supplemented with 0.5% BSA (Sigma-Aldrich Corp., St. Louis, MO, USA) containing 2.8 mM glucose (pH 7.4) and preincubated for 30 min at 37°C in air in the same buffer. KRB was then discarded and replaced with fresh buffer containing 2.8 mM glucose, and islets were incubated for 1 h to assess basal secretion, followed by additional 1-h incubation in KRB containing 16.7 mM glucose. Incubates were collected and frozen for insulin assays. Islets were washed with PBS and extracted with 0.18 N HCl in 70% ethanol for 24 h at 4°C. Acid-ethanol extracts were collected for determination of insulin content. Insulin was determined by a human insulin RIA kit (CIS Bio International, Gif-Sur-Yvette, France).

Evaluation and statistical analysis

Samples were evaluated in a randomized manner by a single investigator (K.M.) who was blinded to the treatment conditions. Care was taken to score islets of similar size. Some larger islets did not completely spread and were several cells thick. Such larger islets were excluded because a monolayer is a prerequisite for single cell evaluation. Saisam software (Microvision Instruments, Evry, France) was used to measure the areas. Data were analyzed by Student's *t* test or by ANOVA with a Bonferroni correction for multiple group comparisons.

RESULTS

JNK inhibitor protects from glucose- and leptin-induced β-cell apoptosis and dysfunction

Human islets were cultured on extracellular matrix-coated plates for 4 days, and the β-cell line INS 832/13 was cultured for 18 h in the presence of increasing glucose concentrations (5.5, 11.1, and 33.3 mM) or 5.5 mM glucose with 10 nM leptin with or without 1 μM JNKi. Glucose dose dependently induced β-cell apoptosis in human islets (**Fig. 1*A, C***). In contrast, in INS 832/13 cells, baseline apoptosis was minimal at 11.1 mM glucose and increased at 5.5 and 33.3 mM (Fig. 1*B*). In both models, leptin induced β-cell apoptosis (2.6-fold in human islets and 3.3-fold in INS832/13 cells, compared with untreated islets at 5.5 mM glucose, $P<0.001$) (Fig. 1*A, B*). Addition of 1 μM JNKi to the culture medium for 1 h before glucose or leptin exposure through the whole culture period protected from glucose- and leptin-induced β-cell apoptosis (Fig. 1*A–C*). A radioactive kinase assay showed that both leptin and high glucose induced JNK activity in human islets, which was prevented when JNKi was present 1 h before addition of either glucose or leptin (Fig. 1*D*).

Induction of JNK activity was increased either by leptin (204.2±24.5%) or by glucose (170.7±15.7%) compared with the control 5.5 mM glucose (100%). Addition of JNKi peptide before exposure to leptin or high glucose decreased the induced JNK activities to 123.7 ± 12.5 and 111.5 ± 9.5% for leptin and high glucose, respectively.

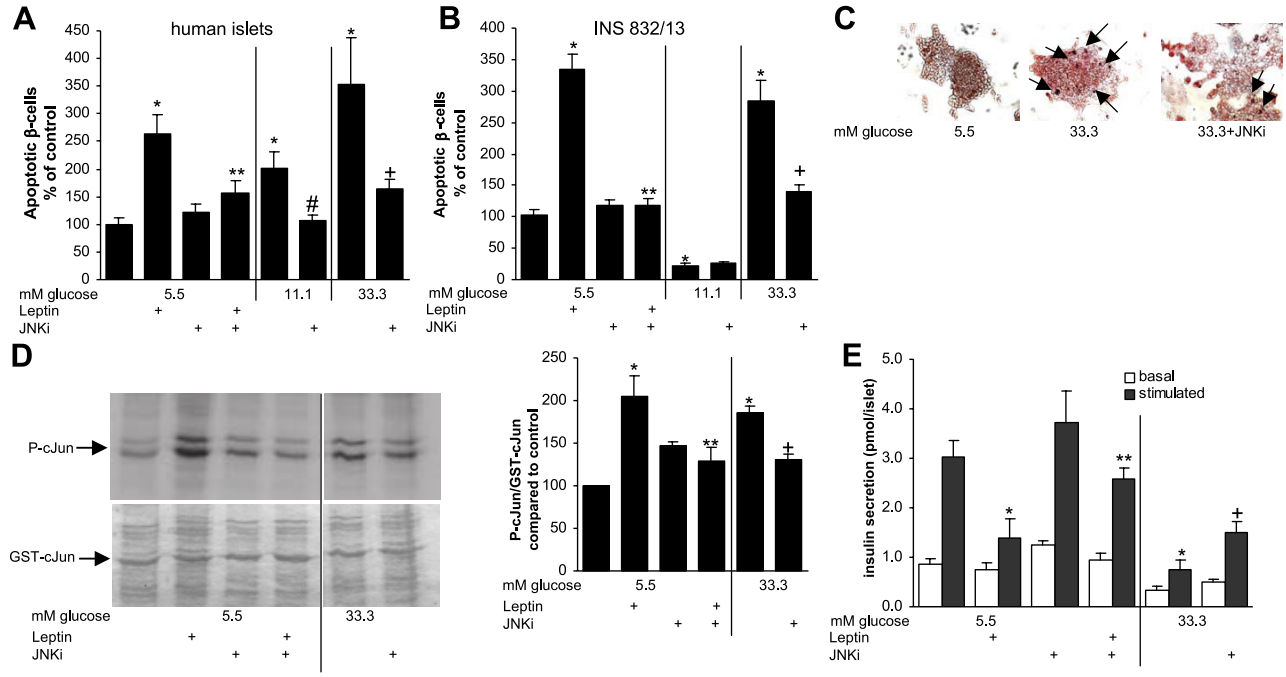

Figure 1. Glucose and leptin induce β-cell apoptosis and impaired function through activation of c-Jun N-terminal kinases. Human islets were cultured on extracellular matrix-coated dishes for 4 days (*A, C*) and INS 832/13 cells were cultured for 18 h (*B*) at 5.5, 11.1, or 33.3 mM glucose or 5.5 mM glucose plus 10 nM recombinant human leptin with or without 1 μM JNKi. *A*) Percentage of TUNEL-positive β-cells normalized to control incubations at 5.5 mM glucose alone (100%; corresponds in absolute values to 0.4±0.05% TUNEL-positive β-cells). The mean number of islets scored was 41 for each treatment condition from each donor. *B*) Percentage of INS 832/13 cells presenting a condensed nuclear chromatin or a fragmented nuclear membrane, visualized by Hoechst staining and normalized to control incubations at 5.5 mM glucose alone. *C*) Double immunostaining for β-cell apoptosis with the TUNEL assay in black and anti-insulin in brown in control (5.5 mM glucose) and glucose-treated human islets with (33.3 mM glucose+JNKi) or without (33.3 mM glucose) addition of 1 μM JNKi. Black arrows mark TUNEL-positive β-cells. *D*) Measurement of JNK activity from human islet lysates cultured for 18 h in suspension at 5.5 or 33.3 mM glucose with or without leptin or JNKi. Upper panel, phosphorylated GST-cJun as revealed by Western blotting radioactive assay; lower panel, input of GST-cJun stained with Coomassie Blue. The blot shows a representative blot of five experiments from five islet donors. The density of the phosphorylated (P) GST-cJun band was quantified by scanning and expressed as a percentage relative to control (5.5 mM glucose) *E*) Basal and glucose-stimulated insulin secretion denote the amount secreted during successive 1-h incubations at 2.8 (basal) and 16.7 (stimulated) mM glucose after the 4-day culture period and expressed as secreted insulin. *F*) Insulin content from the same islets. Islets were isolated from five organ donors (*A, C, E, F*) or results are from three separate experiments (*B*). Results are means ± SE. $^{*}P < 0.05$ compared with untreated controls; $^{**}P < 0.05$ compared with leptin-treated islets; $^{\#}P < 0.05$ compared with 11.1 mM glucose-treated islets; $^{+}P < 0.05$ compared with 33.3 mM glucose-treated islets.

In human islets, the changes in β-cell apoptosis were accompanied by leptin (2.2-fold) and glucose (4-fold) -induced reductions in glucose-stimulated insulin secretion (GSIS) compared with baseline glucose at 5.5 mM (Fig. 1*E*) together with 2.6- and 3.3-fold reductions in islet insulin content by leptin and 33.3 mM glucose, respectively (Fig. 1*F*). Addition of JNKi to the culture medium protected the impairing effects of leptin and 33.3 mM glucose on both GSIS and islet insulin content (Fig. 1*E, F*) ($P<0.01$).

Inhibition of JNK protects from caspase 1 activation in β-cells

The mechanisms leading to JNK-dependent β-cell apoptosis induced by high glucose and leptin were studied by analyzing expression levels of caspase 1, also named interleukin-1β-converting enzyme (ICE). Human isolated islets were cultured for 48 h at 5.5, 11.1, or 33.3 mM glucose or in the presence of 10 nM leptin with or without 1 μM JNKi. High glucose and leptin increased caspase 1 on the mRNA level (1.7-fold at 11.1 mM and 2.2-fold at 33.3 mM glucose and 1.5-fold at 10 nM leptin, $P<0.05$) (**Fig. 2*A***). Additional treatment of the islets with 1 μM JNKi completely blocked the increase of caspase 1 mRNA (Fig. 2*A*). Expression of caspase 1 protein was increased after exposure of human islets to high glucose and to leptin (Fig. 2*B*), and inhibition of JNK activity restored basal levels of caspase 1 in glucose-treated islets and reduced the effect of leptin on caspase 1 expression (Fig. 2*B, C*). To analyze cellular localization of caspase 1 in islets, we used sections from

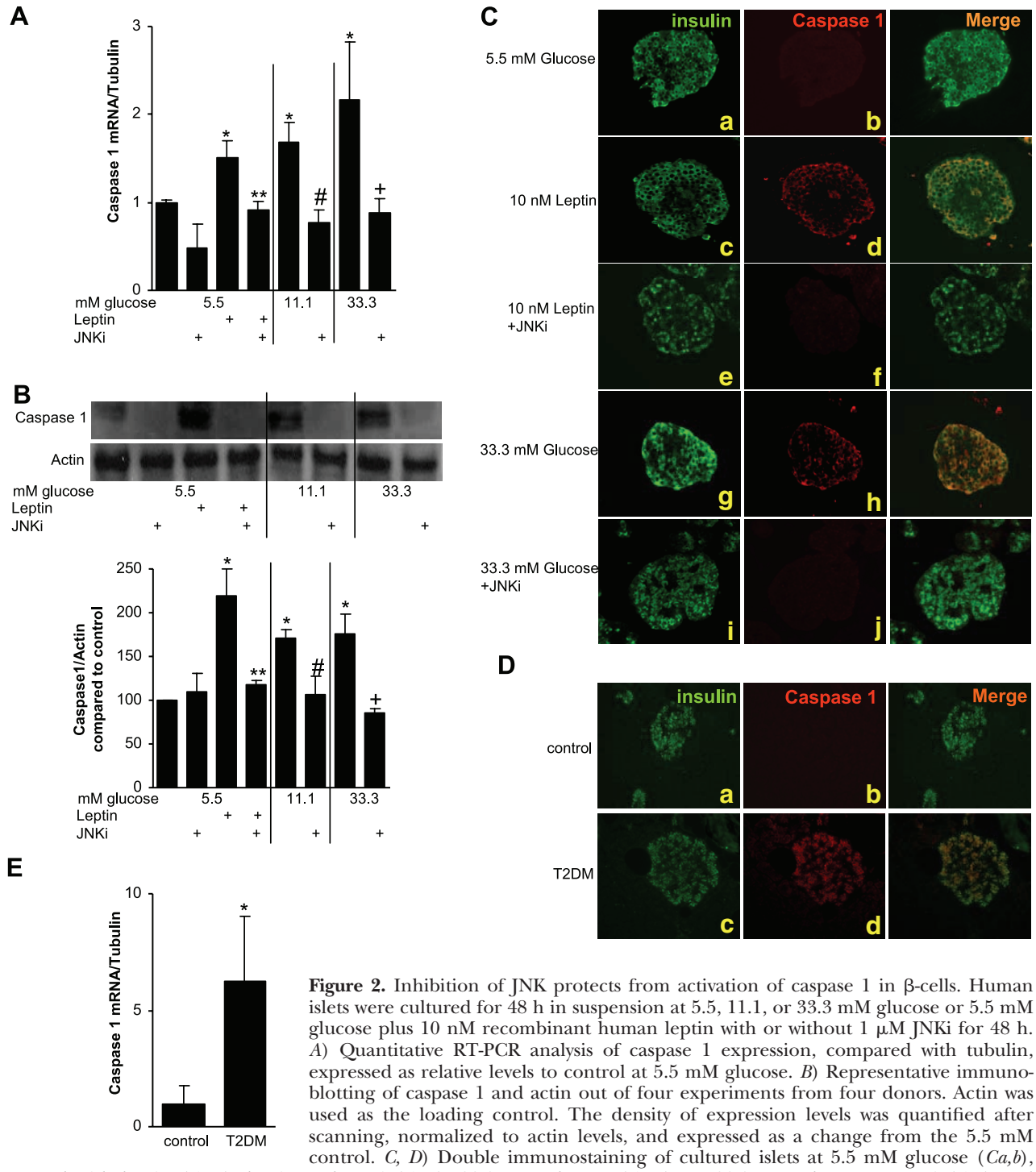

Figure 2. Inhibition of JNK protects from activation of caspase 1 in β-cells. Human islets were cultured for 48 h in suspension at 5.5, 11.1, or 33.3 mM glucose or 5.5 mM glucose plus 10 nM recombinant human leptin with or without 1 μM JNKi for 48 h. *A*) Quantitative RT-PCR analysis of caspase 1 expression, compared with tubulin, expressed as relative levels to control at 5.5 mM glucose. *B*) Representative immunoblotting of caspase 1 and actin out of four experiments from four donors. Actin was used as the loading control. The density of expression levels was quantified after scanning, normalized to actin levels, and expressed as a change from the 5.5 mM control. *C, D*) Double immunostaining of cultured islets at 5.5 mM glucose (*Ca,b*), treated with leptin (*Cc,d*), leptin and JNKi (*Ce,f*), 33.3 mM glucose (*Cg,h*), or 33.3 mM glucose + JNKi (*Ci,j*) and pancreatic sections from a nondiabetic control (*Da,b*) or a patient with T2DM (*Dc,d*) with anti-insulin (*Ca,c,e*; *Da,c*) and anti-caspase 1 (*Cb,d,f*; *Db,d*) antibodies. *E*) Quantitative RT-PCR analysis of caspase 1 expression in isolated islets from three controls and from three patients with T2DM and expressed relative to controls. Results are means ± SE. $^{*}P < 0.05$ compared with untreated (*A*) or nondiabetic (*E*) controls (5.5 mM glucose); $^{**}P < 0.05$ compared with leptin-treated islets; $^{\#}P < 0.05$ compared with 11.1 mM glucose-treated islets; $^{+}P < 0.05$ compared with 33.3 mM glucose-treated islets.

cultured human islets and double-stained them for caspase 1 and insulin. Figure 2*Ca–f*, shows representative images from one experiment of five from five separate donors. No caspase 1 staining was observed in control islets cultured at 5.5 mM glucose, but 48 h of culture with leptin or 33.3 mM glucose induced expression of caspase 1 in β-cells. On the basis of these *in vitro* studies, we expected caspase 1 expression also in islets of patients with T2DM, as a result of hyperglycemia. Caspase 1 expression was studied in sections of pancreases from four patients with poorly controlled type 2 diabetes, all with documented fasting blood glucose higher than 8 mM. Double immunostaining of the pancreatic sections for caspase 1 and insulin revealed

expression of the caspase in almost all β-cells in the T2DM sections of pancreases of T2DM, but no signal was observed in the control sections (Fig 2*D*). In parallel, we analyzed caspase 1 levels in isolated islets from three control and three age- and weight-matched organ donors with T2DM. Caspase 1 mRNA expression was 6.23-fold increased in the diabetic islets (Fig. 2*E*) ($P<0.05$).

Glucose but not leptin up-regulates c-*fos* and c-*myc* transcripts and enhances activator protein-1 (AP-1) and NF-κB activities in β-cells

Although we found similar activation of JNK induced by high glucose and leptin, downstream targets were differently modulated. We measured mRNA expression levels of downstream targets of JNK, c-*fos*, and c-*myc* in cultured human islets. Glucose dose dependently induced c-*fos* up-regulation; there were 1.9 ± 0.1- and 3.1 ± 0.5-fold increases in c-*fos* mRNA by 11.1 and 33.3 mM glucose, respectively ($P<0.05$), which were prevented by JNKi (**Fig. 3*A***). In contrast, leptin treatment of the islets had no effect on c-*fos* expression. Then, we measured c-*myc* in islets and found 2.2 ± 0.2- and 3.2 ± 0.6-fold increases in c-*myc* mRNA by 11.1 and 33.3 mM glucose, respectively ($P<0.05$), which were inhibited by JNKi. Leptin showed no significant effect on c-*myc* (Fig. 3*B*). Results obtained in the INS 832/13 cells line support the hypothesis of diverse downstream activators for glucose and leptin. AP-1 activity, performed by dual luciferase assay, was 5.9-fold increased by glucose and decreased in the presence of JNKi ($P<0.05$). In contrast, leptin failed to induce AP-1 activity (Fig. 3*C*). Finally, activation of the transcription factor NF-κB was assessed. This nuclear factor was 2.3 ± 0.4- and 2.1 ± 0.4-fold activated by 33.3 mM glucose in human islets (Fig. 3*D*) and INS 832/13 cells (Fig. 3*E*), respectively, but JNKi failed to inhibit the activation. Again, leptin did not activate NF-κB in both cell types. Altogether these results are compatible with the possibility that AP-1, c-*myc*, and NF-κB are implicated in β-cell glucotoxicity but not in the action of leptin.

DISCUSSION

The deleterious effects of chronically elevated glucose levels (11, 12, 35–47) as well as repetitively high postprandial glucose (48) on the pancreatic β-cell and its role in the pathophysiology of diabetes are well estab-

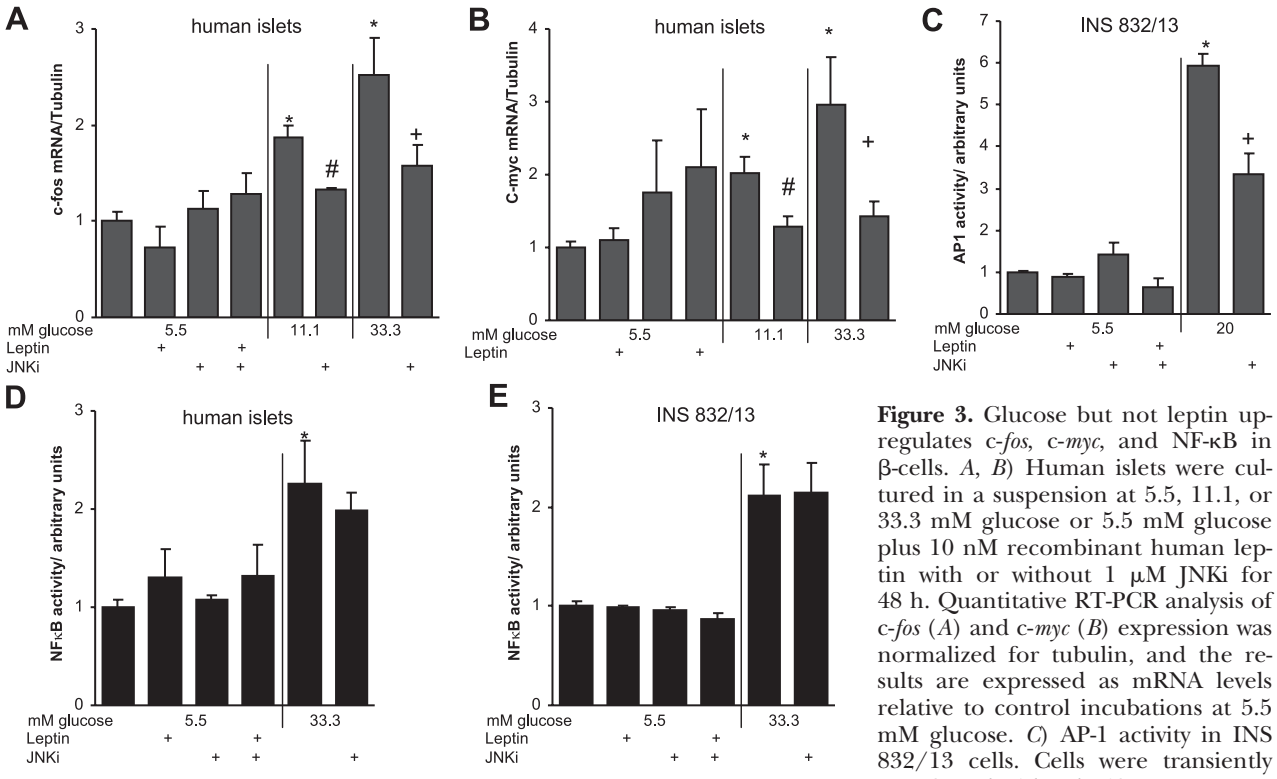

Figure 3. Glucose but not leptin up-regulates c-*fos*, c-*myc*, and NF-κB in β-cells. *A, B*) Human islets were cultured in a suspension at 5.5, 11.1, or 33.3 mM glucose or 5.5 mM glucose plus 10 nM recombinant human leptin with or without 1 μM JNKi for 48 h. Quantitative RT-PCR analysis of c-*fos* (*A*) and c-*myc* (*B*) expression was normalized for tubulin, and the results are expressed as mRNA levels relative to control incubations at 5.5 mM glucose. *C*) AP-1 activity in INS 832/13 cells. Cells were transiently transfected with a luciferase reporter gene fused to an AP-1 consensus binding site. Then cells were cultured in 5.5 or 20 mM glucose or 5.5 mM glucose plus 10 nM recombinant human leptin with or without 1 μM JNKi for 18 h. Luciferase expression was assessed, and results are expressed as mean ± SE normalized to control incubations at 5.5 mM glucose. Results represent data from three independent experiments. *D, E*) Relative NF-κB activity detected by an anti-p65 subunit antibody in human islets (*D*) and INS832 cells (*E*). Results are means ± SE from five experiments from five different donors for human islets experiments and from three distinct experiments for INS 832/13 cells. $^{*}P < 0.05$ compared with untreated controls; $^{\#}P < 0.05$ compared with 11.1 mM glucose-treated islets; $^{+}P < 0.05$ compared with to 33.3 mM glucose-treated islets.

lished, causing impaired β-cell function as well as increased apoptosis. Therefore, preventing the β-cell from these effects is a target to treat diabetes. Previously we have shown that glucose induces production of the proinflammatory cytokine IL-1β and activation of Fas in human islets (12, 13). IL-1β and Fas induce β-cell death and activate the JNK signaling cascade (49, 50). This study shows that high glucose-induced apoptosis is also related to JNK activation. One explanation of JNK activation could be that isolation of the islets renders them susceptible to activation of stress pathways (51). Baseline JNK activation was variable in the different human islet isolation procedures used, possibly dependent on the islet isolation procedure itself. Nevertheless, JNK activity was always further up-regulated by glucose not only in isolated islets but also in the pancreatic β-cell line INS 832/13 (data not shown). Interestingly, treatment of islets with a specific inhibitor of JNK blocked glucose-induced β-cell apoptosis and normalized glucose-stimulated insulin secretion, which was markedly impaired by glucotoxicity.

Leptin is also a proinflammatory cytokine (52). It has a proapoptotic effect on human β-cells with chronic exposure (17, 53), and it shifts the balance of IL-1β/IL-1Ra toward the proinflammatory IL-1β (17). The concentration of leptin used is similar to that used in other *in vitro* studies (54, 55) and is in the upper range of those measured in obese people (56). As for chronic high glucose, similar deleterious effects were induced by exposure of human islets to leptin, and JNK inhibition also restored normal β-cell function and reduced apoptosis. This observation is of interest in view of the fact that recent studies suggest that hyperleptinemia plays a role in obesity-associated diseases (57). Leptin induces stimulation of inflammatory reactions as well as oxidative stress in many cell types, including β-cells. Leptin also impairs islet function in rat, mouse, and human β-cells (19, 21, 58–60) and is ineffective in leptin receptor-deficient *db/db* mice (58) and *fa/fa* rats (19). In contrast, others have documented an antiapoptotic effect of leptin with respect to free fatty acid-induced apoptosis of rodent islets *in vitro* (61). This result might be due to the lipid-lowering effect of leptin, which is anticipated to reduce "lipotoxicity." Chronically hyperleptinemic rats develop hypoglycemia with reversible β-cell dysfunction by depletion of tissue lipids (62). Nonetheless, there is evidence for a toxic rather than a beneficial effect of hyperleptinemia in the obese/diabetic situation (63). Obesity in humans is the main risk factor for the development of diabetes; it is accompanied by increased circulating leptin and cytokine levels. Interestingly, leptin administration accelerates the onset and progression of autoimmune diabetes in NOD mice (64), providing a link between type 1 and type 2 diabetes. Furthermore, leptin levels are increased in T2DM as a stress response, independently of obesity and sex (65).

In this study, we detected JNK activation as a common pathway, which mediates glucose- and leptin-induced β-cell apoptosis and impaired function in human isolated islets as well as in a β-cell line. Both compounds also induced up-regulation of caspase 1, the enzyme that promotes the maturation of pro-IL-1β into its biologically active proinflammatory form. Addition of a cell-permeable specific JNK inhibitory peptide inhibited apoptosis, restored β-cell function, and prevented caspase 1 up-regulation. This suggests a link between JNK and caspase 1, at least in part *via* the transcription factor AP-1, which may target the response elements in the caspase 1 gene promoter. On JNK activation, in the presence of high levels of caspase 1, pro-IL-1β could be processed into its active form. In contrast, blocking of JNK would lead to down-regulation of caspase 1 and IL-1β production. IL-1β itself induces JNK (49) and caspase 1 (66) up-regulation in β-cells. This leads to further IL-1β maturation and apoptosis (67). An important substrate of JNK is the cJun protein, which is phosphorylated on JNK activation. cJun protein is a component of transcription factor AP-1, which regulates many cellular processes, including apoptosis. Interestingly, two potential AP-1 binding sites (68) are present in the caspase 1 promoter and, if active, could provide a link between glucose-induced JNK activation and up-regulation of caspase 1. It will be of interest to determine whether these putative AP-1 binding sites are implicated in the mechanisms of glucose-induced β-cell apoptosis. As AP-1 is not increased by leptin, another transcription factor may be involved in the regulation of the caspase 1 gene.

In conclusion, our results provide new insights into the mechanisms of glucotoxicity and leptin-induced β-cell apoptosis, by showing that both chronic high glucose and leptin activate JNK and induce caspase 1/ICE, the enzyme that converts pro-IL-1β to IL-1β. In addition, we show that ICE is induced in islets from patients with T2DM. Because JNK inhibition reversed the toxic actions of high glucose and leptin on the β-cell, the data support the concept that therapeutic approaches designed to block JNK activation could be helpful not only to improve insulin resistance (26) but also to prevent a progressive decrease of β-cell mass and to restore β-cell function. FJ

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