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Glucose potentiates interleukin-1b (IL-1b)-induced p38 mitogen-activated protein kinase activity in rat pancreatic islets of Langerhans

Abstract

The cytokine interleukin-1b (IL-1b) is cytotoxic to rat pancreatic b-cells and has been implicated in the pathogenesis of insulin-dependent diabetes mellitus. IL-1b causes expression of inducible nitric oxide synthase (iNOS) and production of nitric oxide (NO). NO may be the mediator of the cytotoxic effect of IL-1b in rat islets and b-cell lines. Glucose has been shown to modulate the effects of IL-1b

on accumulated insulin release and potentiate NO production in rat islets, but the biochemical mechanism is unknown. IL-1b activates the mitogen-activated protein kinases (MAPK) extracellular signal-regulated kinase 1 and 2 (ERK1/2), p38 and c-jun NH2-terminal kinase (JNK) in rat islets and b-cells. Glucose may modulate MAPK activity although contrasting data have been published. The aim of this study was to investigate whether glucose potentiated IL-1b-induced p38 and ERK1/2 activity in rat islets. It was shown that glucose alone increased the phosphorylation of the MAPK substrates Elk-1 and activating transcription factor 2 (ATF2). D-glucose potentiated the p38 activity induced by a low concentration of IL-1b, whereas no effect was seen at high concentrations of IL-1b. Inhibition of p38 activity prevented IL-1b-induced nitrite production in the presence of D-glucose. We conclude that IL-1b-induced NO production in the presence of glucose is signalled by the p38 pathway.

Full text

INTRODUCTION

The cytokine IL-1beta is cytotoxic to rat pancreatic beta-cells, and together with tumor necrosis factor-alpha (TNF-alpha) and interferon-gamma (IFN-gamma) also to human beta-cells *in vitro* [1], and has been implicated in the pathogenesis of insulin-dependent diabetes mellitus. IL-1beta causes expression of iNOS and production of NO. NO may be a mediator of the cytotoxic effect of IL-1beta in rat islets and beta-cell lines [2]. Glucose has been shown to modulate the effects of IL-1beta on accumulated insulin release and to potentiate NO production in rat islets [3-5], but the biochemical mechanism is unknown.

MAPKs are serine/threonine protein kinases involved in transmission of extracellular signals to the cytosol and nucleus [6]. The MAPK family includes ERK1/2, p38 and JNK/stress activated protein kinases (SAPK) [7, 8], and the balance between the activation of these kinases determines cellular proliferation, differentiation and apoptosis [9]. We have previously demonstrated that IL-1beta activates the MAPK ERK1/2, JNK and p38 in islets and beta-cells, and that inhibition of ERK1/2 and p38 blocked islet iNOS expression and NO synthesis [10]. Glucose can also modulate MAPK activity in the absence of cytokines, although contrasting data have been reported in different experimental systems [11-15]. Since glucose and IL-1beta both induce MAPK

(p38 and ERK1/2) activation in beta-cells, glucose modulates the effect of IL-1beta on beta-cells, and p38 and ERK1/2 are necessary for IL-1beta-induced iNOS expression and NO synthesis, we were prompted to investigate whether glucose potentiated IL-1beta-induced p38 and ERK1/2 activity in rat pancreatic islets of Langerhans. We report that IL-1-induced NO production in the presence of glucose is signalled through the p38 pathway.

MATERIALS AND METHODS

Reagents

All reagents were from Sigma (St. Louis, MO, USA) unless otherwise specified.

Pancreatic islet isolation and culture

Islets from 5-8 day old Wistar Furth rats (Charles River, Sultzfeldt, Germany) were isolated by handpicking after collagenase A (Boehringer Mannheim, Mannheim, Germany) digestion of the pancreata [16]. Islets were cultured in 5-ml dishes for 4-7 days at 37° C in humidified, atmospheric air in complete RPMI-1-640 medium containing 11 mM D-glucose and supplemented with 10% FCS, 100 U/ml penicillin, 100 mug/ml streptomycin, 20 mM HEPES buffer, 2 mM L-glutamine, 0.038% $NaHCO_3$ (all from Life Technologies, Paisley, Scotland), prior to experiments.

Islet experiments

Hundred fifty randomly picked islets were precultured in 300 mul RPMI (Life Technologies, Paisley, Scotland) + 0.5% HS with D- or L-glucose (3.3-17 mM) for 4 hours prior to exposure to IL-1beta (4-60 U/ml recombinant human IL-1beta 400 U/ng, Novo Nordisk, Bagsvaerd, Denmark) and D- or L-glucose (3.3-17 mM). Preculturing islets for 4 hours has been shown earlier to reduce the basal phosphorylation of the SAPK substrates ATF2 and Hsp25 caused by handling of the islets [17], and this preculture period allows the islets to adjust to changes in the glucose concentration. In experiments with p38 inhibitor (p38i) SB203580 (Calbiochem, La Jolla, CA, USA), 10 muM p38i was added to the preculture medium as well as the culture media. DMSO (final concentration 0.04% (v/v)) was added to all conditions, as a control for the DMSO used to dissolve the p38i.

Islets for whole cell lysate kinase and immunoprecipitation assays were exposed to IL-1beta and glucose in a volume of 300 mul for 20 min, the medium was removed and the islets were lysed on ice for 45 min in 25 mul lysis buffer (20 mM Tris pH 7.0, 0.27 M sucrose, 1 mM EDTA, 1 mM EGTA, 1 mM Na_3VO_4, 50 mM NaF, 1% Triton X-100, 5 mM sodium pyrophosphate, 10 mM beta-glycerophosphate, 1 mM dithiothretiol (DTT), 1 mM benzamidine and 4 mug/ml leupeptin). The supernatants were stored at - 80° C.

Islets for nitrite and insulin measurements were exposed to cytokines and glucose for 24 hours in 300 mul and samples of media were collected for nitrite and insulin analysis.

The concentrations of IL-1beta and time points used were chosen according to previous dose and time-response studies [10, 17].

Protein concentration in lysates

The BioRad protein assay (BioRad, Richmond, CA, USA) was performed according to the manufacturers description using the Dye Reagent concentrate.

Whole cell lysate assay

Whole cell lysate and immunoprecipitation assay, were performed as previously described [10]. In brief, for the whole cell lysate assay, 3-5 mul whole islet lysate (the volume of whole islet lysate was adjusted to contain the same amount of protein), was added to 17 mul of reaction buffer (2 mug GST-Elk-1 (a gift from Klaus Seedorf Hagedorn Research Institute, Gentofte, Denmark), 2 mug GST-ATF-2 (1-109) (a gift from Roger J. Davis, Howard Hughes Medical Institute, University of Massachusetts Medical School, Worcester, MA, USA), 1 mug of Hsp25 (Stressgen, Victoria, Canada), 25 mM Tris pH 7.5, 0.1 mM EGTA, 0.1 mM Na_3VO_4, 1 muM of cAMP-dependent protein kinase inhibitor peptide, 10 mM Mg-acetate and 3 mul of ATP mixture (3 muCi [gamma-^{32}P]ATP (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom) and 1 mM ATP)). The phosphotransferase reaction was carried out for 30 min and terminated on ice, adding 25 mul of ice-cold SDS sample buffer (125 mM Tris pH 6.8, 4% SDS, 0.1 M DTT, 10% glycerol and 0.02% bromphenol blue) followed by boiling for 5 min. SDS-polyacrylamide gel electrophoresis (PAGE) using 4% stacking gels and 12% separation gels was performed (all from BioRad, Richmond, CA, USA). The gels

were fixed in a mixture of 10% acetic acid and 40% methanol. The gels were dried, and phosphorylated proteins were visualized by autoradiography using PhospholmagerTM (Molecular Dynamics, Sunnyvale, CA, USA) and quantified using Image Quant software (Molecular Dynamics, Sunnyvale, CA, USA).

For the immunoprecipitation assay 50 mul whole cell lysate (the volume of whole islet lysate was adjusted to contain the same amount of protein) was diluted to a final volume of 500 mul in washing buffer (20 mM Tris pH 7.0, 0.27 M sucrose, 1 mM EDTA, 1 mM EGTA, 1 mM Na_3VO_4, 50 mM NaF, 0.1% Triton X-100, 5 mM sodium pyrophosphate, 10 mM beta-glycerophosphate, 1 mM DTT, 1 mM benzamidine and 4 mug/ml leupeptin) and immunoprecipitated by incubation for 2 hours at 4° C with 2 mul anti ERK1/2 (New England Biolabs, Hitchin Hertfordshire, United Kingdom) or 2 mul of anti-MAPKAPK2 (Upstate Biotechnology, Lake Placid, NY, USA) followed by incubation at 4° C for 2 hours with 10 mul of Sepharose-Protein A beads (Pharmacia Biotech AB, Uppsala, Sweden). The beads were washed four times in washing buffer and three times in kinase buffer (20 mM HEPES pH 7.5, 20 mM beta-glycerophosphate, 10 mM $MgCl_2$, 1mM DTT and 50 muM Na_3VO_4). Kinase reactions were carried out for 30 min at 30° C in 35 mul of kinase buffer containing 10 muCi [gamma-^{32}P]ATP (Amersham Pharmacia Biotech, Buckinghamshire, United-Kingdom), and 5 mug of GST-ATF-2 (a gift from Roger J. Davis, Howard Hughes Medical Institute, University of Massachusetts Medical School, Worcester, MA, USA) or 5 mug of Hsp25 (Stressgen, Victoria, Canada). The reactions were terminated on ice, adding 30 mul of ice-cold SDS sample buffer and boiling for 5 min. The samples were then analysed for kinase activity as described above.

Nitrite and insulin measurements

Islet NO production was measured as nitrite accumulation in media after incubation by the Griess reaction [18]. Hundred fifty mul of medium were mixed with an equal volume of Griess reagent (one part 0.1% naphthylene diamine dihydrochloride and one part 1% sulfanilamide in 5% H_3PO_4 (Merck, Darmstadt, Germany)), and incubated for 10 min at room temperature. Absorbance was measured on an immuno-reader (Nippon Inter Med., Tokyo, Japan). The detection limit of the assay was 1 muM equal to 2 pmol/islet in our conditions. All values below the detection limit were assigned a value of 2 pmol/islet.

Accumulated insulin release in the media was measured by a solid-phase competitive enzyme-linked immunosorbent assay, as described previously [19].

Statistical analysis

Results are presented as means ± SEM. Results were analysed by one- and two-way analysis of variation (ANOVA). Significant differences were evaluated using Student's t-test. Additionally, Cuzick's test for trends across ordered groups [20] and Wilcoxon's matched-pair test was used as the non-parametric statistical test.

RESULTS

Glucose-induced potentiation of IL-1 action on beta*-cell function*

To compare the priming and acute effects of glucose on IL-1-induced alterations in insulin release and NO synthesis, we measured the insulin release and nitrite production in islets treated with IL-1beta and glucose after a 4 hours preculture period with either low or higher concentrations of glucose (Table 1). The time course and dose effects of IL-1beta and glucose on insulin release and NO production in islets have been extensively studied [3-5, 21, 22]. Briefly, a short exposure to low concentrations of D-glucose and/or IL-1beta has a stimulating effect, while an increase in any of those factors led to inhibition of insulin release.

In islets precultured in 3.3, 5.5, 11 or 17 mM D-glucose and exposed to IL-1beta at the same concentration of glucose, there was a stimulatory, although not significant effect of IL-1 at low concentrations of glucose (3.3 and 5.5 mM), while at 11 and 17 mM IL-1beta had a highly significant inhibitory effect on the accumulated insulin release (t-test, $p < 0.0001$ and $p < 0.00004$ respectively). Two-way ANOVA analysis showed a statistically significant interaction between the concentrations of glucose and IL-1beta ($p < 0.0001$).

In islets precultured in 3.3 mM D-glucose and exposed to IL-1beta at 3.3, 5.5, 11 or 17 mM D-glucose, there was a stimulatory effect of IL-1beta on the accumulated insulin release at 3.3 and 5.5 mM D-glucose (t-test, $p < 0.04$ and $p < 0.04$ respectively). At the high concentrations of D-glucose, IL-1beta had a highly significant inhibitory effect on the accumulated insulin release (t-test, p <

0.0001 and $p < 0.00004$ respectively). There was a statistically significant interaction between the concentrations of glucose and IL-1beta (two-way ANOVA, $p < 0.001$).

Increasing the D-glucose concentration in the absence of IL-1beta did not induce nitrite production above the detection limit of the assay. In islets precultured in 3.3, 5.5, 11 or 17 mM D-glucose there was a significant increase in the IL-1beta-induced accumulated nitrite at 5.5 mM D-glucose compared with 3.3 mM (t-test, $p < 0.05$), but there was no effect of increasing the D-glucose concentration above 5.5 mM D-glucose. In islets precultured in 3.3 mM D-glucose, the IL-1beta-induced accumulated nitrite production was increased when the D-glucose concentration in the experiment was increased from 3.3 to 11 mM ($p < 0.05$), but no effect was seen from further increasing the glucose concentration. Control experiments with L-glucose, a non-metabolizable glucose enantiomer, showed that the potentiating effect of glucose on IL-1beta induced nitrite production was dependent on the metabolism of glucose and not due to an osmotic effect of glucose (Table 2). IL-1beta-induced NO production was only about half of that observed in islets cultured in D-glucose-containing medium, supporting the notion that glucose metabolism is linked to iNOS activity.

Immunoprecipitation of ERK1/2 and MAPKAPK2

We first examined the activity of ERK1/2 and MAPKAPK2 (downstream from p38) in islets precultured in 3.3, 5.5, 11 or 17 mM D-glucose and thereafter exposed to 60 U/ml IL-1beta for 20 min at the same glucose concentration, by immunoprecipitation (Figure 1). In an earlier time response study, a 20 min IL-1beta exposure led to maximal phosphorylation of the substrates [10]. ERK1/2 but not MAPKAPK2 was constitutively activated in the islets, but there was no clear effect of increasing medium glucose on either MAPKAPK2 or ERK1/2 activity. Therefore, the same lysates were analyzed by the whole cell lysate kinase assay, which detects the net phosphorylation of the substrate, *i.e.* the combined activity of phosphatases as well as kinases in the lysate.

D-glucose increases the phosphorylation of Elk-1 and ATF2

To determine whether glucose alone increased the MAPK activity in islets, we measured the phosphorylation of the MAPK substrates Elk-1, ATF2 and Hsp25 in

whole cell lysates of islets exposed to D-glucose under the different experimental conditions (Figure 2 and Table 3). In islets, a specific inhibitor of MEK blocked the phosphorylation of Elk-1, suggesting that Elk-1 is a specific substrate of ERK1/2 in islets [10]. Inhibition of p38 blocked ATF2 and Hsp25 phosphorylation, indicating that both substrates are phosphorylated by p38. Hsp25 is phosphorylated by MAPKAPK2, which is a specific substrate of p38. Combining the two inhibitors blocked the phosphorylation of Elk-1 and ATF2, suggesting that JNK in islets does not phosphorylate these substrates.

Increasing the glucose concentration in the experimental medium from 3.3 to 17 mM in islets precultured in 3.3, 5.5, 11 or 17 mM D-glucose, led to an increase in the phosphorylation of Elk-1 and ATF2 (Table 3) (Cuzick's test, $p < 0.00006$ and $p < 0.009$ respectively). In islets precultured in 3.3 mM D-glucose, only the phosphorylation of Elk-1 was increased by increasing the D-glucose concentration in the experiment from 3.3 to 17 mM (Cuzick's test, $p < 0.001$).

Control experiments with L-glucose showed no effect on the phosphorylation of the three substrates (Table 4).

*D-glucose does not potentiate phosphorylation of MAPK substrates induced by a high concentration of IL-1*beta

To determine whether D-glucose could potentiate IL-1beta-induced MAPK activity in rat islets, we determined the phosphorylation of the MAPK substrates Elk-1, ATF2 and Hsp25 in whole cell lysates from islets exposed to 60 U/ml of IL-1beta in different glucose concentrations (Table 3). In both islets precultured in 3.3 mM D-glucose and islets precultured in 3.3, 5.5, 11 or 17 mM D-glucose, IL-1beta induced increases in the phosphorylation of all three MAPK substrates, but D-glucose failed to modulate this response. The IL-1beta-induced phosphorylation of the substrates was, however, dependent on D-glucose, as IL-1beta in the L-glucose control experiments only induced about half the phosphorylation of MAPK substrates seen in experiments with D-glucose (Tables 3 and 4).

*D-glucose potentiates p38 activity induced by a low concentration of IL-1*beta

Since we did not find a modulating effect of glucose on MAPK activity induced by 60 U/ml of IL-1beta which is the concentration giving maximal MAPK activity response, we performed the same experiments with 20 U/ml, which gave a half maximal MAPK activity response and 4 U/ml of IL-1beta, which was the lowest concentration to induce MAPK activity in earlier dose-response studies (data not shown). We chose to perform these experiments with islets precultured in 3.3 mM D-glucose as these islets showed a submaximal IL-1beta (60 U/ml)-induced phosphorylation of Hsp25 compared to islets precultured in 3.3-17 mM D-glucose, thus providing a better opportunity to detect a modulating effect of glucose.

Firstly, we asked if glucose at low concentrations of IL-1beta could modulate inhibition of insulin release and induction of NO production (Table 5). Four U/ml of IL-1beta at 3.3, 5.5 and 11 mM D-glucose had no effect on islet accumulated insulin release. However, at 17 mM D-glucose there was a significantly inhibitory effect of IL-1beta (t-test, $p < 0.03$). Twenty U/ml of IL-1beta had no effect on accumulated insulin release at 3.3 and 5.5 mM D-glucose, whereas an inhibitory effect was observed at 11 and 17 mM D-glucose (t-test, $p < 0.01$ and $p < 0.02$). Four U/ml of IL-1beta did not induce nitrite production at 3.3 mM D-glucose, but increasing the D-glucose concentration from 3.3 to 11 mM increased the accumulated nitrite release. Twenty U/ml of IL-1beta induced nitrite production at all D-glucose concentrations. The nitrite production was not further augmented by increasing the D-glucose concentration above 5.5 mM.

Next we determined the MAPK activity under the same experimental conditions (Figure 3). Glucose alone increased the phosphorylation of Elk-1 (Cuzick's test, $p < 0.0003$) as seen in the first experiment (Table 3), albeit starting at 11 mM D-glucose. Increasing the glucose concentration from 3.3 to 17 mM D-glucose at 4 U/ml of IL-1beta induced a significant increase in the phosphorylation of Hsp25 (Cuzick's test, $p < 0.02$). The increase in phosphorylation of Elk-1 induced by glucose alone was not changed at 4 U/ml of IL-1. There was no effect on MAPK activity of increasing the D-glucose concentration at 20 U/ml of IL-1beta.

*Inhibition of p38 activity prevents IL-1*beta*-induced nitrite production in the presence of D-glucose*

We next wished to investigate whether the observed potentiating effect of D-glucose on the phosphorylation of Hsp25 at 4 U/ml of IL-1beta could explain the

modulating effect of D-glucose on the induction of nitrite release. Islets were treated with 4 U/ml of IL-1beta, in combination with p38 inhibitor, at different D-glucose concentrations and the accumulated nitrite production was measured. Inhibition of p38 blocked glucose modulation of the IL-1beta-induced nitrite production completely (Figure 4).

DISCUSSION

The clarification of the molecular mechanisms and signaling pathways involved in the previously demonstrated modulating effect of glucose on IL-1-mediated induction of pancreatic islet NO synthesis and inhibition of insulin release is important considering the potential importance of NO in immune mediated beta-cell damage in IDDM. Here we show that D-glucose potentiates IL-1-induced p38 activity and that IL-1-induced islet NO production in the presence of D-glucose was completely blocked by a p38 inhibitor, indicating that this effect is signalled through the p38 pathway.

IL-1 signalling in beta-cells involves binding of IL-1 to the type I IL-1 receptor [23-26]. In other cells it has been shown that IL-1 receptor binding leads to formation of a receptor complex consisting of IL-1 receptor-associated protein, MyD88, IL-1 receptor associated kinase 1 and 2 (IRAK1/2) [27-32]. In mouse beta-cells, IL-1beta-induced effects were blocked by MyD88 dominant negative proteins [33]. IRAK is phosphorylated and activates TNF receptor-associated factor 6 (TRAF6) [34]. TRAF6 interacts with nuclear factor kappaB (NFkappaB)-inducing kinase (NIK), and is crucial for NFkappaB activation [35]. NIK shares sequence similarity with several MAPKK kinases and may thus be involved in activation of MAPKs by IL-1 [36]. IL-1 may also activate MAP kinases through phosphatidylinositol 3-kinase (PI3K) activation and ceramide production. There are data showing that PI3K interacts with IL-1R1, and that this may be an early part of the signaling pathway [37].

In beta-cells the signalling pathway from glucose to p38 activity may include PI3K, as glucose has been shown to signal to p38 through a Wortmannin/LY 294002, sensitive pathway in MIN6 cells. The activation of p38 by other stimulators was not blocked by the PI3K inhibitors. This indicates that in beta-cells-glucose may activate p38 by a novel pathway [15]. As L-glucose (a non-metabolizable glucose enantiomer) in our experiments did not potentiate p38

activity induced by IL-1, a metabolite of glucose may activate PI3K, which then activates p38.

PKC is known to interact with MAPK and NFkappaB pathways in other cell systems. In rat aortic smooth muscle cells, glucose activation of p38 is prevented by a protein kinase C (PKC) inhibitor, while activation with other agonists are not [38]. However, indirect evidence has questioned the role of PKC in beta-cells, as phorbol 12-myristate 13-acetate, a phorbol ester which activates PKC, had only a minor effect on NFkappaB activation in RIN cells [39]. However, IL-1 stimulated the generation of diacylglycerol [39] which is produced by phospholipase C and which activates PKC. Thus, taken together PKC may play a role in IL-1-induced MAPK activity in beta-cells.

The sphingomyelinase/ceramide pathway may signal the effects of IL-1beta on beta-cells. In other cell types, IL-1beta has been shown to activate the sphingomyelinase pathway [40, 41]. In beta-cells, degradation of sphingomyelin has been shown to occur within 20 min of cytokine stimulation [39]. However, another study has found that although ceramide analogs mimic the cytotoxic effect of cytokines on beta-cells, cytokines did not activate the sphingomyelin/ceramide signaling pathway [42]. Activation of the ceramide pathway led to increases in the activities of ERK1/2, p38 and JNK in a human mammary epithelial cell line and human skin fibroblasts [43, 44]; in other cell lines ceramide activated only p38 and JNK [45]. The MAPK cascades have been suggested to be activated by ceramide at the level of MAP-kinases or through PKCdelta [44, 46].

We found no priming effect of preincubation of the islets in high glucose concentrations before the addition of a high concentration of IL-1, suggesting that glucose metabolism and glucose-regulated gene expression may not affect MAPK activity induced by high concentrations of IL-1. In contrast in islets precultured in high glucose concentrations, the phosphorylation of Elk-1 and ATF2 was increased, while in islets precultured in 3.3 mM D-glucose, only the phosphorylation of Elk-1 was increased by glucose. This effect was not seen when performing identical experiments with L-glucose. This indicates that glucose may prime MAPK activity either by glucose metabolism in itself or by glucose-regulated gene expression, but that this effect is hidden by the massive IL-1beta-induced activation of MAPK.

The MAPK activities were measured by a phosphotransferase assay to determine the net activity of the kinases. Thus, the observed effects may be affected by the level of phosphatase activity, in addition to the level of activated kinase activity. In HeLa cells, IL-1 has been suggested to repress phosphoprotein phosphatase 2A [47]. A downregulation of phosphatases caused by IL-1 will lead to a sustained activation of MAPK. Sustained activation of particularly the JNK/p38 SAPK is known to lead to apoptosis in other cells. This issue deserves to be addressed in future experimental studies.

No correlation between IL-1beta-induced NO production and inhibition of insulin release was found in this study in accordance with previous findings [5, 48, 49]. Similarly, an apparent discrepancy between MAPK signaling towards NO-dependent and independent destruction of beta-cells has previously been suggested since inhibition of p38 partially reversed IL-1 induced inhibition of insulin release despite significant NO production, whereas inhibition of ERK did not affect IL-1-induced inhibition of insulin release at similar NO levels [10]. Taken together these findings suggest that the modulating effects of glucose on IL-1beta induced NO production are not the cause of the inhibition of insulin release.

CONCLUSION

In summary, we have demonstrated that IL-1beta-induced NO production in the presence of D-glucose is signalled by the p38 pathway. We hypothesize that IL-1-induced p38 activity may be potentiated in beta-cells in inflamed islets during early development of type 1 diabetes, where a reduced beta-cell mass may lead to slightly elevated blood glucose levels and thereby potentiation of IL-1-induced p38 activity in beta-cells.

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References

1. Mandrup-Poulsen T. 1996. The role of interleukin-1 in the pathogenesis of IDDM. (Review) *Diabetologia* 39: 1005.

2. Eizirik D L, Pavlovic D. 1997. Is there a role for nitric oxide in beta-cell dysfunction and damage in IDDM. (Review) *Diabetes Metab. Rev.* 13: 293.

3. Spinas G A, Palmer J P, Mandrup-Poulsen T, Andersen H, Nielsen J H, Nerup J. 1988. The bimodal effect of interleukin-1 on rat pancreatic beta-cells - stimulation followed by inhibition - depends upon dose, duration of exposure, and ambient glucose concentration. *Acta Endocrinol. (Copenh)* 119: 307.

4. Palmer J P, Helqvist S, Spinas G A, Mølvig J, Mandrup-Poulsen T, Andersen H, Nerup J. 1989. Interaction of beta-cell activity and IL-1 concentration and exposure time in isolated rat islets of Langerhans. *Diabetes* 38: 1211.

5. Andersen H, Mauricio D, Karlsen A E, Mandrup-Poulsen T, Nielsen J H, Nerup J. 1996. Interleukin-1beta-induced nitric oxide production from isolated rat islets is modulated by D-glucose and 3-isobutyl-1-methyl xanthine. *Eur. J. Endocrinol.* 134: 251.

6. Whitmarsh A J, Davis R J. 1996. Transcription factor AP-1 regulation by mitogen-activated protein kinase signal transduction pathways. (Review) *J. Mol. Med.* 74: 589.

7. Kyriakis J M, Avruch J. 1996. Protein kinase cascades activated by stress and inflammatory cytokines. (Review) *Bioessays* 18: 567.

8. Su B, Karin M. 1996. Mitogen-activated protein kinase cascades and regulation of gene expression. (Review) *Curr. Opin. Immunol.* 8: 402.

9. Xia Z, Dickens M, Raingeaud J, Davis R J, Greenberg M E. 1995. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. *Science* 270: 1326.

10. Larsen C M, Wadt K A, Juhl L F, Andersen H, Karlsen A E, Su M S, Seedorf K, Shapiro L, Dinarello C A, Mandrup-Poulsen T. 1998. Interleukin-1beta-induced rat pancreatic islet nitric oxide synthesis requires both the p38 and extracellular signal-regulated kinase 1/2 mitogen activated protein kinases. *J. Biol. Chem.* 273: 15294.

11. Khoo S, Cobb M. 1997. Activation of mitogen-activating protein kinase by glucose is not required for insulin secretion. *Proc. Natl. Acad. Sci. USA* 94: 5599.

12. Benes C, Roisin M P, van Tan H, Creuzet C, Miyazaki J, Fagard R. 1998. Rapid activation and nuclear translocation of mitogen-activated protein kinases in response to physiological concentration of glucose in the MIN6 pancreatic beta-cell line. *J. Biol. Chem.* 273: 15507.

13. Persaud S J, Wheeler-Jones C P, Jones P M. 1996. The mitogen-activated protein kinase pathway in rat islets of Langerhans: studies on the regulation of insulin secretion. *Biochem. J.* 313: 119.

14. Macfarlane W M, Smith S B, James R F, Clifton A D, Doza Y N, Cohen P, Docherty K. 1997. The p38/reactivating kinase mitogen-activated protein kinase cascade mediates the activation of the transcription factor insulin upstream factor-1 and insulin gene transcription by high glucose in pancreatic beta-cells. *J. Biol. Chem.* 272: 20936.

15. Mac farlane W M, McKinnon C M, Felton-Edkins Z A, Cragg H, James R F, Docherty K. 1999. Glucose stimulates translocation of the homeodomain transcription factor PDX1 from the cytoplasm to the nucleus in pancreatic beta-cells. *J. Biol. Chem.* 274: 1011.

16. Brunstedt J, Nielsen J H, Lernmark Å and the Hagedorn Study Group. 1984. Isolation of islets from mice and rats. In: Larner J, Pohl S L, eds *Methods in diabetes research.* New York: John Wiley and Sons, vol 1: 254.

17. Andersen N A, Larsen C M, Mandrup-Poulsen T. 2000. TNF-alpha and IFN-gamma potentiate IL-1beta induced mitogen activated protein kinase activity in rat pancreatic islets of Langerhans. *Diabetologia* 43: 1389.

18. Green L C, Wagner D A, Glogowski J, Skipper P L, Wishnok J S, Tannenbaum S R. 1982. Analysis of nitrate, nitrite and [^{15}N]nitrate in biological fluids. *Anal. Biochem.* 126: 131.

19. Nielsen K, Karlsen A E, Deckert M, Madsen O D, Serup P, Mandrup-Poulsen T, Nerup J. 1999. beta-Cell maturation leads to *in vitro* sensitivity to cytotoxins. *Diabetes* 48: 2324.

20. Pee D Y, Freedman L S. 1990. A stratified Wilcoxon-type test for trend. *Stat. Med.* 9: 829.

21. Wadt K A, Larsen C M, Andersen H, Nielsen K, Karlsen A E, Mandrup-Poulsen T. 1998. Ciliary neurotrophic factor potentiates the beta-cell inhibitory effect of IL-1beta in rat pancreatic islets associated with increased nitric oxide synthesis and increased expression of inducible nitric oxide synthase. *Diabetes* 47: 1602.

22. Andersen H U, Jørgensen K H, Egeberg J, Mandrup-Poulsen T, Nerup J. 1994. Nicotinamide prevents interleukin-1 effects on accumulated insulin release and nitric oxide production in rat islets of Langerhans. *Diabetes* 43: 770.

23. Hammonds P, Beggs M, Beresford G, Espinal J, Clarke J, Mertz R J. 1990. Insulin-secreting beta-cells possess specific receptors for interleukin-1beta. *FEBS Lett.* 261: 97.

24. Eizirik D L, Bjorklund A, Welsh N. 1993. Interleukin-1 induced expression of nitric oxide synthase in insulin-producing cells is preceded by c-fos induction and depends on gene transcription and protein synthesis. *FEBS Lett.* 317: 62.

25. Jafarian-Tehrani M, Amrani A, Homo-Delarche F, Marquette C, Dardenne M, Haour F. 1995. Localization and characterization of interleukin-1 receptors in the islets of Langerhans from control and nonobese diabetic mice. *Endocrinology* 136: 609.

26. Scarim A L, Arnush M, Hill J R, Marshall C A, Baldwin A, McDaniel M L, Corbett J A. 1997. Evidence for the presence of type I IL-1 receptors on beta-cells of islets of Langerhans. *Biochim. Biophys. Acta.* 1361: 313.

27. Cao Z, Henzel W J, Gao X. 1996. IRAK: a kinase associated with the interleukin-1 receptor. *Science* 271: 1128.

28. Dinarello C A. 1997. Interleukin-1. (Review) *Cytokine Growth Factor Rev.* 8: 253.

29. Wesche H, Henzel W J, Shillinglaw W, Li S, Cao Z. 1997. MyD88: an adapter that recruits IRAK to the IL-1 receptor complex. *Immunity* 7: 837.

30. Burns K, Martinon F, Esslinger C, Pahl H, Schneider P, Bodmer J L, Di Marco F, French L, Tschopp J. 1998. MyD88, an adapter protein involved in interleukin-1 signaling. *J. Biol. Chem.* 273: 12203.

31. Wesche H, Korherr C, Kracht M, Falk W, Resch K, Martin M U. 1997. The interleukin-1 receptor accessory protein (IL-1RAcP) is essential for IL-1 induced activation of interleukin-1 receptor-associated kinase (IRAK) and stress-activated protein kinases (SAP kinases). *J. Biol. Chem.* 272: 7727.

32. Roux-Lombard P. 1998. The interleukin-1 family. (Review) *Eur. Cytokine Netw.* 9: 565.

33. Dupraz P, Cottet S, Hamburger F, Dolci W, Felley-Bosco E, Thorens B. 2000. Dominant negative MyD88 proteins inhibit interleukin-1beta/interferon-mediated induction of nuclear factor kappaB-dependent nitrite production and apoptosis in beta-cells. *J. Biol. Chem.* 275: 37672.

34. Cao Z, Xiong J, Takeuchi R, Kurama T, Goeddel D V. 1996. TRAF6 is a signal transducer for interleukin-1. *Nature* 383: 443.

35. Kanakaraj P, Schafer P H, Cavender D E, Wu Y, Ngo K, Grealish P F, Wadsworth S A, Peterson P A, Siekierka J J, Harris C A, Fung-Leung W P. 1998. Interleukin (IL)-1 receptor associated kinase (IRAK) requirement for optimal induction of multiple IL-1 signaling pathways and IL-6 production. *J. Exp. Med.* 187: 2073.

36. Malinin N L, Boldin M P, Kovalenko A V, Wallach D. 1997. MAP3K-related kinase involved in NFkappaB induction by TNF, CD95 and IL-1. *Nature* 385: 540.

37. Reddy S A, Huang J H, Liao W S. 1997. Phosphatidylinositol 3-kinase in interleukin-1 signaling. Physical interaction with the interleukin-1 receptor and requirement in NFkappaB and AP-1 activation. *J. Biol. Chem.* 272: 29167.

38. Igarashi M, Wakasaki H, Takahara N, Ishii H, Jiang Z Y, Yamauchi T, Kuboki K, Meier M, Rhodes C J, King G L. 1999. Glucose or diabetes activates p38 mitogen-activated protein kinase *via* different pathways. *J. Clin. Invest.* 103: 185.

39. Welsh N. 1996. Interleukin-1beta-induced ceramide and diacylglycerol generation may lead to activation of the c-jun NH_2-terminal kinase and the transcription factor ATF2 in the insulin-producing cell line RINm5F. *J. Biol. Chem.* 271: 8307.

40. Ballou L R, Chao C, Holness M A, Barker S C, Raghow R. 1992. Interleukin-1-mediated PGE_2 production and sphingomyelin metabolism. Evidence for the regulation of cyclooxygenase gene expression by sphingosine and ceramide. *J. Biol. Chem.* 267: 20044.

41. Mathias S, Younes A, Kan C C, Orlow I, Joseph C, Kolesnick R N. 1993. Activation of the sphingomyelin signaling pathway in intact EL4 cells and in a cell-free system by IL-1beta. *Science* 259: 519.

42. Major C D, Gao Z, Wolf B A. 1999. Activation of the sphingomyelinase/ceramide signal transduction pathway in insulin-secreting beta-cells. *Diabetes* 48: 1372.

43. Subbaramaiah K, Chung W J, Dannenberg A J. 1998. Ceramide regulates the transcription of cyclooxygenase-2. Evidence for involvement of extracellular signal-regulated kinase/c-Jun-terminal kinase and p38 mitogen-activated protein kinase pathways. *J. Biol. Chem.* 273: 32943.

44. Reunanen N, Westermarck J, Häkkinen L, Holmström T H, Elo I, Eriksson J E, Kähäri V M. 1998. Enhancement of fibroblast collagenase (matrix metalloproteinase-1) gene expression by ceramide is mediated by extracellular signal-regulated and stress-activated protein kinase pathways. *J. Biol. Chem.* 273: 5137.

45. Cuvillier O, Pirianov G, Kleuser B, Vanek P G, Coso O A, Gutkind J S, Spiegel S. 1996. Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate. *Nature* 381: 800.

46. Berra E, Díaz-Meco M T, Lozano J, Frutos S, Municio M M, Sánchez P, Sanz L, Moscat J. 1995. Evidence for a role of MEK and MAPK during signal transduction by protein kinase C zeta.*EMBO J.* 14: 6157.

47. Appel A, Kracht M, Petersen K, Resch K, Szamel M. 1996. Interleukin-1 induced signalling: biphasic activation of mitogen-activated protein kinase kinase and mitogen-activated protein kinases in HeLa cells. Involvement of phosphoprotein phosphatases. *Eur. Cytokine. Netw.* 7: 775.

48. Southern C, Schulster D, Green I C. 1990. Inhibition of insulin secretion by interleukin-1beta and tumour necrosis factor-alpha *via* an L-arginine-dependent nitric oxide generating mechanism. *FEBS Lett.* 276: 42.

49. Sternesjö J, Bendtzen K, Sandler S. 1995. Effects of prolonged exposure to interferon-gamma and tumour necrosis factor-alpha on nitric oxide and insulin production of rat pancreatic islets. *Autoimmunity* 20: 185.

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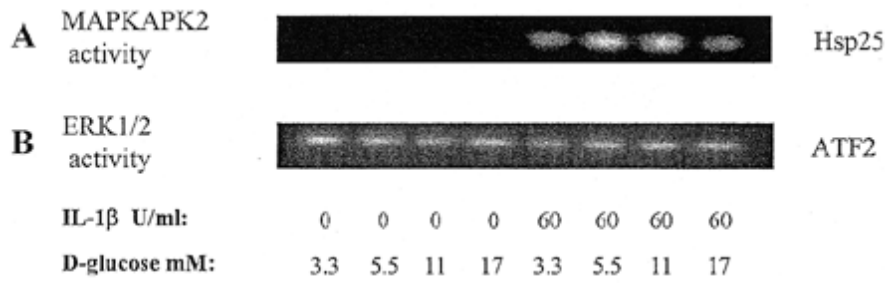


Figure 1

Immunoprecipitated MAPKAPK2 and ERK1/2 activity in lysates from islets exposed to glucose and IL-1beta for 20 min.

The islets were precultured for 4 hours in 3.3-17 mM D-glucose. MAPKAPK2 and ERK1/2 were immunoprecipitated with specific antibodies and their activities were measured in an immunocomplex kinase assay with Hsp25 and ATF2 as substrates. Phosphorylation reactions were initiated by the addition of [gamma-^{32}P]ATP. Proteins were separated by SDS-PAGE and phosphorylation quantified by PhosphorImager analysis. The immunoprecipitations were performed in duplicate on a pool of lysates from 6 experiments, and representative autoradiograms are shown.

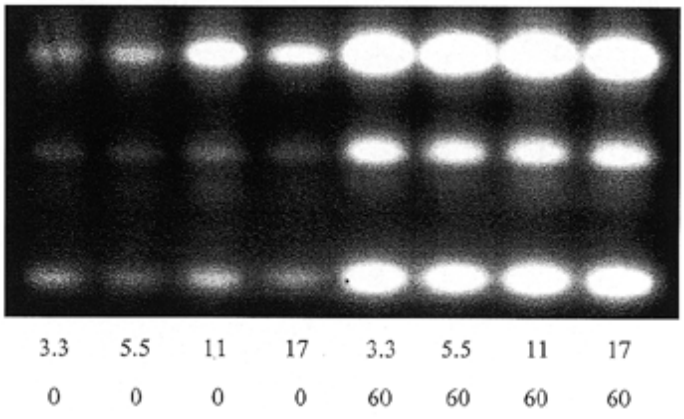


Figure 2

A representative gel from the experiment depicted in table 3 showing MAPK activity towards Elk-1, ATF2 and Hsp25 in whole cell lysates from islets exposed to D-glucose and IL-1beta.

Hundred fifty islets were precultured for 4 hours in 3.3-17 mM D-glucose, followed by incubation for 20 min with or without 60 U/ml IL-1beta in D-glucose concentrations as indicated. MAPK activity against the substrates Elk-1, ATF2 and Hsp25 was determined by incubating whole cell lysates from the islets with the substrates, in the presence of radioactive ATP. Proteins were separated by SDS-PAGE and visualized by PhosphorImager.

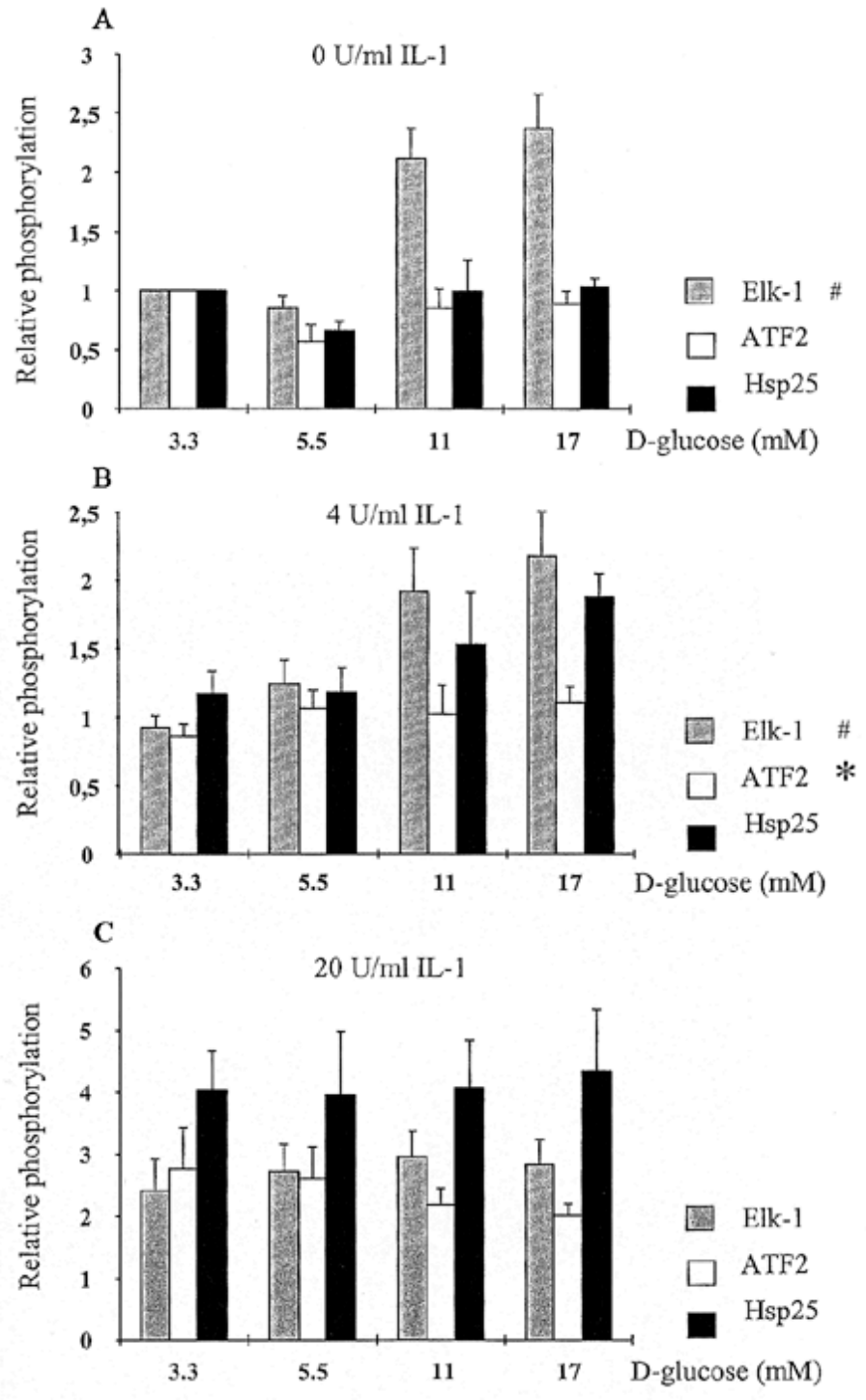


Figure 3

MAPK activity towards Elk-1, ATF2 and Hsp25 in whole cell lysates from islets exposed to D-glucose and IL-1beta for 20 min.

The islets were precultured for 4 hours in 3.3 mM D-glucose prior to exposure to glucose and IL-1. Islets in

panel A were not exposed to IL-1beta, islets in panel B to 4 U/ml IL-1beta and islets in panel C to 20 U/ml IL-1beta. MAPK activity was measured by incubating whole cell lysates from the islets with the substrates, in the presence of radioactive ATP. Proteins were separated by SDS-PAGE and phosphorylation was quantified by PhosphorImager analysis and presented as means (n = 7-8 independent experiments) ± SEM relative to values for lysates of islets incubated in 3.3 mM glucose without IL-1. # P < 0.003 (Cuzick's test). * P < 0.02 (Cuzick's test).

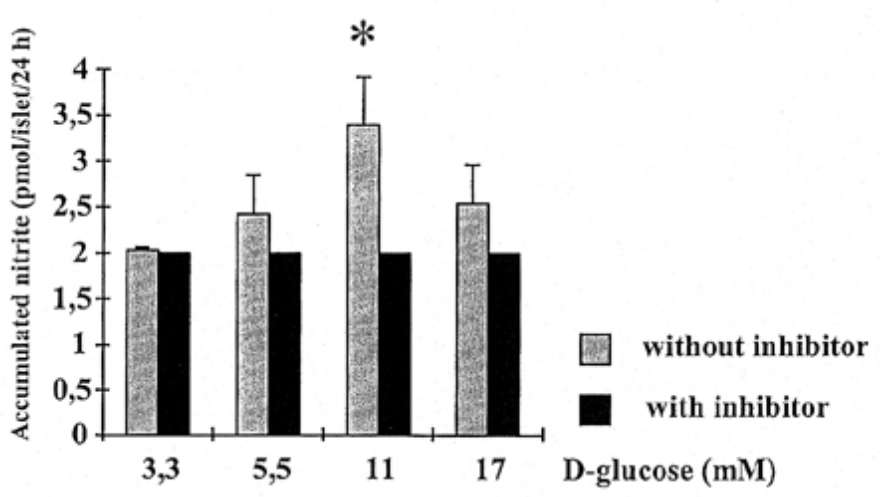


Figure 4

Accumulated nitrite release in whole cell lysates from islets exposed to D-glucose and 4 U/ml IL-1beta with or without 10 muM p38 inhibitor for 24 hours.

The islets were precultured for 4 hours in 3.3 mM D-glucose with or without 10 mu M p38 inhibitor, prior to exposure to glucose, IL-1beta and p38 inhibitor. Medium was sampled after 24 hours for determination of nitrite. Values presented are means ± SEM of n = 6 independent experiments. * P < 0.05 *versus* control (Wilcoxon).

Table 1
Effects of D-glucose on IL-1beta-induced inhibition of islet insulin and induction of NO production

Hundred fifty islets were precultured for 4 hours in the D-glucose concentrations indicated, followed by incubation for 24 hours with 0 or 60 U/ml IL-1beta in D-glucose concentrations as indicated. Medium was sampled for insulin and nitrite determinations. Values presented are means ± SEM of n = 8 independent experiments.

Culture conditions		Insulin (ng/islet/24 hours)		Nitrite (pmol/islet/24 hours)	
Preculture	Incubation	0 U/ml IL-1beta	60 U/ml IL-1beta	0 U/ml IL-1beta	60 U/ml IL-1beta
3.3 mM D-glucose	3.3 mM D-glucose	1.9 ± 0.4	2.7 ± 0.7	2	8.5 ± 0.7
5.5 mM D-glucose	5.5 mM D-glucose	1.3 ± 0.3	3.6 ± 1.0	2	11.2 ± 0.7[b]
11 mM D-glucose	11 mM D-glucose	12.3 ± 0,9	6.3 ± 0.3[a]	2	11.4 ± 0.7[b]
17 mM D-glucose	17 mM D-glucose	25.4 ± 1.9	8.5 ± 0.6[a]	2	11.9 ± 0.7[b]
3.3 mM D-glucose	3.3 mM D-glucose	1.0 ± 0.2	1.6 ± 0.2[a]	2	9.9 ± 0.5
3.3 mM D-glucose	5.5 mM D-glucose	1.2 ± 0.2	2.3 ± 0.4[a]	2	12.6 ± 1.0
3.3 mM D-glucose	11 mM D-glucose	13.5 ± 1.2	7.4 ± 1.0[a]	2	13.6 ± 1.2[b]
3.3 mM D-glucose	17 mM D-glucose	20.3 ± 1.6	8.8 ± 0.7[a]	2	13.0 ± 0.7[b]

[a] $P < 0.05$ *versus* 0 U/ml IL-1 (for exact p-values see text). [b] $P < 0.05$ *versus* 3.3 mM D-glucose (for exact p-values see text).

Table 2
Effects of L-glucose on IL-1beta-induced inhibition of insulin release and induction of NO production

Hundred fifty islets were precultured 4 hours in the L-glucose concentrations indicated, followed by incubation for 24 hours with 0 or 60 U/ml IL-1beta in L-glucose concentrations as indicated. Medium was sampled for insulin and nitrite determinations. Values presented are means ± SEM of n = 3 independent experiments.

Culture conditions		Insulin (ng/islet/24 hours)		Nitrite (pmol/islet/24 hours)	
Preculture	Incubation	0 U/ml IL-1beta	60 U/ml IL-1beta	0 U/ml IL-1beta	60 U/ml IL-1beta
3.3 mM L-glucose	3.3 mM L-glucose	1.1 ± 0.2	1.0 ± 0.5	2	4.1 ± 1.1
5.5 mM L-glucose	5.5 mM L-glucose	0.9 ± 0.3	0.8 ± 0.3	2	3.4 ± 0.7
11 mM L-glucose	11 mM L-glucose	0.7 ± 0.2	1.0 ± 0.4	2	4.3 ± 1.1
17 mM L-glucose	17 mM L-glucose	0.5 ± 0.1	0.8 ± 0.4	2	3.4 ± 0.7
3.3 mM L-glucose	3.3 mM L-glucose	0.9 ± 0.3	1.2 ± 0.2	2	5.2 ± 0.7
3.3 mM L-glucose	5.5 mM L-glucose	0.8 ± 0.2	1.1 ± 0.2	2	5.4 ± 0.9
3.3 mM L-glucose	11 mM L-glucose	0.7 ± 0.2	0.9 ± 0.1	2	5.2 ± 0.7
3.3 mM L-glucose	17 mM L-glucose	0.7 ± 0.2	1.2 ± 0.3	2	5.8 ± 0.8

Table 3
Effects of D-glucose on IL-1beta-induced phosphorylation of the MAPK substrates Elk-1, ATF2 and Hsp25

Hundred fifty islets were precultured for 4 hours in D-glucose concentrations as indicated, followed by incubation for 20 min with, or without 60 U/ml IL-1 beta in D-glucose concentrations as indicated. MAPK activity against the substrates Elk-1, ATF2 and Hsp25 was determined by incubating whole cell lysates from the islets, with the substrates in the presence of radioactive ATP. Proteins were separated by SDS-PAGE and phosphorylation was quantified by PhosphorImager analysis and presented as means ± SEM of n = 8 independent experiments relative to control islets.

Culture conditions		Elk-1 phosphorylation		ATF2 phosphorylation		Hsp25 phosphorylation	
Preculture	Incubation	0 U/ml IL-1ß	60 U/ml IL-1ß	0 U/ml IL-1ß	60 U/ml IL-1ß	0 U/ml IL-1ß	60 U/ml IL-1ß
3.3 mM D-glucose	3.3 mM D-glucose	1	7.7 ± 1.4	1	5.8 ± 1.0	1	6.6 ± 1.6
5.5 mM D-glucose	5.5 mM D-glucose	1.8 ± 0.3	9.0 ± 1.7	2.2 ± 0.6	6.6 ± 1.0	3.1 ± 0.8	7.4 ± 1.6
11 mM D-glucose	11 mM D-glucose	3.0 ± 0.7	8.7 ± 1.6	1.7 ± 0.3	7.8 ± 1.2	2.2 ± 0.4	8.8 ± 1.9
17 mM D-glucose	17 mM D-glucose	3.3 ± 0.5	7.7 ± 1.5	2.5 ± 0.5	7.33 ± 1.3	4.1 ± 1.7	8.4 ± 1.8
3.3 mM D-glucose	3.3 mM D-glucose	1	8.5 ± 2.0	1	8.7 ± 3.9	1	4.4 ± 1.1
3.3 mM D-glucose	5.5 mM D-glucose	1.7 ± 0.2	8.3 ± 1.7	1.8 ± 1.0	7.5 ± 3.1	1.7 ± 0.8	4.1 ± 1.0
3.3 mM D-glucose	11 mM D-glucose	3.6 ± 0.6	8,7 ± 2.3	1.7 ± 0.7	9.9 ± 5.6	1.5 ± 0.4	5.2 ± 2.1
3.3 mM D-glucose	17 mM D-glucose	3.0 ± 0.5	7.6 ± 1.5	1.3 ± 0.3	6.1 ± 1.9	1.4 ± 0.4	4.2 ± 0.8

Table 4
Effects of L-glucose on IL-1beta-induced phosphorylation of the MAPK substrates Elk-1, ATF2 and Hsp25

Hundred fifty islets were precultured for 4 hours in L-glucose concentrations as indicated, followed by incubation for 20 min with, or without 60 U/ml IL-1 beta in L-glucose concentrations as indicated. MAPK activity against the substrates Elk-1, ATF2 and Hsp25 was determined by incubating whole cell lysates from the islets, with the substrates in the presence of radioactive ATP. Proteins were separated by SDS-PAGE and phosphorylation was quantified by PhosphorImager analysis and presented as means ± SEM of n = 3 independent experiments relative to control islets.

Culture conditions		Elk-1 phosphorylation		ATF2 phosphorylation		Hsp25 phosphorylation	
Preculture	Incubation	0 U/ml IL-1β	60 U/ml IL-1β	0 U/ml IL-1β	60 U/ml IL-1β	0 U/ml IL-1β	60 U/ml IL-1β
3.3 mM L-glucose	3.3 mM L-glucose	1	2.7 ± 0.7	1	5.1 ± 1.8	1	4.5 ± 1.1
5.5 mM L-glucose	5.5 mM L-glucose	0.9 ± 0.2	2.6 ± 0.6	1.1 ± 0.3	5.2 ± 1.7	1.0 ± 0.1	4.2 ± 0.2
11 mM L-glucose	11 mM L-glucose	0.9 ± 0.1	2.6 ± 0.6	0.9 ± 0.1	4.4 ± 1.2	1.1 ± 0.2	3.7 ± 0.0
17 mM L-glucose	17 mM L-glucose	0.7 ± 0.1	3.4 ± 0.8	0.9 ± 0.2	6.1 ± 1.9	1.0 ± 0.2	5.6 ± 0.8
3.3 mM L-glucose	3.3 mM L-glucose	1	6.5 ± 2.0	1	5.2 ± 1.2	1	4.9 ± 1.8
3.3 mM L-glucose	5.5 mM L-glucose	1.4 ± 0.1	5.5 ± 1.5	1.1 ± 0.0	3.9 ± 0.7	1.5 ± 0.3	4.3 ± 1.8
3.3 mM L-glucose	11 mM L-glucose	1.1 ± 0.4	6.7 ± 1.4	0.5 ± 0.0	4.5 ± 0.3	0.5 ± 0.2	4.8 ± 1.9
3.3 mM L-glucose	17 mM L-glucose	1.2 ± 0.5	5.5 ± 1.3	0.7 ± 0.2	3.6 ± 0.2	0.6 ± 0.2	3.8 ± 1.5

Table 5
Effects of D-glucose on IL-1beta-induced of islet insulin and induction of NO (prod)
Hundred fifty islets were precultured for 4 hours in D-glucose concentrations as indicated in the table, followed by incubation for 24 hours with 0, 4 or 20 U/ml IL-1beta in D-glucose concentrations as indicated.
Medium was sampled for insulin and nitrite determinations. Values presented are means ± SEM of n = 6 independent experiments.

Culture conditions		Insulin (ng/islet/24 h)			Nitrite (pmol/islet/24 h)		
Preculture	Incubation	0 U/ml IL-1β	4 U/ml IL-1β	20 U/ml IL-1β	0 U/ml IL-1β	4 U/ml IL-1β	20 U/ml IL-1β
3.3 mM D-glucose	3.3 mM D-glucose	1.2 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	2	2	9.3 ± 0.5
3.3 mM D-glucose	5.5 mM D-glucose	1.6 ± 0.2	1.5 ± 0.2	1.6 ± 0.1	2	4.4 ± 0.2	12.5 ± 0.8[b]
3.3 mM D-glucose	11 mM D-glucose	9.8 ± 1.3	10.7 ± 1.2	5.7 ± 0.8 [a]	2	9.1 ± 1.0[b]	13.4 ± 0.9[b]
3.3 mM D-glucose	17 mM D-glucose	18.5 ± 2.2	14.9 ± 1.0[a]	8.9 ± 0.9 [a]	2	6.2 ± 0.9[b]	13.9 ± 0.7[b]

[a] $P < 0.05$ *versus* 0 U/ml IL-1beta (for precise p-values see text).
[b] $P < 0.05$ *versus* 3.3 mM D-glucose at the same IL-1beta concentration (for precise p-values see text).

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