Glucose- and Interleukin-1β–Induced β-Cell Apoptosis Requires Ca^{2+} Influx and Extracellular Signal–Regulated Kinase (ERK) 1/2 Activation and Is Prevented by a Sulfonylurea Receptor 1/Inwardly Rectifying K^+ Channel 6.2 (SUR/Kir6.2) Selective Potassium Channel Opener in Human Islets

Kathrin Maedler,[1] Joachim Størling,[2] Jeppe Sturis,[3] Richard A. Zuellig,[1] Giatgen A. Spinas,[1] Per O.G. Arkhammar,[3] Thomas Mandrup-Poulsen,[2,4] and Marc Y. Donath[1]

Increasing evidence indicates that a progressive decrease in the functional β-cell mass is the hallmark of both type 1 and type 2 diabetes. The underlying causes, β-cell apoptosis and impaired secretory function, seem to be partly mediated by macrophage production of interleukin (IL)-1β and/or high-glucose–induced β-cell production of IL-1β. Treatment of type 1 and type 2 diabetic patients with the potassium channel opener diazoxide partially restores insulin secretion. Therefore, we studied the effect of diazoxide and of the novel potassium channel opener NN414, selective for the β-cell potassium channel SUR1/Kir6.2, on glucose- and IL-1β–induced apoptosis and impaired function in human β-cells. Exposure of human islets for 4 days to 11.1 and 33.3 mmol/l glucose, 2 ng/ml IL-1β, or 10 and 100 μmol/l of the sulfonylurea tolbutamide induced β-cell apoptosis and impaired glucose-stimulated insulin secretion. The deleterious effects of glucose and IL-1β were blocked by 200 μmol/l diazoxide as well as by 3 and 30 μmol/l NN414. By Western blotting with phosphospecific antibodies, glucose and IL-1β were shown to activate the extracellular signal–regulated kinase (ERK) 1/2, an effect that was abrogated by 3 μmol/l NN414. Similarly, 1 μmol/l of the mitogen-activated protein kinase/ERK kinase 1/2 inhibitor PD098059 or 1 μmol/l of the L-type Ca^{2+} channel blocker nimodipine prevented glucose- and IL-1β–induced ERK activation, β-cell apoptosis, and impaired function. Finally, islet release of IL-1β in response to high glucose could be abrogated by nimodipine, NN414, or PD098059. Thus, in human islets, glucose- and IL-1β–induced β-cell secretory dysfunction and apoptosis are Ca^{2+} influx and ERK dependent and can be prevented by the β-cell selective potassium channel opener NN414. *Diabetes* 53:1706–1713, 2004

There is now general agreement that both impaired β-cell function and decreased β-cell mass contribute to the insulin deficiency observed in patients with type 2 diabetes (1–11). Therefore, in the rationale behind new therapeutic approaches to both type 2 and type 1 diabetes maintenance or even improvement of β-cell functional mass should be considered. A key regulator of β-cell function and survival is the β-cell ATP-sensitive K^+ (K_{ATP}) channel. It is a heterooctamer composed of four inwardly rectifying K^+ channels (Kir) and four sulfonylurea receptors (SUR1). Increased intracellular glucose concentrations result in the production of ATP, raising the ATP-to-ADP ratio, with subsequent closure of K_{ATP} channels. A decrease of K^+ conductance leads to membrane depolarization, opening of voltage-operated Ca^{2+} channels, mainly of the L-type, Ca^{2+} influx, and a rise in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$), which induces insulin secretion (12). Modulation of the K_{ATP} channel also influences β-cell survival. Indeed, blocking of β-cell K_{ATP} channels with the sulfonylureas tolbutamide and glibenclamide induces Ca^{2+} influx–dependent β-cell apoptosis, as shown in a β-cell line (13) in rodent (14) and human (15) islets. In contrast, K_{ATP} channel openers may have protective effects in β-cells. In 1976, Greenwood et al. (16) were the first to report an improvement in insulin secretion after administration for 7 days of the K_{ATP} channel opener diazoxide to diabetic subjects. Preservation of residual insulin secretion by treatment with diazoxide was then shown in patients with type 1 diabetes (17) and more recently in subjects with type 2 diabetes (18). Similar protective effects were observed in the VDF Zucker rat and in a type 1 diabetic rat model treated with the recently developed β-cell–selective

From the [1]Division of Endocrinology and Diabetes, University Hospital, Zurich, Switzerland; [2]Steno Diabetes Center, Gentofte, Denmark; [3]Novo Nordisk, Måløv, Denmark; and the [4]Department of Molecular Medicine, Rolf Luft Center for Diabetes Research, Karolinska Institute, Stockholm, Sweden.

Address correspondence and reprint requests to Marc Y. Donath, MD, Division of Endocrinology and Diabetes, Department of Medicine, University Hospital, CH-8091 Zurich, Switzerland. E-mail: marc.donath@usz.ch.

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J.S., P.O.G.A., and T.M.-P are employed by Novo Nordisk A/S, Bagsværd, Denmark, at Steno Diabetes Center. The clinical activities of this center are paid by the public health care system, but Novo Nordisk strongly subsidizes the research activities of the center. Novo Nordisk manufactures and markets pharmaceuticals related to the treatment of diabetes.

ERK, extracellular signal–regulated kinase; IL, interleukin; K_{ATP}, ATP-sensitive K^+; iNOS, inducible nitric oxide synthase; Kir, inwardly rectifying K^+ channel; MAPK, mitogen-activated protein kinase; SUR, sulfonylurea receptor; TUNEL, transferase-mediated dUTP nick-end labeling.

SUR1/Kir6.2 potassium channel opener NN414 (19,20). Finally, activation of K_{ATP} channels also preserves β-cell pulsatile insulin secretion in human islets cultured at high glucose (21). Although these beneficial effects may be due to inducing β-cell "rest," they may also reflect in part an antiapoptotic effect of such drugs (14).

Chronic elevation of the blood glucose concentration impairs β-cell function (22–31) and induces β-cell apoptosis (14,32–34). Furthermore, in cultured human pancreatic islets, overstimulation by high glucose leads to a rise in $[Ca^{2+}]_i$, which persists after normalization of the glucose concentration (35,36). Therefore, we hypothesized that parts of these toxic effects of glucose are mediated via a reduction in K_{ATP} channel activity and elevated $[Ca^{2+}]_i$ and thus may be counteracted by potassium channel openers and/or calcium channel antagonists.

Interleukin (IL)-1β is thought to be a key mediator of both impaired function and destruction of pancreatic β-cells during the development of autoimmune type 1 diabetes (37). Furthermore, IL-1β seems to contribute to β-cell glucotoxicity in the pathogenesis of type 2 diabetes. Indeed, exposure of cultured human islets to elevated glucose levels leads to β-cell production and release of IL-1β (38). In turn, IL-1β feeds back on the β-cell to induce impaired function and apoptosis. Among several signaling pathways activated by IL-1β in the β-cell is the activation of extracellular signal–regulated kinase (ERK) 1/2, a mitogen-activated protein kinase (MAPK). This has been shown in β-cell lines, rat islets, and purified primary rat β-cells (39–41). ERK activation has been demonstrated to be required both for cytokine-induced expression of inducible nitric oxide (NO) synthase (iNOS) (39) and β-cell apoptosis (40). Glucose also stimulates activation of ERK1/2 in β-cells (42–47), and in macrophages, ERK1/2 activation was recently shown (48) to be required for double-stranded RNA- and virus-induced IL-1β expression. However, whether glucose-induced β-cell production of IL-1β as well as IL-1β–induced β-cell toxicity in human islets require ERK1/2 is unknown. Therefore, we investigated the effect of diazoxide and NN414 on glucose- and IL-1β–induced impaired function and apoptosis in human β-cells and to what extent their mechanisms of action involved modulation of $[Ca^{2+}]_i$ and ERK1/2 activity.

RESEARCH DESIGN AND METHODS

Islet isolation and cell culture. Islets were isolated from pancreata of nine organ donors at the Department of Endocrinology and Diabetes, University Hospital Zurich, as described (49–51). The islet purity was >95%, as judged by dithizone staining. When this degree of purity was not primarily achieved by routine isolation, islets were handpicked. The brain-dead, heart-beating organ donors were aged 45–72 years, and none had a previous history of diabetes or metabolic disorders. For short-term studies, the islets were cultured in nonadherent plastic dishes and for long-term studies on extracellular matrix–coated plates derived from bovine corneal endothelial cells (Novamed, Jerusalem, Israel), allowing the cells to attach to the dishes and spread, preserving their functional integrity (27,28). Islets were cultured in CMRL 1066 (5.5 mmol/l glucose) medium that contained 100 units/ml penicillin, 100 μg/ml streptomycin, and 10% FCS (Invitrogen, Paisley, U.K.), 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 that contained 5.5, 11.1, or 33.3 mmol/l glucose. In some experiments, islets were additionally cultured with 3, 30, or 100 μmol/l NN414 (Novo Nordisk, Bagsværd, Denmark); 200 μmol/l diazoxide; 10 or 100 μmol/l tolbutamide; 1 or 10 μmol/l nimodipine; 1 μmol/l nitrendipine (Sigma); 1 or 10 μmol/l PD098059; 1 μmol/l UO126 (Calbiochem, San Diego, CA); 2 ng/ml recombinant human IL-1β (ReproTech EC, London, U.K.); or solvent. NN414, nimodipine, nitrendipine, PD098059, and UO126 were dissolved in DMSO (Fluka, Buchs, Switzerland) to stock solutions. Stock solutions of diazoxide and tolbutamide were prepared in a sodium carbonate/sodium hydrogen carbonate (1:3) solution.

β-Cell replication and apoptosis. For β-cell proliferation studies, a monoclonal antibody against the human Ki-67 antigen was used (Zymed, San Francisco, CA). The free 3′-OH strand breaks resulting from DNA degradation were detected by the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) technique (52) according to the manufacturer's instructions (In Situ Cell Death Detection Kit, AP; Boehringer, Mannheim, Germany) and as described previously in detail (32,38,53). Thereafter, islets were incubated for 30 min at 37°C with a guinea pig anti-insulin antibody (Dako, Carpinteria, CA), followed by detection using the streptavidin-biotin-peroxidase complex (Zymed Laboratories, South San Francisco, CA). 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 cultured on extracellular matrix–coated plates of similar size. Some larger islets did not completely spread and were several cell layers thick. Such larger islets were excluded because a monolayer is a prerequisite for single-cell evaluation. The mean surface of the evaluated islet monolayers has been determined previously in islets cultured at 5.5 and 33.3 mmol/l glucose, respectively, and found to be similar (38,53). Thus, the exclusion of larger islets occurred to a similar extent in each dish independent of the treatment.

Western blot analysis. At the end of the incubations, islets (a total of 150 islets/condition) were washed in PBS, suspended, and lysed for 40 min on ice in 30 μl of lysis buffer that contained 20 mmol/l Tris acetate (pH 7.0), 0.27 mol/l sucrose, 1 mmol/l EDTA, 1 mmol/l EGTA, 1 mmol/l Na_3VO_4, 50 mmol/l NaF, 1% Triton X-100, 5 mmol/l sodium pyrophosphate, 10 mmol/l β-glycerophosphate, 1 mmol/l dithiothreitol, 1 mmol/l benzamidine, and 4 μg/ml leupeptin. The detergent-insoluble material was pelleted by centrifugation at 15,000*g* for 5 min at 4°C. The supernatants that contained whole-cell lysate were stored at −80°C until assayed. Equivalent amounts of cell extracts were mixed with SDS sample buffer, boiled for 10 min, and subjected to 10% SDS-PAGE. Proteins were electrically transferred to nitrocellulose filter membranes. Blocking of nonspecific protein binding was done by incubating the filter membrane in blocking buffer (1× TBS [pH 7.6], 0.1% Tween-20, and 5% nonfat dry milk) for 1 h. After washing in TBST (1× TBS [pH 7.6] and 0.1% Tween-20), filters were incubated at 4°C overnight with anti-phosphospecific (Thr202/Tyr204) ERK1/2 (1:1,000), total anti-ERK1/2 (1:1,000) (Cell Signaling, Beverly, MA), or mouse anti-actin (C-2; Santa Cruz Biotechnology, Santa Cruz, CA) antibodies. Filter membranes were then washed in TBST and incubated for 1 h with horseradish peroxidase–conjugated secondary antibodies (1:4,000). Immune complexes were detected by chemiluminescence using Lumi-GLO (Cell Signaling). Light emission was captured on X-ray film.

IL-1β release. IL-1β release was evaluated in the culture medium collected before the termination of experiment using a human IL-1β enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN).

Insulin release and content. For determining the acute insulin release in response to glucose stimulation, islets were washed in Krebs-Ringer bicarbonate buffer (KRB-HEPES [pH 7.4]: 4.8 mmol/l KCl, 134 mmol/l NaCl, 5 mmol/l $NaHCO_3$, 1.2 mmol/l $MgSO_4$, 1.2 mmol/l KH_2PO_4, 1 mmol/l $CaCl_2$, 0.5% BSA, and 10 mmol/l HEPES) that contained 3.3 mmol/l glucose and preincubated for 30 min in the same buffer. The Krebs-Ringer buffer was then discarded and replaced by fresh buffer that contained 3.3 mmol/l glucose for 1 h for basal secretion, followed by an additional 1-h incubation in Krebs-Ringer buffer that contained 16.7 mmol/l glucose. Supernatants were collected and frozen for insulin assays. Thereafter, islets were washed with PBS and extracted with 0.18 N HCl in 70% ethanol for 24 h at 4°C. The acid-ethanol extracts were collected for determination of insulin content. Insulin was determined by a human insulin radioimmunoassay kit (CIS Bio International, Gif-Sur-Yvette, France).

Statistical analysis. Data were analyzed by Student's *t* test or by ANOVA with a Bonferroni correction for multiple-group comparisons.

RESULTS

K_{ATP} channel openers protect against glucose-induced apoptosis and impaired function, whereas tolbutamide augments the deleterious effect of glucose in human β-cells. Human islets were cultured on extracellular matrix–coated dishes and exposed for 4 days to increasing concentrations of glucose in the presence or absence of NN414, diazoxide, or tolbutamide. Analysis of β-cell nuclei for DNA fragmentation (TUNEL positive) revealed a 1.9- and 3.9-fold increase in islets cultured at

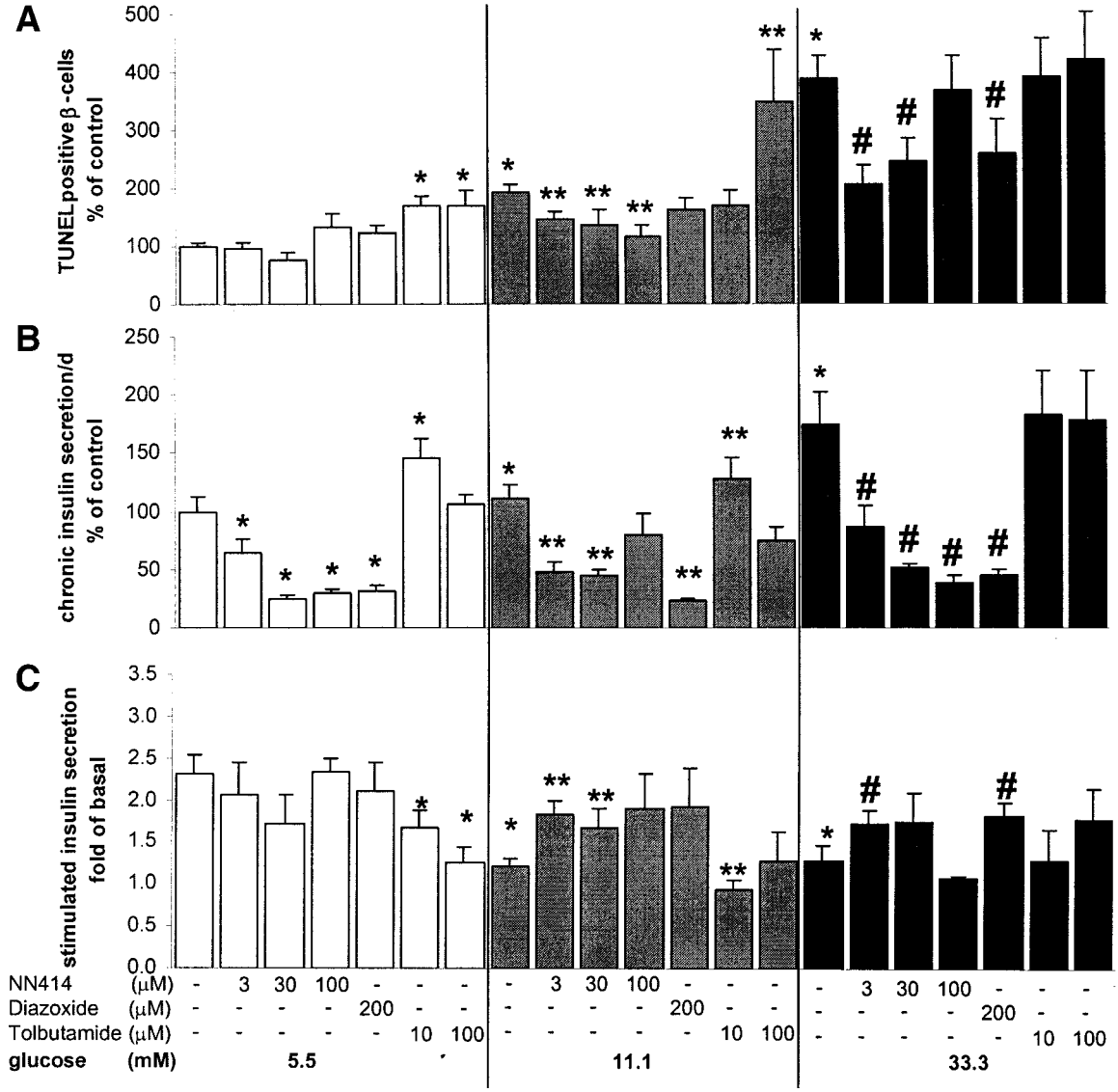

FIG. 1. K_{ATP} channel openers protect from glucose-induced β-cell apoptosis and impaired secretory function in human islets. Human islets were cultured on extracellular matrix–coated dishes for 4 days in 5.5, 11.1, and 33.3 mmol/l glucose alone or with 3, 30, and 100 μmol/l NN414; 200 μmol/l diazoxide; or 10 and 100 μmol/l tolbutamide. *A*: Results are means ± SE of the percentage of TUNEL-positive β-cells per islet normalized to control incubations at 5.5 mmol/l glucose alone (100%; in absolute value, 0.32 ± 0.03 TUNEL-positive β-cells per islet). Islets were isolated from five organ donors. The mean number of islets scored from each donor was 28 (range 21–45) for each treatment condition. *B*: Chronic insulin release into the culture medium during the 4-day incubation period. Results are means ± SE of the insulin secreted per islet relative to control incubations at 5.5 mmol/l glucose alone (100%; in absolute value, 3.45 ± 0.59 pmol · islet^{-1} · day^{-1}). *C*: Ratio of stimulated to basal insulin secretion during successive 1-h incubation at 3.3 (basal) and 16.7 (stimulated) mmol/l glucose after the 4-day culture period. Results are means ± SE. Islets were isolated from three organ donors. In each experiment, the data were collected from three plates per treatment. *$P < 0.05$ vs. control islets at 5.5 mmol/l glucose alone; **$P < 0.05$ vs. islets at 11.1 mmol/l glucose alone; #$P < 0.05$ vs. islets at 33.3 mmol/l glucose alone.

medium glucose concentration of 11.1 and 33.3 mmol/l, respectively, relative to islets at 5.5 mmol/l glucose (Fig. 1*A*). The K_{ATP} channel openers NN414 and diazoxide did not significantly change baseline apoptosis at 5.5 mmol/l glucose. However, 3, 30, and 100 μmol/l NN414 partially protected the β-cells from apoptosis induced by 11.1 mmol/l glucose, whereas only 3 and 30 μmol/l NN414 were protective at 33.3 mmol/l glucose. Diazoxide had no significant effect in the presence of 11.1 mmol/l glucose, but 200 μmol/l diazoxide did protect from 33.3-mmol/l glucose–induced apoptosis.

The effect of closure of K_{ATP} channels by the sulfonylurea tolbutamide was also tested. At 5.5 mmol/l glucose, exposure to 10 or 100 μmol/l tolbutamide induced a 1.7-fold increase in the number of TUNEL-positive β-cells, relative to islets at 5.5 mmol/l glucose alone. At 11.1 mmol/l glucose, 10 μmol/l tolbutamide had no additive effect on β-cell apoptosis induced by the closure of K_{ATP} channels by glucose itself, whereas 100 μmol/l tolbutamide induced a similar deleterious effect to that of 33.3 mmol/l glucose alone. Tolbutamide had no additive effect to 33.3-mmol/l glucose–induced DNA fragmentation.

Exposure of cultured human islets to elevated glucose concentrations for 4 days decreased the number of proliferating (Ki-67$^+$) β-cells. Proliferation was reduced by 34 and 61% in medium that contained 11.1 and 33.3 mmol/l glucose, respectively, relative to islets at 5.5 mmol/l glucose alone (100%; in absolute value: 0.89 ± 0.2% Ki-67$^+$

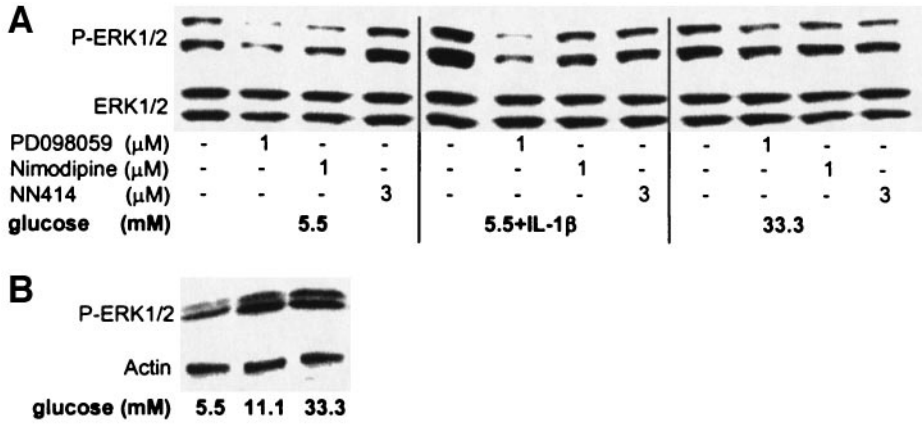

FIG. 2. Glucose and IL-1β activate ERK1/2 in human islets. Human islets were cultured in suspension for 30 min in 5.5 mmol/l glucose alone or in the presence of 2 ng/ml IL-1β or in 11.1 or 33.3 mmol/l glucose with or without preincubation for 1 h with 1 μmol/l PD098059, 1 μmol/l nimodipine, or 3 μmol/l NN414. Islet extracts were analyzed by Western blotting for Thr^{202}/Tyr^{204}-phosphorylated ERK1/2 (P-ERK1/2) and total ERK1/2 (ERK1/2) or actin. One of three experiments with all conditions, except for the presence of PD098059 and 11.1 mmol/l glucose (two experiments), from six organ donors is shown. All experiments gave similar results.

β-cells; $P < 0.01$). Tolbutamide, diazoxide, and NN414 had no significant influence on β-cell proliferation at those glucose concentrations (data not shown).

Accumulated insulin release into the culture medium was measured after the 4-day culture period. Glucose (11.1 and 33.3 mmol/l) as well as 10 μmol/l tolbutamide increased accumulated insulin release, which was decreased by the K_{ATP} channel openers NN414 and diazoxide (Fig. 1*B*).

Exposure of human islets to 11.1 or 33.3 mmol/l glucose for 4 days completely abolished acute glucose-stimulated insulin release (Fig. 1*C*). Coincubation with 3 and 30 μmol/l NN414 or 200 μmol/l diazoxide partially restored glucose responsiveness. Similar to elevated glucose concentrations, chronic exposure to tolbutamide also impaired β-cell secretory function.

Insulin content of islets cultured for 4 days in high glucose or in the presence of tolbutamide was decreased. Insulin content was reduced by 12.9, 47.9, and 55.8% in medium that contained 11.1 mmol/l glucose, 33.3 mmol/l glucose, and 100 μmol/l tolbutamide, respectively, relative to islets at 5.5 mmol/l glucose alone ($P < 0.01$). Coincubation with 3 μmol/l NN414 prevented the decrease in insulin content by 30.2% ($P < 0.05$) and 111.2% ($P < 0.01$) compared with 11.1 and 33.3 mmol/l glucose alone, respectively, whereas 200 μmol/l diazoxide prevented the decrease by 33.9% at 33.3 mmol/l ($P < 0.05$) but had no significant effect at 11.1 mmol/l glucose.

Glucose-induced β-cell apoptosis and impaired function require L-type Ca^{2+} influx and activation of ERK MAPK. To investigate whether ERK1/2 is activated by glucose and IL-1β, we performed Western blotting with phosphospecific antibodies that recognize only Thr^{202}/Tyr^{204}-phosphorylated ERK1/2 (39,54). As seen in Fig. 2, exposure of cultured human islets to 11.1 and 33.3 mmol/l glucose or 2 ng/ml IL-1β for 30 min enhanced the phosphorylation of ERK1/2 (P-ERK1/2), whereas total ERK1/2 remained unchanged. In contrast, baseline as well as IL-1β– and glucose-induced ERK1/2 phosphorylation were decreased by preincubation for 1 h with 1 μmol/l of the inhibitor of MAPK/ERK1/2 PD098059. Similarly, ERK1/2 phosphorylation was also prevented by preincubation for 1 h with 1 μmol/l of the L-type Ca^{2+} channel blocker nimodipine. It is interesting and in contrast to PD098059 and nimodipine that preincubation for 1 h with 3 μmol/l of the β-cell–selective potassium channel opener NN414 had no influence on baseline ERK1/2 phosphorylation but decreased glucose- and IL-1β–induced ERK activation.

We next studied the functional role of glucose-induced, calcium-mediated, ERK activation in human β-cells; specifically, we investigated the ability of nimodipine and PD098059 to protect β-cells from glucose-induced apoptosis and impaired function. Exposure of human islets to 11.1 and 33.3 mmol/l glucose in the presence of 1 μmol/l nimodipine as well as 1 μmol/l PD098059 protected the β-cells from 11.1 and 33.3 mmol/l glucose–induced apoptosis and restored acute glucose-stimulated insulin secretion (Fig. 3). Furthermore, 1 μmol/l nimodipine and 1 μmol/l PD098059 induced a 2.4- and 1.9-fold increase of insulin content, respectively, in islets cultured at 33.3 mmol/l glucose ($P < 0.05$). A total of 1 μmol/l of each compound had no significant effect at 5.5 mmol/l glucose (Fig. 3), whereas higher concentrations of nimodipine (10 μmol/l) and PD098059 (10 μmol/l) by themselves induced β-cell apoptosis at 5.5 mmol/l glucose (data not shown). To rule out possible nonspecific actions, we confirmed the protective effects of PD098059 and nimodipine using an additional MAPK inhibitor (UO126) and Ca^{2+} channel blocker (nitrendipine) (Fig. 3*D*). Glucose-induced IL-1β release and IL-1β–induced β-cell apoptosis are also Ca^{2+} influx and ERK dependent and can be prevented by potassium channel openers.

The ability of nimodipine, PD098059, diazoxide, and NN414 to protect from the deleterious effects of IL-1β was tested. Exposure of human islets cultured on extracellular matrix–coated dishes to 2 ng/ml IL-1β for 4 days resulted in a 4.8-fold increase in β-cell apoptosis compared with control at 5.5 mmol/l (Fig. 4*A*). This was strongly reduced by coincubation with 1 μmol/l nimodipine, 1 μmol/l PD098059, 200 μmol/l diazoxide, or 3 μmol/l NN414. Similarly, these compounds restored glucose-stimulated insulin secretion (Fig. 4*B* and *C*). Furthermore, IL-1β decreased insulin content by 26.0% compared with islets at 5.5 mmol/l glucose alone, a decrease that was prevented by 61.6, 79.4, 62.1, and 70.0% by 200 μmol/l diazoxide, 3

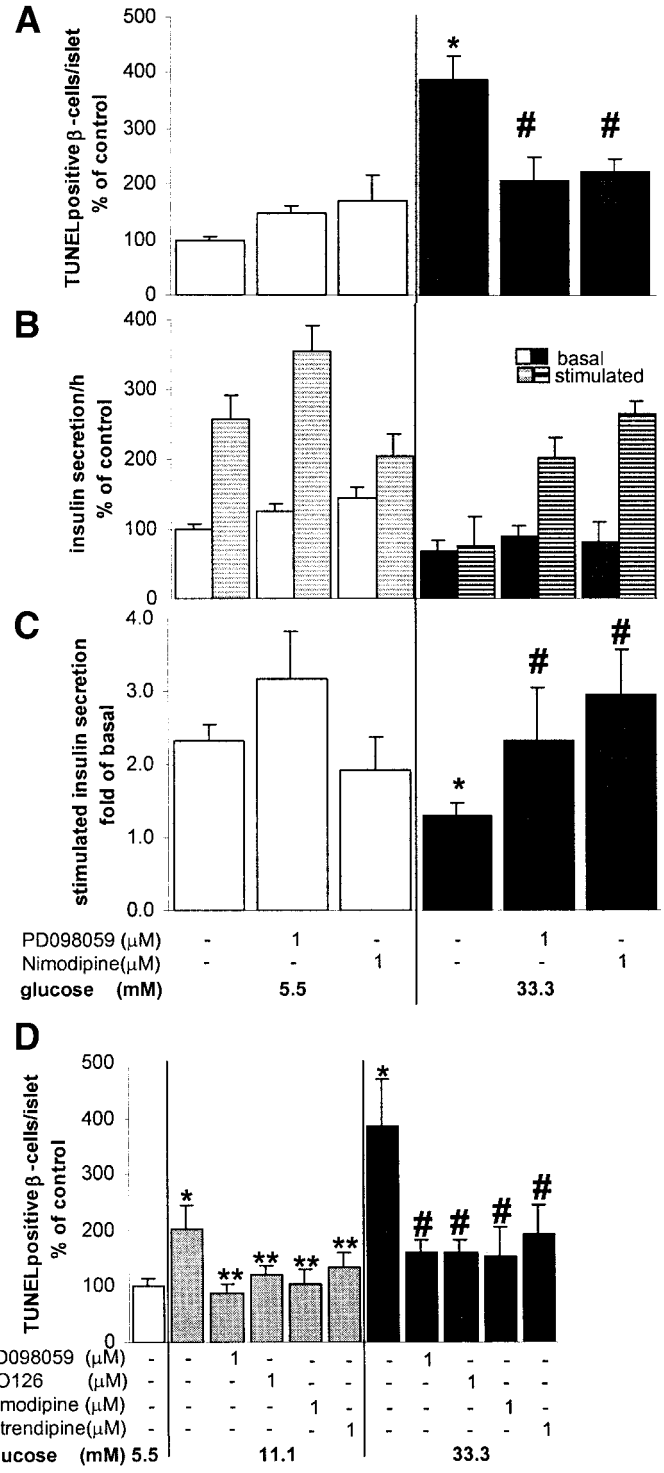

FIG. 3. Glucose-induced β-cell apoptosis and impaired function require L-type Ca^{2+} influx and ERK1/2 activation. Human islets were cultured on extracellular matrix–coated dishes for 4 days in 5.5, 11.1, and 33.3 mmol/l glucose alone or with 1 μmol/l nimodipine, 1 μmol/l nitrendipine, 1 μmol/l PD098059, or 1 μmol/l UO126. *A*: Results are means ± SE of the percentage of TUNEL-positive β-cells per islet normalized to control incubations at 5.5 mmol/l glucose alone (100%; in absolute value, 0.32 ± 0.03 TUNEL-positive β-cells per islet). The mean number of islets scored from each donor was 32 (range 25–43) for each treatment condition. *B* and *C*: Basal and stimulated insulin secretion during successive 1-h incubation at 3.3 (basal) and 16.7 (stimulated) mmol/l glucose after the 4-day culture period. Results are means ± SE of the insulin secreted per islet relative to control incubations at 5.5 mmol/l glucose alone (100%; in absolute value, 0.79 ± 0.08 pmol/islet for basal insulin). *D*: Results are means ± SE of the percentage of TUNEL-positive β-cells per islet normalized to control incubations at 5.5 mmol/l glucose alone. Islets were isolated from three (*A–C*) or one (*D*) organ donor. In each experiment, the data were collected from three plates per treatment. *$P < 0.05$ vs. control islets at 5.5 mmol/l glucose alone; **$P < 0.05$ vs. islets at 11.1 mmol/l glucose alone; #$P < 0.05$ vs. islets at 33.3 mmol/l glucose alone.

μmol/l NN414, 1 μmol/l nimodipine, and 1 μmol/l PD098059, respectively ($P < 0.05$). Finally, the role of K_{ATP} and Ca^{2+} channels and ERK in glucose-induced IL-1β release was tested. IL-1β secretion to the culture medium after a 36-h culture period at 11.1 and 33.3 mmol/l glucose increased 1.5- and 3.8-fold, respectively, and was prevented by 1 μmol/l nimodipine, 1 μmol/l PD098059, and 3 μmol/l NN414 (Fig. 5) as well as by 1 μmol/l nitrendipine and 1 μmol/l UO126, although this prevention reached statistical significance only at 33.3 mmol/l glucose (data not shown).

DISCUSSION

The central role of the K_{ATP} channel in mediating acute glucose-stimulated insulin release is well established. In the present study, we described an additional role of this channel as a key mediator of β-cell survival/apoptosis. Indeed, long-term (4 days) exposure to either glucose or IL-1β led not only to impaired β-cell secretory function but also to β-cell apoptosis. These effects were mediated by closure of K_{ATP} channels and therefore could be prevented by the potassium channel openers diazoxide and NN414. Furthermore, our data demonstrated that both glucose and IL-1β effects were mediated by Ca^{2+} influx and activation of ERK1/2.

The protective mechanisms of potassium channel openers have been associated with β-cell "rest." However, defective insulin secretion may be secondary to decreased β-cell mass (23,55–57). Therefore, the beneficial effect of potassium channel openers could be due to the antiapoptotic effects of these compounds described in the present study. After a 4-day exposure to high glucose or IL-1β, ~1% of the β-cells undergo apoptosis. Therefore, it is unlikely that this apoptotic process has a significant impact on β-cell function in vitro during this time period, whereas the direct toxic effects of chronic exposure to high glucose and to IL-1β on β-cell function can clearly be reversed by potassium channel openers. Nevertheless, the potent anti-apoptotic effect of these compounds may be of potential importance in vivo, when one considers that a decrease in β-cell mass is the hallmark of both type 1 and type 2 diabetes and the long-term consequence of even a small daily turnover of apoptotic β-cells.

Both glucose- and IL-1β–induced apoptosis and impaired function were dependent on Ca^{2+} influx and ERK1/2 activation and could be counteracted by the action of K_{ATP} channel openers. The similarity of the effects induced by the nutrient glucose and the cytokine IL-1β is explained by the ability of glucose to induce β-cell production of IL-1β (38). Surprisingly, the K_{ATP} channel opener NN414, the L-type Ca^{2+} channel blocker nimodipine, and the MAPK/ERK1/2 inhibitor PD098059 also prevented glucose-induced IL-1β release. It follows that the pathway that leads to islet production of IL-1β is also used by IL-1β itself to induce its toxic effects. That glucose-induced β-cell release

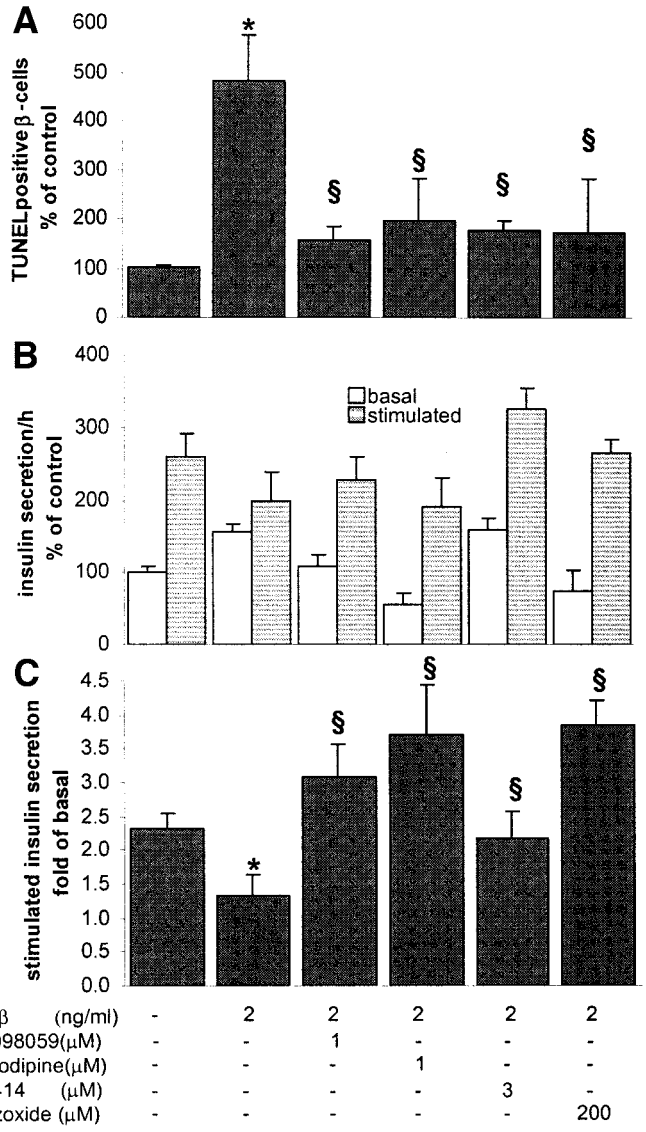

FIG. 4. IL-1β–induced β-cell apoptosis and impaired function require L-type Ca^{2+} influx and ERK1/2 activation and can be prevented by potassium channel openers. Human islets were cultured on extracellular matrix–coated dishes for 4 days in 5.5 mmol/l glucose or in the presence of 2 ng/ml IL-1β alone or with 1 μmol/l nimodipine, 1 μmol/l PD098059, 200 μmol/l diazoxide, or 3 μmol/l NN414. *A*: Results are means ± SE of the percentage of TUNEL-positive β-cells per islet normalized to control incubations at 5.5 mmol/l glucose alone (100%; in absolute value, 0.32 ± 0.03 TUNEL-positive β-cells per islet). Islets were isolated from three organ donors. The mean number of islets scored from each donor was 35 (range 31–41) for each treatment condition. *B* and *C*: Basal and stimulated insulin secretion during successive 1-h incubation at 3.3 (basal) and 16.7 (stimulated) mmol/l glucose after the 4-day culture period. Results are means ± SE of the insulin secreted per islet relative to control incubations at 5.5 mmol/l glucose alone (100%; in absolute value, 0.79 ± 0.08 pmol/islet for basal insulin). Islets were isolated from three organ donors. In each experiment, the data were collected from three plates per treatment. *$P < 0.05$ vs. control islets at 5.5 mmol/l glucose alone; §$P < 0.05$ vs. islets exposed to IL-1β alone.

of IL-1β is dependent on ERK1/2 is in line with a recent report (48) showing that ERK activation is required for virus-induced IL-1β expression in macrophages.

In the present study, we observed phosphorylation of ERK1/2 by glucose and IL-1β (already) after a 30-min incubation, whereas apoptosis and impaired function were investigated after 4-day exposures. Detection of ERK1/2 activation was investigated after such a short incubation time because it is shown to be rapidly activated. However, in rat islets exposed to IL-1β, ERK1/2 activation is still detectable after 24 h (39). Moreover, PD098059 inhibited ERK1/2 phosphorylation not only in the short-time experiment but also apoptosis and impaired function in the 4-day incubations. Therefore, ERK1/2 is also a mediator of the long-term effects of glucose and IL-1β.

IL-1β activation of ERK1/2 has previously been observed in rat islets cells (39–41). Moreover, in rat islets, IL-1β–induced expression of iNOS requires ERK1/2 activation (39). Although IL-1β also induces ERK1/2 activation in human islets, IL-1β alone does not induce iNOS, as has been shown repeatedly (38,58,59). Because of this difference between human and rat islets, it was important to demonstrate that IL-1β indeed activates ERK1/2 in human islets. Furthermore, we show that ERK1/2 mediates the deleterious effects of IL-1β on β-cell function and is required for IL-1β–induced apoptosis.

The TUNEL assay detects DNA fragmentation associated with both apoptotic and necrotic cell death. Therefore, the observation of glucose-induced TUNEL positivity and its antagonism by NN414, nimodipine, and PD098059 could reflect a modulation of necrosis and not apoptosis. Nevertheless, in several previous studies, we tested, in parallel to the TUNEL reaction, the DNA-binding dye propidium iodide, triple immunostaining for DNA fragmentation, insulin, and cleaved caspase 3 and were able to demonstrate solely an apoptotic process in the presence of high glucose (34,38,53).

The beneficial effect of NN414 against glucotoxic effects was maximal at a concentration of 3 μmol/l, whereas 100 μmol/l was much less effective. Accordingly, it was shown recently (60) in HEK293 cells stably expressing the pancreatic β-cell type K_{ATP} channel that the EC_{50} of NN414 is 0.45 μmol/l and that very high concentrations may be toxic.

β-Cell mass depends not only on β-cell apoptosis but also on the rate of neogenesis or replication. In the present study, tolbutamide, diazoxide, and NN414 had no significant influence on β-cell proliferation. However, our in vitro system does not allow for detection of neogenesis. Nevertheless, in type 2 diabetes, increased apoptosis seems of greater importance in the reduced β-cell mass than impaired neogenesis/proliferation (2).

In conclusion, this study suggests that in human islets, glucose- and IL-1β–induced β-cell secretory dysfunction and apoptosis are K_{ATP} channel, Ca^{2+} influx, and ERK dependent. Glucotoxicity and IL-1β are suggested to be major driving forces for β-cell death and impaired β-cell function in both type 1 and type 2 diabetes. Therefore, the use of orally active K_{ATP} channel openers may be a promising novel therapeutic strategy to preserve β-cell mass and function in type 1 and 2 diabetes.

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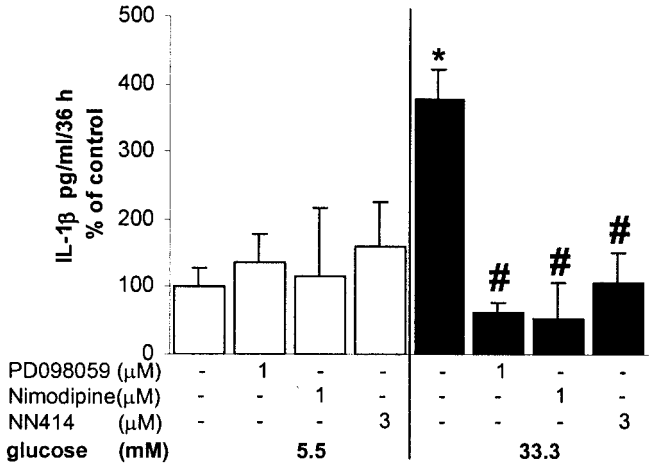

FIG. 5. Glucose-induced IL-1β release requires L-type Ca^{2+} influx and ERK1/2 activation and can be prevented by a potassium channel opener. Secretion of human islets cultured in suspension for 36 h in 5.5 or 33.3 mmol/l glucose alone or in the presence of 1 μmol/l PD098059, 1 μmol/l nimodipine, or 3 μmol/l NN414. Each bar represents the means ± SE of four separate experiments with islets from four organ donors relative to control incubations at 5.5 mmol/l glucose alone (100%; in absolute values, 0.95 ± 0.22 pg · 20 islets^{-1} · 2 ml^{-1}). *P < 0.05 vs. control islets at 5.5 mmol/l glucose; #P < 0.05 vs. islets at 33.3 mmol/l glucose alone.

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