Is There a Role for Locally Produced Interleukin-1 in the Deleterious Effects of High Glucose or the Type 2 Diabetes Milieu to Human Pancreatic Islets?

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Different degrees of β-cell failure and apoptosis are present in type 1 and type 2 diabetes. It has been recently suggested that high glucose–induced β-cell apoptosis in type 2 diabetes shares a common pathway with type 1 diabetes, involving interleukin-1β (IL-1β) production by β-cells, nuclear factor-κB (NF-κB) activation, and death via Fas-FasL. The aim of this study was to test whether human islet exposure to high glucose in vitro, or to the type 2 diabetes environment in vivo, induces IL-1β expression and consequent activation of NF-κB–dependent genes. Human islets were isolated from five normoglycemic organ donors. The islets were cultured for 48 h to 7 days at 5.6, 11, or 28 mmol/l glucose. For comparative purposes, islets were also exposed to IL-1β. Gene mRNA expression levels were assessed by real-time RT-PCR in a blinded fashion. Culture of the human islets at 11 and 28 mmol/l glucose induced a four- to fivefold increase in medium insulin as compared with 5.6 mmol/l glucose, but neither IL-1β nor IL-1 receptor antagonist (IL-1ra) expression changed. IL-1β and IL-1ra protein release to the medium was also unchanged. Stimulated human monococytes, studied in parallel, released >50-fold more IL-1β than the islets. There was also no glucose-induced islet Fas expression. Expression of the NF-κB–dependent genes ICβ-α and monocyte chemoattractant protein (MCP)-1 was induced in human islets by IL-1β but not by high glucose. In a second set of experiments, human islets were isolated from seven type 2 diabetic patients and eight control subjects. The findings on mRNA levels were essentially the same as in the in vitro experiments, namely the in vivo diabetic state did not induce IL-1β, Fas, or MCP-1 expression in human islets, and also did not modify IL-1ra expression. The present findings suggest that high glucose in vitro, or the diabetic milieu in vivo, does not induce IL-1β production or NF-κB activation in human islets. This makes it unlikely that locally produced IL-1β is an important mediator of glucotoxicity to human islets and argues against the IL-1β–NF-κB–Fas pathway as a common mediator for β-cell death in type 1 and type 2 diabetes.

Chronic β-cell exposure to hyperglycemia and/or elevated free fatty acids may lead to dysfunction and death of β-cells in type 2 diabetes, a phenomenon denominated by glucotoxicity or glucolipotoxicity (1–3). This progressive loss of pancreatic β-cells (4–6) probably contributes to the worsening of glycemic control in type 2 diabetic patients (7).

It has been recently suggested that prolonged exposure of human islets to high glucose triggers interleukin-1β (IL-1β) production by the β-cells themselves (8,9), leading to nuclear factor-κB (NF-κB) activation and upregulation of Fas signaling (8,10,11), thus triggering “autocrine apoptosis” (8–12 and rev. in 13,14). Since both IL-1β and NF-κB are important mediators for cytokine-induced β-cell death in type 1 diabetes (3,15), it was proposed that IL-1β–NF-κB is a common final pathway for β-cell death in both type 1 and type 2 diabetes (13) and that the classification and treatment of type 1 and type 2 diabetes should be revisited accordingly (14).

Most of the data supporting this novel hypothesis have been generated by one research group (rev. in 13), and, based on these findings, a clinical trial has been initiated using interleukin-1 receptor antagonist (IL-1ra) to prevent β-cell death in type 2 diabetic patients (14). Of concern is, however, that other groups have failed to reproduce in rodent models the key findings of this unifying hypothesis for β-cell death in diabetes. Specifically, we and others failed to detect glucose-induced NF-κB activation or IL-1β production in whole rat islets, in fluorescence-activated cell sorter–purified primary rat β-cells, in the β-cell line INS-1E (16), or in islets isolated from prediabetic Psammomys obesus (3). In addition, islets isolated from mice that lack the IL-1 receptor or Fas were not protected against β-cell dysfunction and death induced by long-term exposure to high glucose (17).

Part of this discrepancy might be related to species differences, and it could not be excluded that high glucose–induced IL-1β production and NF-κB activation is a phenomenon specific for human pancreatic islets. To evaluate this, and to further test the intriguing hypothesis that glucotoxicity is IL-1β-NF-κB-mediated, we have presently utilized the following two approaches: J) To measure the expression of IL-1β, IL-1ra, and other NF-κB–dependent genes in human islets exposed for 2–7 days in vitro to...
5.6, 11, or 28 mmol/l glucose; and 2) To study the expression of these same genes in human islets isolated from either type 2 diabetic patients or from nondiabetic organ donors (control subjects). To avoid any potential bias, all determinations of mRNA expression were done blindly in a central laboratory (Brussels), using cDNA obtained from human islets isolated in Uppsala (in vitro studies) or in Pisa (islets obtained from type 2 diabetic patients and nondiabetic controls). The data unequivocally show that neither high glucose in vitro nor the diabetic state in vivo induces IL-1β production or NF-κB activation in human islets, suggesting that locally produced IL-1β is not a mediator of glucotoxicity to human islets.

RESEARCH DESIGN AND METHODS

Islet isolation, cell culture, and exposure to different glucose concentrations. Human pancreatic islets were kindly provided by Prof. Olle Korsgren (Uppsala Akademiska Sjukhuset, Uppsala, Sweden). This study was approved by the Uppsala University Research Ethical Committee. The human pancreatic islets were isolated from the pancreas of five brain-dead organ donors (Table 1) using collagenase digestion and Biocoll gradient centrifugation (18). After isolation, the islets were cultured free-floating in Sterilin dishes approved by the Uppsala University Research Ethical Committee. The human islets were kindly provided by Prof. Kenneth Nilsson (Uppsala University, Uppsala, Sweden). The cells were cultured in RPMI-1640 medium containing 10% FCS and one of the following supplements: 5.6, 11, or 28 mmol/l glucose; 5.6 mmol/l glucose + recombinant human IL-1β (25 units/ml) (PeproTech, London, U.K.); or 5.6 mmol/l glucose + IL-1β (25 units/ml) and γ-interferon (IFN-γ) (1,000 units/ml) (PeproTech). The culture media were changed on days 2 and 5.

The human U937 monocyte cell line was kindly provided by Prof. Kenneth Nilsson (Uppsala University, Uppsala, Sweden). The cells were cultured in RPMI-1640 medium containing 10% heat-inactivated FCS and 2 mmol/l glucose. To induce differentiation into a macrophage phenotype (20), 50% confluent U937 cells were treated with tetradecanoylphorbol acetate (TPA) (50 nmol/ml) for 4 h. The TPA was then replaced by lipopolysaccharide (LPS) (10 μg/ml; Sigma) and the cells maintained in culture for another 20 h. Human dendritic cells were isolated from peripheral blood mononuclear cells from two healthy donors as previously described (21). They were cultured in RPMI-1640 medium supplemented with 2 mmol/l glutamine, 20 μg/ml gentamicin, 50 μg/ml 2-mercaptoethanol, 1% nonessential amino acids (Gibco), and 10% FCS. The dendritic cells were stimulated or not (control) by 1 μg/ml LPS Escherichia coli (Sigma) and 10 ng/ml IFN-γ (Biosource) for 6 h. The experiments were performed in duplicate for each donor.

Table 1 shows the impact of the in vivo diabetic environment on human islet gene expression, pancreatic islets were isolated in Pisa, Italy, from seven type 2 diabetic and eight nondiabetic cadaveric organ donors (Table 2). The nondiabetic organ donors were matched for age and BMI with the diabetic donors. The diagnosis of diabetes was made by family physicians, and the diabetic organ donors had been known to have diabetes for 6.7 ± 2.9 years. The pancreata were obtained and processed with the approval of the local ethics committee. The islets were isolated by enzymatic digestion and density gradient purification, as previously described (22–24). At the end of the isolation procedure, the islets were placed in M199 medium containing 5.5 mmol/l glucose and 10% adult bovine serum and studied within 3–4 days from isolation. Electron microscopy was performed to assess β-cell purity as previously described (22). Briefly, samples were fixed with glutaraldehyde, postfixed with osmium tetroxide, dehydrated in ethanol, and embedded in Epon-Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate. The cell composition was counted in micrographs and used to calculate the percentage of β-cells per total endocrine cell number (24).

Table 1
Characteristics of five normoglycemic organ donors

<table>
<thead>
<tr>
<th>Donor</th>
<th>Age (years)</th>
<th>Sex</th>
<th>BMI (kg/m²)</th>
<th>Cause of death</th>
<th>Cold ischemia time (h)</th>
<th>β-Cell purity (%)</th>
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</thead>
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<tr>
<td>Control</td>
<td>52</td>
<td>M</td>
<td>24.2</td>
<td>Cerebral hemorrhage</td>
<td>15</td>
<td>64</td>
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<td>Control</td>
<td>54</td>
<td>M</td>
<td>29.4</td>
<td>Cerebral hemorrhage</td>
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<tr>
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<td>F</td>
<td>20.2</td>
<td>Cerebral hemorrhage</td>
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<td>58</td>
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<tr>
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<td>M</td>
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<td>10</td>
<td>60</td>
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<tr>
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<td>22.6</td>
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<td>F</td>
<td>22.5</td>
<td>Cerebral hemorrhage</td>
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<tr>
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<td>28.9</td>
<td>Cerebral hemorrhage</td>
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**IL-1β DOES NOT MEDIATE GLUCOTOXICITY**

Real-time RT-PCR analysis. Total islet RNA was purified in Uppsala using the Ultraspec RNA reagent (Biotech, Houston, TX). cDNA was synthesized using 1 unit reverse transcriptase-Moloney murine leukemia virus (Finnzymes, Espoo, Finland) and 50 μmol/l oligo(dT)primers at 42°C for 60 min. The cDNA was then precipitated by adding ammonium acetate and ethanol, washed with 70% ethanol, and dried before real-time PCR analysis. In Pisa, total RNA was extracted from human islets and handled as previously described (25) using the RNeasy protect mini kit (QIAGEN, Santa Clarita, CA). cDNA was synthesized from 2 μg RNA. All human islet cDNA material was sent coded to the central laboratory in Brussels, where the real-time analyses were performed in a blinded fashion. Following the real-time PCR analysis, the results were sent to Uppsala or Pisa, after which the code of the samples was broken. Poly(A)^+ RNA was isolated from human dendritic cells in Brussels, using oligo(dT)25-coated polystyrene Dynabeads (Dynal, Oslo), and reverse transcribed as previously described (26).

The real-time PCR amplification reaction was done in 20 μl containing 3 mmol/l MgCl2, 0.5 μmol/l forward and reverse primers, 2 μl FastStart SYBR Green mix (Roche), and 2 μl cDNA. Standards for each gene were prepared using appropriate primers in a conventional PCR and purified for subsequent analyses. PCR products were quantified fluorometrically using SYBR Green, using appropriate primers in a conventional PCR and purified for subsequent analyses. PCR products were quantified fluorometrically using SYBR Green, using appropriate primers in a conventional PCR and purified for subsequent analyses.

**Enzyme-linked immunosorbent assay (ELISA) for insulin, IL-1β, and IL-1ra.** Insulin was determined in the perfusion fractions and in the medium following static incubation using the high-range human insulin ELISA (Merckodia, Uppsala, Sweden). Culture medium contents of human IL-1β and human IL-1ra were determined in 100-μl fractions using commercial ELISA kits from R&D Systems (Minneapolis, MN) according to the instructions of the manufacturer.

**Statistical analysis.** Data are shown as means ± SE, and comparisons between groups were carried out by Student’s t test or by Mann-Whitney test. A P value of ≤0.05 was considered statistically significant.

RESULTS

In the first series of experiments, we studied human islets obtained from five normoglycemic organ donors. Information about the islet donors is provided in Table 1. These preparations contained on average 47.4 ± 4.1% β-cells, as determined by immunohistochemistry for insulin. To confirm the good functional status of the islets, insulin secretion was evaluated in perfusion experiments (Fig. 1). All human islet preparations had a well-preserved biphasic insulin release in response to glucose, with a >15-fold increase in insulin secretion at 16.7 mmol/l glucose as compared with basal insulin release. These islets were then cultured for 48 h or 7 days in the presence of low, medium, or high glucose concentrations. As positive controls, we exposed islets to IL-1β and, in some experiments, IL-1β + IFN-γ. In agreement with our previous observations (19), culture of human islets for 48 h at high glucose concentrations increased insulin release. Thus, insulin accumulation in the medium at 5.6 mmol/l glucose was 28 ± 2 ng/ml × 50 islets × 48 h, and it increased by 3.8 ± 1.8-fold at 11 mmol/l glucose and 5.2 ± 1.2-fold at 28 mmol/l glucose ($P < 0.05$ vs. 5.6 mmol/l glucose for 11 and 28 mmol/l glucose by Mann-Whitney test; $n = 4$). This increased insulin release was maintained between days 2 and 7, with a >10-fold medium insulin accumulation at 11 or 28 mmol/l glucose as compared with 5.6 mmol/l glucose...
Exposure of human islets to IL-1 tended to increase insulin accumulation, with a 2.1–0.6 and 3.0–1.5–fold increase in medium insulin as compared with control subjects after, respectively, 48 h and 7 days. This is in agreement with our previous observations that a 48-h exposure of human islets to IL-1 alone increases both medium insulin accumulation and acute glucose-induced insulin release (28). In summary, the human islet preparations used in this study were rich in β-cells and showed excellent acute glucose responsiveness and maintained insulin secretory function during in vitro culture.

We next determined whether high glucose, or the positive control IL-1, induced expression of iNOS, IL-1, or other NF-κB–dependent mRNAs (Fig. 2). Culture at different glucose concentrations did not affect expression of the housekeeping gene β-actin. Thus, as compared with 5.6 mmol/l glucose (considered as 100%), β-actin expression was 120 ± 41% at 11 mmol/l glucose, 114 ± 32% at 28 mmol/l glucose, and 80 ± 32% in the presence of IL-1 after 48 h. Similar results were observed after 7 days (data not shown). Culture at 11 or 28 mmol/l glucose did not affect iNOS expression, while IL-1 induced a mild and transitory (48 h) increase in iNOS mRNA (Fig. 2). In agreement with these observations, medium nitrite accumulation after 48 h was 2.4 ± 2.0, 1.7 ± 0.9, 3.5 ± 2.6 and 3.1 ± 1.6 × 10^-2 pmol/islet × h at, respectively, 5.6, 11, or 28 mmol/l glucose or in the presence of IL-1. On the other hand, a combination of IL-1 + IFN-γ induced a 90-fold increase in iNOS expression and a 50-fold increase in medium nitrite accumulation, as compared with human islets cultured at 5.6 mmol/l glucose for 48 h (n = 3). This is in line with our previous findings that IL-1 + IFN-γ, but not IL-1 alone, induces iNOS expression and nitrite production by human islets (28–30).

Culture of human islets for 48 h or 7 days at 11 or 28 mmol/l glucose did not modify expression of IL-1 or IL-1ra, while IL-1 induced a nonsignificant trend for an increased IL-1 expression at 48 h (Fig. 2). To confirm these findings at the protein level, we measured IL-1 and IL-1ra protein release into the medium (Fig. 3). In agreement with the mRNA data, culture at different glucose concentrations did not modify IL-1 or IL-1ra medium accumulation. Similar results were obtained after 7 days.

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Expression of different mRNAs was analyzed by real-time PCR, and the control islets (\(n=10\)) secreted 50-fold more than the average IL-1\(\beta\) production by human islets. We probably underestimated IL-1\(\beta\) production by the monocytes, because the 250 pg/ml upper limit of the assay was exceeded in all human monocyte samples. On the other hand, basal IL-1ra production by the human islets was triplefold higher than of the human islets, with IL-1ra medium accumulation of 398 \(\pm\) 33 pg/ml \times 10\(^6\) cells \times 24 h. This increased to 1,163 \(\pm\) 120 pg/ml \times 10\(^6\) cells \times 24 h following monocyte stimulation \((P<0.05\) vs. basal state, \(n=3\)).

Fas expression in human islets was neither modified by culture at different glucose concentrations nor by exposure to IL-1\(\beta\) (Fig. 2). The nonsignificant trend to an increase in Fas expression at 28 mmol/l glucose after 7 days was driven by a fourfold increase in Fas expression in a single human islet preparation. We have previously shown that Fas mRNA expression in human islets is induced by IFN-\(\gamma\), but not by IL-1\(\beta\) (31). In agreement with these findings, a combination of IL-1\(\beta\) + IFN-\(\gamma\) induced Fas mRNA expression by 2.9- and 3.2-fold compared with islets exposed to IL-1\(\beta\) alone after 48 h treatment (in two experiments performed in parallel with the experiments shown in Fig. 2).

We next evaluated expression of two other genes previously shown to be NF-\(\kappa\)B-dependent in rodent \(\beta\)-cells, namely MCP-1 (32) and I\(\kappa\)B-\(\alpha\) (33). Culture at different glucose concentrations neither modified MCP-1 nor I\(\kappa\)B-\(\alpha\) expression, while IL-1\(\beta\) increased expression of both genes by two- to fivefold (Fig. 2). These discrepant effects of high glucose and IL-1\(\beta\) on the expression of two well-known NF-\(\kappa\)B-dependent genes are in line with the lack of glucose-induced IL-1\(\beta\) production by human islets (Fig. 3).

Since hyperglycemia is not the sole component of the diabetic milieu, we examined in a second series of experiments whether human islets isolated from type 2 diabetic patients express IL-1\(\beta\) or other NF-\(\kappa\)B-dependent genes. For this purpose, islets were obtained from seven type 2 diabetic patients and eight normoglycemic organ donors (Table 2). Except for the expected difference in glycemia in the intensive care unit (control 130 \(\pm\) 10 mg/dl \([n=8]\), type 2 diabetes 219 \(\pm\) 30 mg/dl \([n=6]\), \(P<0.05\) by unpaired \(t\) test), there were no significant differences between the groups regarding age (control 59 \(\pm\) 4 years, type 2 diabetes 66 \(\pm\) 2 years), sex, BMI (control 26 \(\pm\) 1 kg/m\(^2\), type 2 diabetes 26 \(\pm\) 1 kg/m\(^2\)) or cause of death (Table 2). Moreover, the mean cold ischemia time was similar in both groups (control 10 \(\pm\) 1 h, type 2 diabetes 13 \(\pm\) 2 h), and in three samples from control or type 2 diabetic patients examined by electron microscopy there was a similar proportion of \(\beta\)-cells, namely 61 \(\pm\) 2\% in the control group and 58 \(\pm\) 2\% in the type 2 diabetic group \((n=3)\). In none of the islets studied by electron microscopy were there detectable infiltrating immune cells. In vivo exposure to the diabetic environment did not affect expression of the housekeeping gene \(\beta\)-actin: islets from type 2 diabetic patients had a \(\beta\)-actin expression level corresponding to 75\% of the values observed in normoglycemic donors (not significant). Expression of IL-1\(\beta\), IL-1ra, Fas, and MCP-1 mRNAs were similar in islets from normoglycemic and diabetic patients, but there was a 30\% increase in I\(\kappa\)B-\(\alpha\) expression in islets isolated from type 2 diabetes \((P<0.05\) by unpaired \(t\) test) (Fig. 4). Similar results were obtained when examining only the three control and type 2 diabetic islet preparations with known \(\beta\)-cell content (data not shown). Previous studies of islets from type 2 diabetic patients by the same laboratory demonstrated molecular and functional islet defects such as decreased glucose-stimulated insulin secretion, reduced GLUT-1 and -2, insulin and glucokinase gene expression, and increased markers of oxidative stress (24), suggesting that alterations in islets obtained from type 2 diabetic donors are maintained following islet isolation and culture and can be detected in vitro. Our present observations are in good agreement with the above described in vitro findings and do not support the concept that type 2 diabetes is associated with increased expression of IL-1\(\beta\) or Fas in the islets.

**DISCUSSION**

There have been conflicting results regarding the putative role for IL-1\(\beta\) production and NF-\(\kappa\)B activation in the deleterious effects of prolonged exposure to high glucose on human pancreatic islets (3,8,13,14,16,17). Because this is an important issue for the understanding of the pathogenesis of type 2 diabetes, we currently tested this hypothesis in well-characterized human islet preparations, containing \(\approx\)50\% \(\beta\)-cells. Adequate \(\beta\)-cell function was confirmed by perfusion studies, which showed a nearly 15-fold biphasic increase in insulin secretion in response to glucose. To exclude potential biases in the data generation and interpretation, human islet isolation and culture was performed in Uppsala, after which the real-time RT-PCR analyses were run blindly in Brussels. Culture of human islets for 48 h or 7 days at 11 and 28 mmol/l glucose induced a 4- to 11-fold increase in medium insulin accumulation as compared with 5.6 mmol/l glucose, but neither IL-1\(\beta\) nor IL-1ra expression varied at the different glucose concentrations tested. There was also no glucose-induced Fas expression, the proposed NF-\(\kappa\)B-dependent mechanism by which glucose causes \(\beta\)-cell death (13,14). Furthermore, expression of the NF-\(\kappa\)B-dependent genes I\(\kappa\)B-\(\alpha\) (33) and MCP-1 (32) was induced in human islets by IL-1\(\beta\), used as a positive control, but not by high glucose. Importantly, and in keeping with the RNA data, IL-1\(\beta\) and IL-1ra protein accumulation in the medium was not modified by the different glucose concentrations. Stimulated human monocytes, studied in parallel, released \(>50\)-fold
more IL-1β than the islets. Since the effects of IL-1β on target cells depend on a balance between IL-1β and IL-1ra, which competes for the IL-1 receptor without transducing signals (34), we calculated the ratio between IL-1ra and IL-1β. This ratio for human islets was not changed by exposure to different glucose concentrations (data not shown). The average human islet ratio, pooling all glucose conditions, was 132 ± 31. In contrast, the IL-1ra-to-IL-1β ratio in activated human monocytes was 4 ± 1. We have previously shown that a 10- to 100-fold excess of IL-1ra over IL-1β suffices to block the effects of IL-1β on pancreatic islets (35), suggesting that the >100-fold excess of IL-1ra/IL-1β observed in our present experiments should block any putative biological effects of the minute amounts of IL-1β produced by human islets.

We have no straightforward explanation for the discrepancy between the reports from Maedler and colleagues (8,13), suggesting that high glucose induces a biologically relevant IL-1β production and NF-κB activation (14) and the negative findings in rat (16) and human islets (present data). Our present data were obtained in free-floating human islets, a culture system that assures adequate preservation of islet function and composition (see above), while Maedler et al. (8) cultured human islets spread on an extracellular matrix. Culture on an extracellular matrix allows long-term survival and proliferation of nonendocrine cells, such as duct cells or fibroblasts, which usually contaminate human islet preparations (36,37). Maedler et al. (8) neither provided information on the cellular composition or function of their human islet preparations cultured on the extracellular matrix nor showed electron microscopy information to exclude image superpositions between cells. It is thus possible that their observed high glucose–mediated IL-1β production and NF-κB activation actually represents an effect of high glucose on contaminating nonendocrine cells.

It is difficult to determine which in vitro model more accurately represents the in vivo situation. We therefore experimentally tested the IL-1β–NF-κB hypothesis in another model, namely human islets isolated from nondiabetic or type 2 diabetic donors. This rare and valuable collection of samples was described in detail in previous publications (24,38,39). The main advantages of utilizing these islets are twofold. First, culture conditions are not an issue, and second, the islets have been exposed to an in vivo type 2 diabetic milieu, which is more relevant for the understanding of human disease than pure in vitro models. mRNA expression was again determined blindly in the Brussels’ central laboratory, and the conclusions were in agreement with the in vitro experiments outlined above. The type 2 diabetic environment did not induce expression of IL-1β, Fas, or other NF-κB-dependent genes, except for a minor (30%) induction of IkB-α, which actually acts as an NF-κB inhibitor (40).

The present study adds up with detailed time-course observations in high glucose–treated rat islets, which showed no IL-1β and Fas expression, nor NF-κB activation (16); recent data obtained in islets from Fas and IL-1 receptor KO mice cultured at high glucose, which were not protected against glucotoxicity (17); and studies in P. obesus using immunohistochemistry (3 and A. Jörns, S. Lenzen, unpublished observations) and in situ PCR (A. Jörns, S. Lenzen, unpublished observations), which did not find evidence for induction of IL-1β or NF-κB–dependent genes in β-cells from prediabetic or early diabetic animals. We conclude that glucose-induced IL-1β production and NF-κB activation is unlikely to be a major mediator of glucotoxicity to human or rodent islet cells.

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