Impact of metabolic activity of beta cells on cytokine-induced damage and recovery of rat pancreatic islets


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Abstract. The influence of beta cell activity on cytokine-induced functional and structural impairments as well as the ability of those damaged cells to recover were investigated. Rat islets cultured for 4 days in the presence of 5, 10, and 30 mmol/l glucose were exposed to interferon-γ (IFN-γ, 500 U/ml) and tumor necrosis factor-α (TNF-α, 250 U/ml) for the last 24 h. After cytokine removal islets were allowed to recover spontaneously in culture medium containing 10 mmol/l glucose for a further 7 days. Cytokines significantly inhibited insulin release into culture medium, insulin storage, glucose-stimulated insulin secretion, protein, and DNA synthesis. In the presence of cytokines there was a six- to eightfold increase in nitrite production by the islets. The functional impairments were more pronounced in metabolically stimulated beta cells. In addition, cytokines caused membrane alterations as indicated by increased spontaneous chromium-51 release. The cytokines specifically induced the synthesis of two proteins (72 and 88 kDa, respectively). By immunoblotting, the 72-kDa protein was identified as heat shock protein. After a 1-week recovery period, insulin storage and stimulated insulin secretion of cytokine-treated islets were still significantly diminished. However, protein and DNA synthesis of cytokine-exposed islets returned to pre-exposure levels. In conclusion, high beta cell activity increases islet susceptibility to TNF+IFN. Cytokine-induced, long-lasting, inhibitory effects are primarily directed to beta-cell-specific functions, while general vital cell functions clearly recover after cytokine removal. The induction of certain proteins and the increased protein synthesis and replication rate after cytokine removal might reflect activated repair processes.

Key words: Cytokine – Islet of Langerhans – Insulin secretion – Nitrite – Heat shock proteins

Introduction

Cytokines released by activated mononuclear cells have been hypothesized to play a critical role in the development of insulin-dependent diabetes [1, 2]. In vitro, interleukin-1β (IL-1β) particularly has been shown to modulate insulin secretion in a concentration- and time-dependent fashion in insulin-producing cells [3] and also to induce islet cell damage [4, 5]. Tumor necrosis factor-α (TNF-α) alone has thus far been shown hardly to affect the functioning in rat and human beta cells; however, it synergistically potentiates the IL-1β-induced inhibition of insulin secretion [6, 7] and cytolytic effects in long-term cultures of rat and human islet cell monolayers [8]. Islet cells are sensitive to interferon-γ (IFN-γ) which is produced by activated T lymphocytes and NK cells. Unlike TNF-α, IFN-γ alone inhibits glucose-stimulated insulin secretion [9]. When added together, TNF-α and IFN-γ have been shown to exert synergistic functional and cytotoxic effects on isolated islets of Langerhans [8, 10]. The mechanisms by which each of the cytokines, either alone or in combination, act to impair beta cell functions or destroy the cells are unknown. Oxygen free radicals have been suggested to be involved in the impairment and destruction of beta cells [2, 11]. More recently, nitric oxide (NO) has been implicated as the cellular effector molecule mediating a number of IL-1β effects on islets [12, 13]. Exposure of non-transformed or transformed beta cells to IL-1β selectively causes the production of NO, as evidenced by an increase in nitrite and accumulation of cGMP [14, 15]. The free radical NO targets iron-sulfur centers of iron-containing enzymes [16], which results in mitochondrial dysfunction. High concentrations of glucose are suggested to prevent alloxan-induced beta cell damage [17], as well as to reduce IL-1β-mediated islet cytotoxicity [18]. Based on these findings, the purposes of this study were: (i) to examine whether the exposure of functionally resting or metabolically stimulated rat islets to a combination of IFN-γ and TNF-α would influence the degree of beta cell susceptibility to damage, (ii) to prove whether NO is involved in mediating the effects of IFN+TNF, and (iii) to inves-
gate the ability of the endocrine pancreas to recover spontaneously from possible functional and/or structural alterations following the removal of cytokines.

**Materials and methods**

**Isolation and preparation of islet cells.** Islets were isolated from pancreases of 10- to 12-day-old BB/OK [19] rats by collagenase (Serva, Heidelberg, Germany) digestion followed by dextran density gradient centrifugation [20] and hand picking under a stereomicroscope. Islets were dispersed into single cells by incubation with dispase (Boehringer, Mannheim, Germany), and viability of the cell preparations was evaluated by ethidium bromide/acridine orange staining [21] and consistently exceeded 95%.

**Islet culture.** Groups of 200 islets were cultured free floating in 6 ml of RPMI 1640 (Flow Laboratories, Irvine, UK) containing 11.1 mmol/l glucose, 10% fetal calf serum (FCS, Serva), 100 U/ml penicillin, and 100 U/ml streptomycin for 2-3 days. After preculture the islets were pooled and transferred in groups of 200 islets into 3 ml of culture medium containing 5, 10, or 30 mmol/l glucose and the supplements described above. The islets were maintained for 4 days at 37°C under an atmosphere of 95% air/5% CO₂. For the last 24 h the islets were either exposed to 500 U/ml recombinant IFN-γ (specific activity 4x 10⁶ U/mg; Bibby Dunn, Asbach, Germany) and 250 U/ml recombinant human TNF-α (specific activity 1x10⁷ U/mg; Bibby Dunn) or remained untreated (controls). Medium samples were taken before and after cytokine treatment for measurement of islet insulin release as well as for the determination of nitrite [22]. Groups of cytokine-exposed and control islets were then either used for functional tests or were cultured in RPMI 1640 containing 10 mmol/l glucose in the absence of cytokines for a further 7 days to allow spontaneous recovery. For some experiments islets were cultured in arginine-free RPMI 1640 medium supplemented with N-nitro-L-arginine methyl ester (L-NAME; Sigma, Deisenhofen, Germany). The other conditions were identical to those described above.

**Glucose-induced insulin secretion, insulin content, and protein synthesis.** The glucose responsiveness of control, cytokine-treated, and recovered islets was estimated in a postculture static incubation as described earlier [23]. Briefly, after preincubation in Krebs-Ringer bicarbonate buffer containing 5 mmol/l glucose, groups of 10 islets were challenged with 2 and 20 mmol/l glucose for 2 h. Duplicate aliquots of the incubation medium were removed for insulin radioimmunoassay (RIA) [24]. For estimation of insulin content, duplicates of 5 islets were sonicated, and aliquots of the homogenates were used for insulin RIA. The protein synthesis was determined by exposing groups of 10 islets for 2 h to 1.85 MBq/µl [3H]methionine (specific activity >37.0 TBq/mmol; Du Pont NEN) in methionine-free RPMI 1640 (Sigma) or in control groups or additionally IFN-γ/TNF-α at 37°C for 3 h. Islets removed after the recovery culture period were labelled at 10 mmol/l glucose in the absence of cytokines. After labelling, the islets were washed in ice-cold phosphate-buffered saline and lysed on ice in 10 mM Tris buffer, pH 7.5, containing 0.5% NP-40, 0.1 mM EDTA, 1 mM phenyl methylsulfonil fluoride. Aliquots of the lysate were taken for measurement of radioactivity as described for protein synthesis and for estimation of protein content [28]. Extracted proteins were subjected to electrophoresis on 10% acrylamide, 0.3% bis-sodium dodecylsulfate slab gels [29]. The gels were either stained with Coomassie blue G250 and used for autoradiography using hyperfilm MP (Amersham International) or used for protein transfer onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). Immunoblotting was performed according to Towbin et al. [30] using an anti-HSP 72 antibody (Amersham International) and anti-HSP 90 antibody (Dianova, Hamburg, Germany) and a secondary antibody conjugated with horseradish peroxidase (Jackson Immunoresearch Laboratories, West Grove, USA).

**Statistical analysis.** Data are presented as the mean±SEM of n different experiments. Statistical analysis was performed using SPSS for windows, release 5.02 (analysis of variance).

We followed the “Principles of laboratory animal care” (NIH publication No. 83–23, revised 1985). The study was licensed by the Ministerium für Landwirtschaft und Naturschutz des Landes Mecklenburg-Vorpommern.

**Results**

Neonatal rat islets cultured in the presence of different glucose concentrations for 4 days responded with a concentration-dependent release of insulin. When exposed to 5, 10, or 30 mmol/l glucose, islets released 1.05±0.32, 4.70±0.57, or 7.26±0.88 pmol insulin/islet in 4 days (n=8) into the culture medium, respectively. Insulin release in the presence of cytokines (last 24 h of the 4-day culture) was significantly inhibited when islets were cultured at 10 mmol/l glucose. The cytokine-induced inhibition of insulin release was more pronounced at a medium glucose concentration of 30 mmol/l (Fig. 1). Islets which have