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TNF-α inhibits glucose-induced insulin secretion in a pancreatic β-cell line (INS-1)

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Abstract Recent studies suggest that TNF-α affects various biochemical and physiological processes which may be linked to the etiology of non-insulin-dependent diabetes mellitus (NIDDM). For example, TNF-α interferes with the signaling of the insulin receptor and the metabolism of glucose transporters. The possibility that TNF-α might directly reduce glucose-stimulated insulin secretion in pancreatic β-cells was examined by using an established pancreatic β-cell line (INS-1). TNF-α did not affect glucose-induced acute insulin secretion (30 min). However, over a longer time period (24 h), TNF-α decreased glucose-induced insulin secretion without affecting the total amount of insulin in the cell. In the presence of TNF-α levels of 0, 10, 100 and 1000 U/ml, the respective 20 mM glucose-induced insulin secretion was 1.736 ± 0.166, 1.750 ± 0.302, 1.550 ± 0.200, and 1.400 ± 0.112 mU/ml per 3×10^5 cells in 24 h.

Key words: TNF-α; Insulin secretion; Cytokine; Acetyl-CoA carboxylase

1. Introduction

Recent studies suggest that the cytokines, interleukin-1 (IL-1) interferon-γ (IFN), and tumor necrosis factor α (TNF), alone or in combination, play a role in the pathogenesis of both type I and type II diabetes mellitus [1]. Although the exact mechanisms and sequence of events by which these cytokines trigger β-cell destruction and type I diabetes are not clear, there is strong collaborative evidence that the cytokines are involved in the onset of insulin-dependent diabetes mellitus (IDDM). TNF-induced β-cell destruction has been well documented [1]. In the case of non-insulin-dependent (type II) diabetes (NIDDM), TNF may act at different levels [2–4]. These multiple sites of action might explain the phenomenon of insulin resistance in the peripheral tissues. If TNF interferes with normal function at several levels, it might lead to both insulin resistance and NIDDM. The clinical manifestations of NIDDM might also be due to TNF action at the level of insulin secretion. The diminished sensitivity of β-cells towards insulin secretagogues, such as glucose, could likewise lead to the clinical manifestations of NIDDM. Indeed, there has been much debate over the years about whether the cause of NIDDM is insulin resistance or insulin deficiency [5].

In the present studies, we have examined the effects of TNF on glucose-induced insulin secretion by using a well-established pancreatic β-cell line (INS-1) [6], so that the effect of TNF on insulin secretion could be directly assessed. Our studies indicate that, over a long time period, TNF diminished glucose-dependent insulin secretion, although it did not affect acute glucose-dependent insulin secretion.

2. Materials and methods

2.1. Materials

Commercial products were obtained from the following sources: RPMI-1640 medium and cell culture plates from Sigma Chemical Company; donor calf serum, trypsin-EDTA, penicillin and streptomycin from GIBCO BRL; Micromedic insulin RIA kit from ICN; sodium pyruvate from Flow Laboratories. All other chemicals were analytical reagent grade.

2.2. Experimental procedure

An insulin secreting cell line (INS-1) was grown as described earlier [7] in 100-mm Petri dishes in RPMI-1640 medium containing 10 mM glucose, 10 mM HEPES, 10% heat-inactivated donor calf serum, 1 mM sodium pyruvate, 50 μM β-mercaptoethanol, 100 IU/ml penicillin and 100 μg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. For trypsinization, INS-1 cells at approximately 80% confluence were washed twice with PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.4, 37°C), and incubated in 1 ml of 0.5% trypsin-EDTA solution at 37°C for 2 min. The detached cells were diluted with medium and dispersed by passing through a 10-ml pipette. The cell suspension was spun at 500 rpm for 5 min, and the cell pellet was resuspended into about 10^5 cell/ml and seeded on 12-well culture plates. Cells were grown for 7 days, and then preincubated for 24 h in medium without glucose. In the short-term experiment, cells were further incubated in glucose-free KRB buffer (pH 7.4) containing 118 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.3 mM $MgSO_4$, 5 mM $NaHCO_3$, 25 mM HEPES and 0.07% BSA. Then freshly prepared KRB buffer containing different concentrations of glucose and TNF was added to the plates and further incubation was continued for 30 min. Supernatant was removed for insulin determination using insulin RIA kit. The cells were washed with cold PBS and extracted overnight at 4°C with 1 ml of acid-ethanol solution (1 M HCl/H_2O/ethanol = 200:10:790, v/v). Cell extract was centrifuged at 14,000 × *g* for 5 min, and the supernatant was diluted 100 times with KRB buffer before insulin was determined. Total protein in the cell extract was determined by using the Bradford reagent. Statistical analysis were performed with Student's *t*-test for unpaired data.

3. Results

3.1. Short- and long-term response of INS-1 cells to glucose

The mean value of the insulin level of this cell line is 15 mU/10^5 cells. The acute response of INS-1 cells to increasing concentrations of glucose is insulin secretion (Fig. 1). The glucose dependency can best be shown when the cells are preincubated in the absence of glucose. After prolonged exposure to high glucose concentrations, the cells become unresponsive to low glucose concentrations (data not shown). Persistent hyperglycemia causes pancreas to lose its ability to respond to glucose [5]. As shown by the results in column 1 of Fig. 1, pretreatment of the cells with glucose-free medium makes the cells particularly glucose sensitive, even at low glucose concentra-

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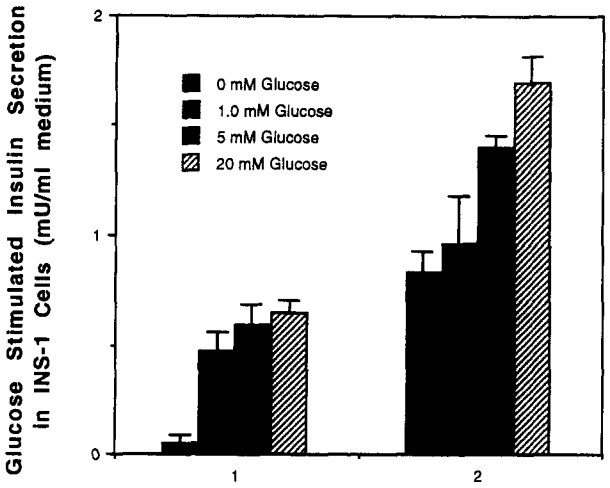

Fig. 1. Glucose and insulin secretion. Following one week of growth in RPMI-1640 medium containing 10 mM glucose, INS-1 cells were incubated for 24 h in the absence of glucose, and then for 2 h in KRB buffer. In the experiment for short-term exposure to glucose (column 1) cells were then incubated in KRB buffer in the absence or presence of 1, 5 or 20 mM glucose, as indicated, for 30 min. For experiments where the cells were exposed for a long term, cells were incubated at the same series of glucose concentration for 24 h (column 2). Insulin secretion was determined as described under section 2. Data shown are mean value ± S.E. of twelve determinations.

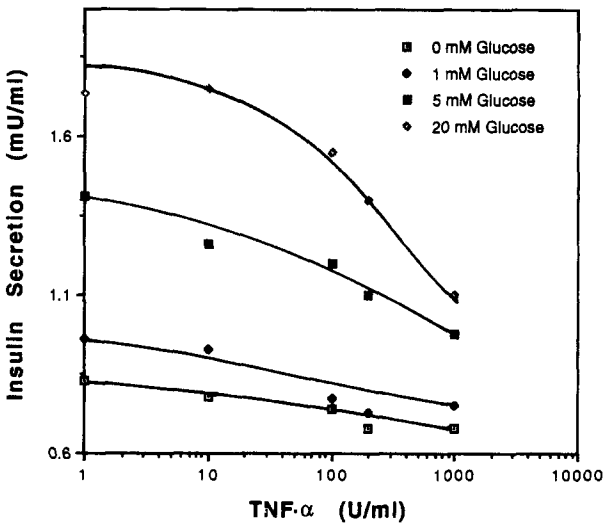

Fig. 2. TNF inhibition of glucose-induced insulin secretion. INS-1 cells were grown and preincubated as described in Fig. 1. During 24 h incubation, TNF and glucose were added to the RPMI-1640 medium. Insulin secretion during the 24 h period was determined as described in section 2. Each well contained 3×10^5 cells in 1 ml of medium. Data shown for each point represent mean value ± S.E. of twelve repeats.

tions (0 vs. 1 mM glucose in column 1, Fig. 1). Although a small increase in insulin secretion occurred in the presence of higher concentrations of glucose, almost maximum insulin secretion occurred at 5 mM glucose, and the effect of 20 mM glucose on further insulin secretion was minimal. This suggests that the insulin level that is affected by glucose in the short term is limited to about 0.6 mU/ml and that this secretion can be affected by glucose concentrations as low as 1 mM. The level of insulin that was secreted over a 24-h period in the absence of glucose was as high as 0.8 mU/ml/24 h. However, further insulin secretion is still glucose concentration dependent, and insulin secretion at 20 mM was as high as at 1.8 mU/ml/24 h (Fig. 1, column 2).

3.2. *Effects of TNF on insulin secretion*

Having established the existence of glucose-induced insulin secretion in our system, we examined the effect of TNF on this phenomenon. First, we tested the effect of TNF on the short-term effect of glucose (30 min). As shown in Table 1, TNF, at a concentration as high as 2000 U/ml, did not affect insulin secretion, at any glucose concentration, in a 30-min time period.

Although TNF had no apparent effect on glucose-induced acute insulin secretion, over longer time periods, it did affect insulin secretion induced by glucose. As shown in Fig. 2, at all concentrations of glucose, increasing amounts of TNF significantly affected the level of insulin secretion. At TNF concentrations of 0, 10, 100 and 1000 U/ml, the glucose-stimulated insulin secretion, when the concentration of glucose was under 20 mM, was: 1.736 ± 0.166, 1.751 ± 0.302, 1.550 ± 0.200, and 1.400 ± 0.112 mU/ml, respectively. These results show that TNF interferes with glucose-induced insulin secretion by β-cells. Several reports suggest that a combination of different cytokines synergistically exert cytotoxic effects that destroy β-cells [8–10]. Therefore, we also examined whether or not the insulin content in the cell was affected by TNF treatment. As shown in Table 2, TNF treatment of the cells at doses as high as 2000 U/ml, for 24 h, did not affect the total insulin content in the cell. This suggests that the observed effect of TNF might be the result of action at the insulin secretion step. Under these experimental conditions, no morphological changes were observed during the incubation of the cells.

4. Discussion

TNF, alone or in combination with other cytokines, is asso-

Table 1
Short-term effect of TNF on insulin secretion in INS-1 cells

Glucose (mM)	TNF-α				
	0 U/ml	100 U/ml	200 U/ml	1000 U/ml	2000 U/ml
0.1	0.20 ± 0.130	0.18 ± 0.035	0.20 ± 0.076	0.20 ± 0.035	0.22 ± 0.032
0.5	0.35 ± 0.019	0.35 ± 0.027	0.38 ± 0.052	0.35 ± 0.061	0.36 ± 0.040
20	0.71 ± 0.012	0.69 ± 0.011	0.69 ± 0.021	0.68 ± 0.050	0.67 ± 0.100

After 7 days growth in RPMI-1640 medium containing 10% bovine serum and 10 mM glucose, INS-1 cells were grown in the same medium without glucose for 24 h and then in Krebs-Ringer bicarbonate buffer for 2 h. Glucose and TNF were added at concentrations indicated for 30 min and insulin secretion (mU/ml) was estimated as described in section 2. The average value of cell insulin content was 15 ± 1.1 mU per 10^5 cells in this experiment.

Table 2
Insulin content in glucose and TNF-treated INS-1 cells

TNF-α (U/ml)	Glucose					
	0 mM	0.1 mM	1.0 mM	5.0 mM	20 mM	30 mM
0	92.6 ± 8.3	93.8 ± 8.6	97.1 ± 20.8	99.4 ± 5.4	98.3 ± 6.8	95.0 ± 14.2
200	92.8 ± 5.6	93.9 ± 21.3	94.2 ± 11.1	98.6 ± 4.3	97.5 ± 17.7	95.2 ± 4.8
1000	93.3 ± 2.7	93.9 ± 6.5	93.8 ± 6.5	96.7 ± 16.9	95.2 ± 22.2	93.8 ± 18.6

After 7 days growth in RPMI-1640 medium containing 10% bovine serum and 10 mM glucose, INS-1 cells were pretreated with the same medium for 24 h at 0 mM glucose which was followed by the treatment with glucose and TNF at concentrations indicated for 24 h. Cells were collected and insulin was extracted and determined (mU per well) as described in section 2. Data shown are mean value ± S.E. from four determinations.

ciated with type I and type II diabetes mellitus [1,8–12]. While destruction of β-cells by cytokines may be responsible for type I diabetes, cytokines appear to play a multifaceted role in type II diabetes. The suppression of insulin-induced tyrosine phosphorylation of insulin receptors and its substrates in insulin target cells has been well established [1–4]. TNF also affects the expression of the glucose transporters gene [4]. These TNF effects alone would be sufficient to make peripheral tissues insulin-resistant and would then lead to NIDDM.

The present studies dealt with another aspect of TNF action in the etiology of type II diabetes. Our observations indicate that TNF inhibits glucose-induced insulin secretion. A recent report [11] suggested that cytokines inhibit insulin secretion by human islets of Langerhans through the elevation of nitric oxide. In that study, TNF alone showed little or no effect on nitric oxide formation. However, Picarella et al. [13] reported that tissue-specific TNF production in transgenic mice led to insulitis. TNF was also reported to alter HLA (human leukocyte antigen) class II antigen expression and to have toxic effects on human β-cells [14]. During our incubation period, no changes in the morphology or insulin content of the INS-1 cells were detected. The mechanism by which TNF inhibits glucose-induced insulin secretion is not known. Although TNF causes Ca^{2+} redistribution between the bound and free forms, it is unlikely that TNF action on insulin secretion is mediated through Ca^{2+} redistribution, as was reported for 30A5 cells [15], because TNF increases the free form of Ca^{2+} transiently and Ca^{2+} is a positive effector in the insulin secretion process [16]. TNF destruction of β-cells is a chronic process [8] and such an effect of TNF on the β-cells would not explain the present observations of TNF action on the secretion of insulin. In our experimental system, TNF has no apparent effect on cell morphology or insulin content. TNF action involves many different nuclear factors which control the transcription of different genes. For example, TNF action involves transcription factors, such as NF-kB, the AP-1 family of transcription factors (c-fos and c-jun), interferon factors IRF-1 and IRF-2 [17], and the C EBP family [18].

Recently, we have shown that TNF inhibited the expression of acetyl-CoA carboxylase (ACC) promoter II, which is the only active promoter for the ACC gene in the β-cell [7]. ACC is the key enzyme for the production of malonyl-CoA, which is thought to be the coupler for glucose-stimulated insulin secretion [19,20]. Further experimental evidence is required as to the mechanism of TNF inhibition of glucose-induced insulin secretion. However, it is attractive to suggest that TNF may affect ACC gene expression, and that the subsequent decrease in malonyl-CoA diminished the effect of glucose in insulin secretion. In the case of glucose-induced acute insulin secretion (30 min), we have recently shown that glucose promoted dephosphorylation of phosphorylated ACC, and that activation of the enzyme was associated with glucose-induced insulin secretion [7]. This process was acute, and thus not likely to be inhibited by TNF. Regardless of the mechanism by which TNF inhibits glucose-induced insulin secretion, it is clear that TNF inhibition of insulin secretion is a contributing factor in NIDDM.

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