Interleukin-6 increases insulin secretion and preproinsulin mRNA expression via Ca^{2+}-dependent mechanism

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Abstract

Interleukin (IL)-6, one of the cytokines released from inflammatory cells, stimulates insulin secretion in a physiological concentration (1–100 pg/ml), but the exact mechanism is still unknown. The present studies were undertaken to investigate the mechanism of IL-6-induced stimulation of insulin secretion in HIT-T 15 cells. The effects of the addition of nifedipine on the IL-6 (100 pg/ml)-induced stimulation of insulin secretion were investigated. We also examined the possibility that IL-6 (1–100 pg/ml) may stimulate insulin messenger ribonucleic acid (mRNA) expression, using the reverse transcription-polymerase chain reaction method. The addition of 100 and 1000 nM nifedipine significantly attenuated the stimulatory effects of 100 pg/ml IL-6 on insulin secretion. The addition of 1–100 pg/ml IL-6 dose-dependently increased preproinsulin mRNA expression relative to β-actin mRNA. IL-6 increased insulin gene promoter activity of fragments A (−2188 to +337 bp) and B (−1782 to +270 bp) but not fragments C (−1275 to +270 bp), D (−1138 to +270 bp), E (−880 to +236 bp) or F (−356 to +252 bp). The addition of 10 nM nifedipine completely abolished the stimulatory effect of 10–100 pg/ml IL-6 on relative preproinsulin mRNA expression. These data raised the possibility that IL-6 increased preproinsulin mRNA expression via the stimulation of Ca^{2+} influx which enhances insulin gene expression.

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Introduction

Interleukin (IL)-6, one of the cytokines, is released from invaded inflammatory cells in various acute phase inflammatory responses (Kishimoto *et al.* 1992). Its complementary DNA has been cloned by Hirano *et al.* in 1986. Several studies have demonstrated that IL-6 stimulates insulin secretion in HIT-T 15 cells and rat pancreatic islets (Buchard *et al.* 1990, Sandler *et al.* 1990, Shimizu *et al.* 1995*a*). IL-6 may be involved in the regulation of insulin secretion from pancreatic β-cells following inflammatory responses. However, the exact mechanism by which IL-6 stimulates insulin secretion still remains to be established.

The expression of hepatic genes is regulated by cytokines during an acute phase inflammatory response (Baumann *et al.* 1987, Gaudlie *et al.* 1987). Both IL-1 and IL-6 are major inflammatory cytokines which influence plasma protein gene expression (Baumann *et al.* 1990). IL-1β has been reported to deplete insulin mRNA by a mechanism dependent on gene transcription and protein biosynthesis, and to increase the heat shock protein hsp 70 in mouse pancreatic islets (Eizirik *et al.* 1990, Eizirik 1991). However, little is known about the regulation of gene expression by IL-6 in pancreatic β-cells. There is a possibility that insulin gene expression may also be regulated by IL-6, resulting in an increase in insulin secretion from β-cells. The present studies were undertaken to investigate the mechanism by which IL-6 stimulates insulin secretion and synthesis in a clonal cell line of hamster β-cells, HIT-T 15 cells (Santerre *et al.* 1981).

Materials and Methods

Chemicals

IL-6 was obtained from Sigma Immuno Chemicals (St Louis, MO, USA). The powder of the incubation medium F-12 K was purchased from Flow Laboratories, Inc. (Irvine, Scotland). ISOGENE was purchased from Nippon Gene (Tokyo, Japan). Fetal bovine serum was purchased from Gibco (Grand Island, NY, USA). Dimethyl sulfoxide (DMSO), and nifedipine were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Cell culture

Sixty-two to sixty-five passages of HIT-T 15 cells were purchased from Flow Laboratories, Inc. (McLean, VA,

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USA). The cells were routinely cultured in the F-12 K medium containing 7 mM glucose supplemented with 10% fetal bovine serum. Insulin secretion from HIT-T 15 cells is glucose-sensitive (Ohtani *et al.* 1998*a*). For the studies on insulin secretion, cells were passaged and seeded (10^5 cells per well) in 24-well multiwell plates. The cells were used for the experiments just before reaching confluence and normally preincubated for 24 h in the F-12 K medium. On the day of the experiment, the culture medium was aspirated and replaced with medium supplemented with the addition of chemicals as described below.

Experimental protocols

The Ca^{2+} channel blocker, nifedipine, dissolved in DMSO, was added in 1 ml of the F-12 K medium to final concentrations of 1 to 1000 nM. The cells were incubated for 10 min prior to the addition of IL-6. The final concentration of DMSO was designed to be below 0·05% to avoid an influence of osmolarity on insulin secretion. Following the addition of 100 pg/ml IL-6, cells were incubated for 2 h at 37 °C. The medium was aspirated and frozen at − 20 °C until immunoreactive insulin assay.

Preproinsulin messenger ribonucleic acid (mRNA) determination

The cells were incubated with 1 ml of the F-12 K medium containing 1–100 pg/ml IL-6 for 2 h at 37 °C. Following the 2-h incubation, the medium was completely removed and the cells were washed three times with fresh F-12 K medium. Then, the cells were sonicated in 0·8 ml ISOGENE and centrifuged at 12 000 r.p.m. for 10 min by microfuge (Sorvall RMC-14). The supernatants containing total RNA were taken from each sample and total RNA was extracted.

Hamster preproinsulin and β-actin mRNA were measured by the reverse transcription-polymerase chain reaction (RT-PCR) method using a GeneAmp EZ rTth RNA PCR kit (Perkin Elmer, Foster City, CA, USA) as we have previously reported (Ohtani *et al.* 1998*b*). The synthetic preproinsulin primer sequences used in the present study were as follows; forward primer: 5′-AGC GTGGCTTCTTCTACACACC-3′; backward primer: 5′-GGTGCAGCACTGATCCACAATG-3′, according to the preproinsulin cDNA sequence obtained by Bell and his associates (1979). According to the results from our preliminary experiment confirming the linear increase of PCR products for preproinsulin and β-actin mRNA, following reverse transcription step at 60 °C for 30 min, PCR was performed for 32 cycles using a 1-min denaturation step at 94 °C and 1-min annealing-extension step at 60 °C. An additional 7-min extension step at 60 °C was added after the 32 cycles. The PCR product was loaded onto an 8% acrylamide gel, and intensity of fluorescence of the band stained by ethidium bromide was calculated using the National Institutes of Health Image 1·56 program (Bethesda, MD, USA). The relative expression of preproinsulin mRNA to β-actin was calculated in each sample.

Next, the cells were incubated with 1 ml of the F-12 K medium containing 10–100 pg/ml IL-6 in the presence or absence of 10 nM nifedipine. Following 2-h incubation, the relative preproinsulin mRNA expression was measured by RT-PCR as described above.

Insulin gene promoter assay

Preproinsulin gene promoter activity was assayed by the method previously reported (Ohtani *et al.* 1998*a*). Human insulin gene, which was inserted into pBR 327, was kindly provided by Dr Graeme I Bell (Howard Hughes Medical Institute, The University of Chicago, USA). The fragment lengths and the restriction enzymes used were as follows. Fragment A, 2052 bp (− 2188 to +337 bp), SpeI and DraIII; fragment B, 2052 bp (− 1782 to +270 bp), HaeII; fragment C, 1545 bp (− 1275 to +270 bp), RsaI and HaeII; fragment D, 1408 bp (− 1138 to +270 bp), ApaLI and HaeII; fragment E, 1116 bp (− 880 to +236 bp), NcoI; fragment F, 608 bp (− 356 to − 252 bp), HhaI. Each insulin gene fragment was inserted into the SmaI site of a plasmid containing a luciferase gene, Pica gene (Tokyo-Ink, Tokyo, Japan). Plasmid pβgal-Control Vector encoding β-galactosidase gene (Clontech, Palo Alto, CA, USA) was used as the normalization plasmid. Changes of promoter activity were measured by luciferase activity after 24-h incubation with 100 pg/ml IL-6 in F-12 K medium. The experiment was repeated four times.

Assays

Immunoreactive insulin concentrations in the incubation medium were assayed by radioimmunoassay using the Phadeceph Insulin Kit (Pharmacia Japan, Tokyo, Japan).

Statistical analysis

All data are expressed as means ± S.E. The statistical analysis of the differences of the means was performed by analysis of variance (ANOVA), followed by Duncan's multiple range test for the individual comparisons of the means.

Results

As shown in Fig. 1, 10 nM nifedipine tended to attenuate the stimulatory effect of 100 pg/ml IL-6 on insulin secretion. The addition of 100 and 1000 nM nifedipine completely abolished the stimulatory effects of IL-6 on insulin secretion. However, 10–1000 nM nifedipine did not affect

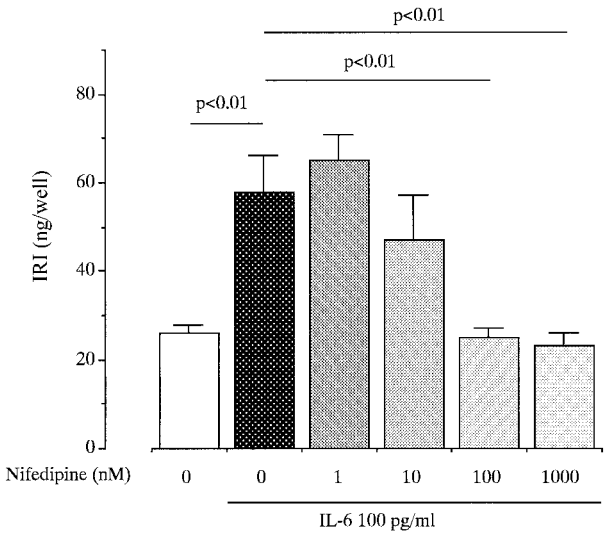

Figure 1 Effect of addition of 1–1000 nM nifedipine on the 100 pg/ml IL-6-induced stimulation of immunoreactive insulin (IRI) secretion in HIT-T 15 cells. $n=6$ in each group. The experiment was repeated twice.

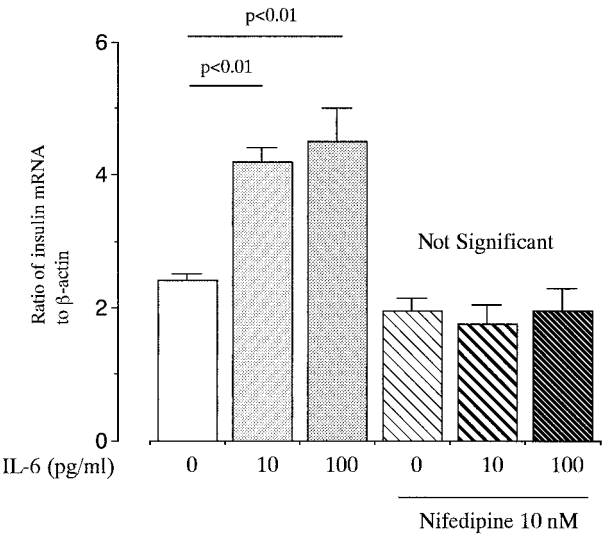

Figure 3 Changes in the effects of IL-6 on preproinsulin mRNA expression relative to β-actin mRNA expression following nifedipine treatment in HIT-T 15 cells. $n=5$ in each group. The experiment was repeated twice.

insulin secretion for 120 min in HIT-T 15 cells (0 nM, 26·0 ± 1·1 ng/well; 10 nM, 26·4 ± 0·4 ng/well; 100 nM, 24·7 ± 0·4 ng/well; 1000 nM, 27·0 ± 0·6 ng/well, not significant). These data indicated a possible involvement of Ca^{2+} influx into β-cells in the effects of IL-6 on insulin secretion.

Figure 2A shows representative PCR products of preproinsulin mRNA and β-actin mRNA in HIT-T 15 cells. The addition of 1 pg/ml IL-6 tended to increase the preproinsulin mRNA expression relative to β-actin mRNA in HIT-T 15 cells, and 10 and 100 pg/ml IL-6 significantly increased the relative preproinsulin expression in a dose-dependent manner (Fig. 2B). The addition of 10 nM nifedipine alone did not show a significant effect on the relative preproinsulin mRNA expression but this dose of nifedipine completely abolished the stimulatory effect of 10–100 pg/ml IL-6 on the relative insulin mRNA expression (Fig. 3).

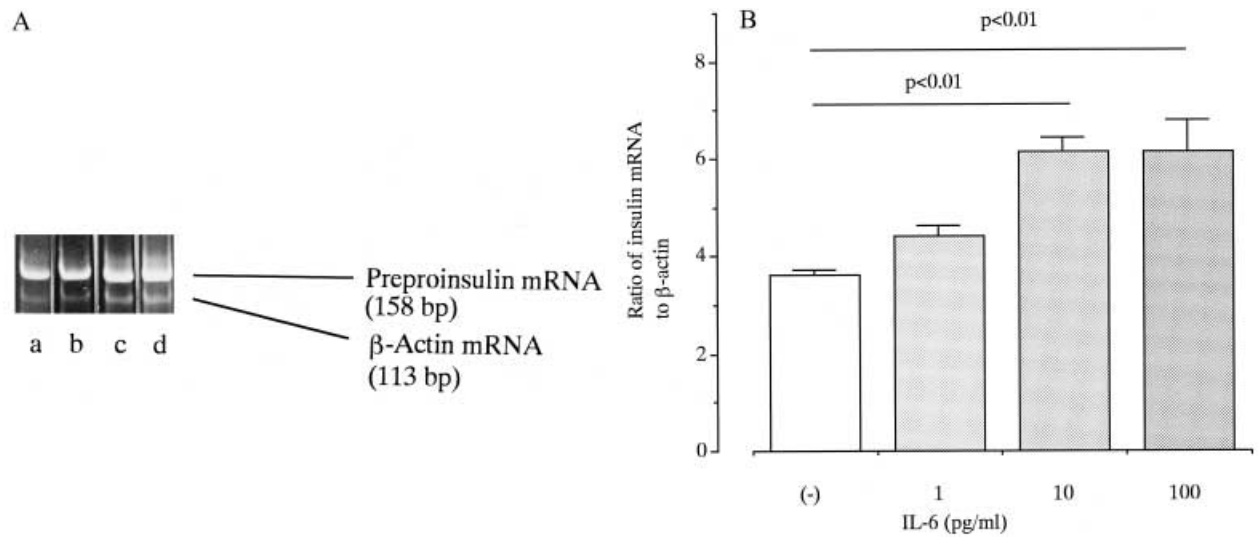

Figure 2 (A) Representative PCR products of preproinsulin mRNA (158 bp) and β-actin mRNA (113 bp) in HIT-T 15 cells. Lane a, non-IL-6-added control; lane b, 1 pg/ml IL-6; lane c, 10 pg/ml IL-6; lane d, 100 pg/ml IL-6. (B) Changes in preproinsulin mRNA expression relative to β-actin mRNA expression in HIT-T 15 cells following the addition of IL-6. $n=5$ in each group. The experiment was repeated twice.

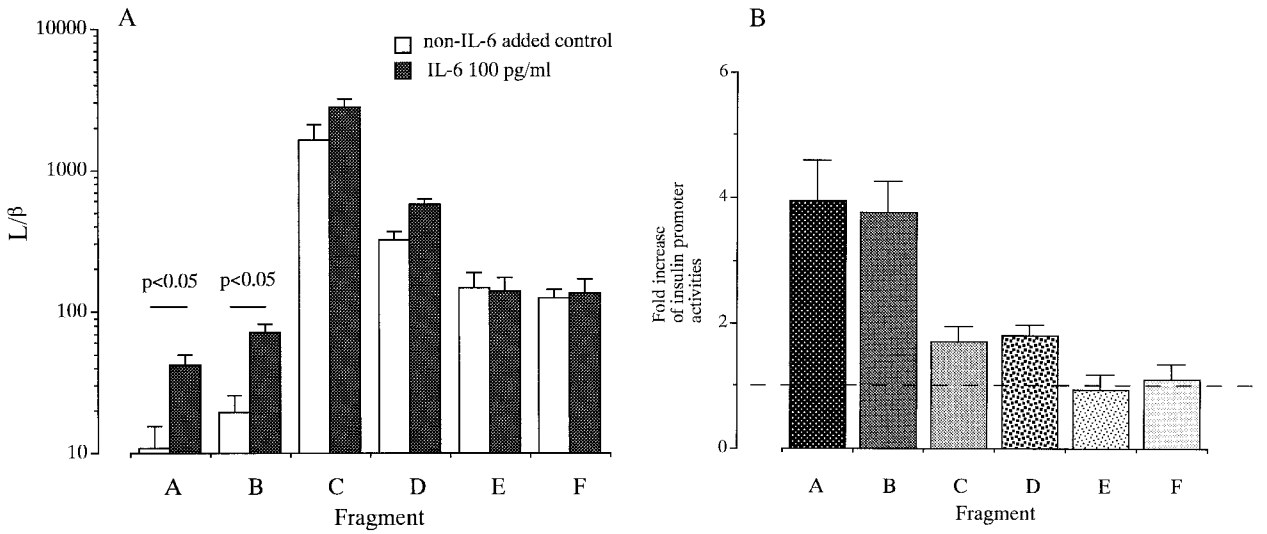

Figure 4 Changes in insulin gene promoter activities, measured by luciferase activities, in each fragment (A) and the fold increase (B) following the addition of 100 pg/ml IL-6. Each value was the average of values obtained from four separate experiments. The y-axis of Fig. 4A shows the ratio of luciferase activity against a cotransfected control plasmid pβgal-control vector (L/β). $n=5$ in each group. Dashed line in (B) represents 1·0.

Figure 4 shows changes of insulin gene promoter activities (A) and their fold increase (B) after the addition of 100 pg/ml IL-6. The addition of IL-6 increased promoter activity in fragments A and B. In contrast, IL-6-induced enhancement of promoter activity was not observed with fragments C, D, E, and F. This result indicates that portions from − 1782 bp to − 1275 bp are important in the expression of the effect of IL-6 on preproinsulin gene expression.

Discussion

The present study demonstrated that the addition of IL-6 increased the relative preproinsulin mRNA expression after 2-h incubation. It is speculated that IL-6 dose-dependently stimulates preproinsulin gene expression, resulting in an increase in insulin secretion in HIT-T 15 cells. The addition of nifedipine significantly antagonized the stimulatory effect of IL-6 on preproinsulin gene expression and secretion, suggesting that changes in intracellular Ca^{2+} concentrations may be involved in the stimulatory effect of IL-6 on insulin synthesis and secretion.

Previous investigators have shown a stimulatory effect of IL-6 on insulin secretion at pharmacological concentrations (500–5000 pg/ml) (Sandler *et al.* 1990) or have failed to show a significant effect of IL-6 on insulin secretion (Eizirik *et al.* 1994). We used lower concentrations of IL-6 in the present studies than those used in previous studies. In our preliminary studies, the stimulatory effects of IL-6 on insulin secretion decreased at higher concentrations (1000 pg/ml) in HIT-T 15 cells. The concentration of IL-6 (1–100 pg/ml) employed in this work appears to be within the physiological range according to our previous observations on the serum of humans after interferon administration for chronic active hepatitis C (Shimizu *et al.* 1995*b*). Therefore, the present data raise the possibility that IL-6 may affect *in vivo* insulin synthesis and secretion in acute inflammatory responses.

The regulation of preproinsulin gene expression has not been well established. A recent study demonstrated that insulin gene expression is regulated immediately by the circulating glucose concentration, e.g. hypoglycemia as well as hyperglycemia (Philippe *et al.* 1994, Redmon *et al.* 1994, Olson *et al.* 1998). The present data add a new finding that IL-6, a cytokine, immediately (within 2 h) stimulates preproinsulin gene expression in HIT-T 15 cells, perhaps via a Ca^{2+}-dependent mechanism.

IL-6 regulates the expression of three fibrinogen genes and CTGGGA is a consensus hexanucleotide sequence of an IL-6 responsive element in the fibrinogen gene on the basis of sequence comparison of the promoter regions (Liu & Fuller 1995, Zhang *et al.* 1995). Signal transducer and activator of transcription (STAT)-3, one of the IL-6-activated transcription factors, has been reported to bind a CTGGGAA site in the α_2-macroglobulin gene, but not in the Aα-fibrinogen (Liu & Fuller 1995). However, a STAT binding site is still not known in the insulin gene promoter.

Our inspection of the promoter region of the preproinsulin gene also revealed two putative consensus sequences of an IL-6 responsive element from −1782 to −1275 bp. In the present studies, we found that IL-6 immediately increased the relative preproinsulin mRNA expression in a dose-dependent manner, in parallel with an increase in insulin secretion for 2 h (Shimizu *et al.* 1995*a*). The promoter activity was increased by IL-6 only by fragments A (−2188 to +337 bp) and B (−1782 to +270 bp), but not by other fragments. These data are compatible with the existence of an IL-6 responsive element in the insulin gene promoter region. It is proposed, therefore, that IL-6 may stimulate preproinsulin gene expression through this part of its promoter region.

Nifedipine (dihydropyridine) is a voltage-dependent Ca^{2+} channel blocker in pancreatic β-cells (Malaisse & Sener 1981). In the present studies, the addition of nifedipine antagonizes the effect of IL-6 on the relative preproinsulin mRNA expression. Possible involvement of the Ca^{2+} channel on gene transcriptional activities has been reported by previous investigators in other cells (Holdstock *et al.* 1996, Raymond & Millhorn 1997, LeHoux & Lefebre 1998). A recent report also demonstrated that Ca^{2+} channel blockers decrease the transcriptional response to glucose in transfected primary islet cultures, suggesting that Ca^{2+} influx may play a critical role in transcription (German *et al.* 1990). These data indicated that Ca^{2+} channels may be involved in the regulation of preproinsulin gene transcription via stimuli such as glucose or IL-6. However, further studies are necessary to clarify the interrelation between this promoter activity and the Ca^{2+}-dependent mechanism in the preproinsulin gene, because we could not negate the possibility that reduction of IL-6-induced insulin secretion by nifedipine may contribute to a reduction in insulin gene promoter activity.

It is concluded that IL-6 stimulates preproinsulin gene expression in a dose-dependent manner in HIT-T 15 cells. The present data raised the possibility that IL-6 increases preproinsulin mRNA expression, perhaps via stimulating Ca^{2+} influx, through its responsive element in the insulin promoter region.

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