

Ciliary Neurotrophic Factor Potentiates the β -Cell Inhibitory Effect of IL-1 β in Rat Pancreatic Islets Associated With Increased Nitric Oxide Synthesis and Increased Expression of Inducible Nitric Oxide Synthase

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Proinflammatory cytokines are implicated as effector molecules in the pathogenesis of IDDM. Interleukin-6 (IL-6) alone or in combination with IL-1 β inhibits glucose-stimulated insulin release from isolated rat pancreatic islets by unknown mechanisms. Here we investigated 1) if the effects of IL-6 are mimicked by ciliary neurotrophic factor (CNTF), another member of the IL-6 family of cytokines signaling via gp130, 2) the possible cellular mechanisms for these effects, and 3) if islet endocrine cells are a source of CNTF. CNTF (20 ng/ml) potentiated IL-1 β -mediated (5–150 pg/ml) nitric oxide (NO) synthesis from neonatal Wistar rat islets by 31–116%, inhibition of accumulated insulin release by 34–49%, and inhibition insulin response to a 2-h glucose challenge by 31–36%. CNTF potentiated IL-1 β -mediated NO synthesis from RIN-5AH cells by 83%, and IL-1 β induced islet inducible NO-synthase (iNOS) mRNA expression fourfold. IL-6 (10 ng/ml) also potentiated IL-1 β -mediated NO synthesis and inhibition of insulin release, whereas β -nerve growth factor (NGF) (5 or 50 ng/ml) had no effect. mRNA for CNTF was expressed in rat islets and in islet cell lines. In conclusion, CNTF is constitutively expressed in pancreatic β -cells and potentiates the β -cell inhibitory effect of IL-1 β in association with increased iNOS expression and NO synthesis, an effect shared by IL-6 but not by β -NGF. These findings indicate that signaling via gp130 influences islet NO synthesis associated with iNOS expression. We hypothesize that CNTF released from destroyed β -cells during the inflammatory islet lesion leading to IDDM may potentiate IL-1 β action on the β -cells. *Diabetes* 47:1602–1608, 1998

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ANOVA, analysis of variance; AP-1, activator protein 1; CM, culture medium; CNTF, ciliary neurotrophic factor; FBS, fetal bovine serum; HBSS, Hanks' balanced salt solution; IFN, interferon; IL, interleukin; iNOS, inducible NO-synthase; IRF-1, interferon response factor-1; NF, nuclear factor; NGF, nerve growth factor; PCR, polymerase chain reaction; PDX-1, pancreatic duodenal homeobox gene 1; RT, reverse transcription; TNF, tumor necrosis factor.

Ciliary neurotrophic factor (CNTF) belongs to the interleukin-6 (IL-6) family of cytokines consisting of IL-6, CNTF, leukemia inhibitory factor, IL-11, cardiotrophin-1, and oncostatin M. The IL-6 family of cytokines all use gp130 as a signal transducing element in the functional receptor (R) complexes (1). The functional CNTF-R complex consists of the specific CNTF-R α (which has no cytoplasmic region and is anchored to the membrane via a glycosyl-phosphatidylinositol linkage), the leukemia inhibitory factor-R, and gp130 (1). CNTF was first identified as a factor that enhanced in vitro survival of embryonic chick parasympathetic ciliary neurons. CNTF also has other neurotrophic effects, e.g., facilitating the survival of sympathetic, sensory, hippocampal, and motor neurons in vitro and in vivo and initiating differentiation of O-2 astrocyte progenitor cells to type 2 astrocytes (2).

CNTF is distributed widely in the adult rodent central nervous system in neurons and glial cells (3). In the peripheral nervous system, CNTF is also found in high concentrations in Schwann cells. CNTF has no signal sequence for exocytosis (2). It has therefore been suggested that CNTF is a cytosolic rather than a secretory protein that is liberated as a consequence of cell damage.

Recently, CNTF was suggested to be a co-mediator of the acute-phase response, sharing actions with tumor necrosis factor- α (TNF- α), IL-1 β , IL-6, and γ -interferon (IFN- γ). Thus, intravenous injection of CNTF causes monophasic fever in rabbits, indicating that CNTF is an endogenous pyrogen (4). Further, CNTF has catabolic effects and induces hepatic acute-phase protein synthesis in mice (5,6). A bolus intravenous injection of CNTF induces hypoglycemia and elevated serum amyloid A and potentiates IL-1-induced corticosterone and IL-6 production in mice (6).

Cytokines, in particular IL-1 β , TNF- α , and IFN- γ , have been implicated as effector molecules in IDDM by causing β -cell destruction via induction of β -cell nitric oxide (NO) synthesis, inhibition of mitochondrial glucose oxidation, DNA damage, and apoptosis (7). IL-6 is known to inhibit glucose-stimulated insulin secretion from rat pancreatic islets (8,9) and to have an additive effect on the inhibitory action of IL-1 β on insulin release by unknown mechanisms (8).

β -Cells express receptors for several nerve growth factors (NGFs) and can undergo a neurotypic response to neuronal differentiation factors (10). The islets of Langerhans are richly innervated (11); and CNTF is found in Schwann cells in the peripheral nervous system, exerts actions on nonneuronal cells, and may have a paracrine function. For these reasons, we studied whether CNTF modulated IL-1 β action on isolated rat islets and on a rat insulinoma cell line, RIN-5AH. Further, to examine whether islet endocrine cells are a source of CNTF, we studied if CNTF mRNA was expressed in islets and in islet cell lines.

RESEARCH DESIGN AND METHODS

Reagents. Recombinant human IL-1 β was provided by Novo Nordisk (Bagsværd, Denmark). The specific activity was 400 IU/ng. Recombinant rat CNTF (activity measured by its ability to support the survival and stimulate neurite outgrowth of cultured embryonic chick dorsal ganglia, ED₅₀ 0.1–0.3 ng/ml), recombinant murine IL-6 (activity measured in a cell proliferation assay using a murine factor-dependent plasmacytoma cell line, ED₅₀ 0.05–0.2 ng/ml), and human β -NGF (activity measured by its ability to support the survival and neurite outgrowth of cultured embryonic chick dorsal root ganglia, ED₅₀ 0.5–1.0 ng/ml), were purchased from R&D systems (Abingdon, U.K.). Collagenase A was from Boehringer Mannheim (Kvistgård, Denmark). RPMI-1640 without glutamine, NaHCO₃ containing 11 mmol/l glucose, RPMI-1640 without glutamine, Hank's balanced salt solution (HBSS), trypsin, EDTA, and fetal bovine serum (FBS) were purchased from GIBCO (Life Technologies, Roskilde, Denmark) (islet experiments) or from HyClone (Logan, Utah) (RIN cell experiments). RPMI-1640 was supplemented with 20 mmol/l HEPES buffer, 100,000 IU/l penicillin, 100 mg/l streptomycin, 2 mmol/l L-glutamine, and 24 mmol/l NaHCO₃ (culture medium [CM]). Human serum albumin was from Behring (Marburg, Germany); oligo(dT) primers were from Invitrogen (Leek, The Netherlands); and 6% acrylamide gel (Gelmix6) was from Life Technologies. The following other reagents were used: naphthylethylene-diamine dihydrochloride and sulfanilamide from SIGMA Bie & Berntsen (Rødovre, Denmark); 8M guanidine and phenol-water-saturated from GIBCO; and Na-acetate, chloroform, NaCl, KCl, CaCl₂, 2H₂O, KH₂PO₄, MgSO₄, 7H₂O, and NaHCO₃ from Merck (Bie & Berntsen).

Islet isolation and preculture. Islets from 4- to 7-day-old Wistar Furth rats (Charles River, Sulzfeldt, Germany) were isolated by hand-picking after collagenase digestion of the pancreatic tissue (12). After isolation, islets were precultured for 4–7 days in CM with 10% FBS at 37°C in four-well multidishes from Nunc (Nuncion, Roskilde, Denmark).

Cells

RIN cells. RIN-5AH-T₂B cells of low passage numbers were cultured at 37°C in 260 ml tissue culture flasks (Nuncion) in RPMI-1640 without glutamine supplemented with 100,000 IU/l penicillin, 100 mg/l streptomycin, 2 mmol/l L-glutamine, and 10% heat-inactivated (56°C, 30 min) FBS (RPMI medium) in a humidified atmosphere of 5% CO₂ and 95% atmospheric air. Cells were detached after washing in Ca²⁺- and Mg²⁺-free HBSS supplemented with 0.5 g trypsin and 0.2 g EDTA by incubation at 37°C for 3 min. Cells pelleted by centrifugation were resuspended in RPMI medium and counted in a Bürger-Türk counting chamber. There were 100,000 cells/200 μ l RPMI medium plated in 96-well plates from Costar (Cambridge, MA).

MSL cells. MSL-G2-cells have previously been characterized and are able to differentiate into all pancreatic endocrine cellular phenotypes (13). A subclone of the MSL-G2 cell line produces mainly glucagon and has been characterized as a pre- β -cell phenotype (NHI-6F glu) (14). After in vivo passage as a subcutaneous tumor in NEDH rats, these cells mature into insulinomas (NHI-6F ins) (15).

The stable transplantable glucagonoma MSL-G-AN can undergo partial β -cell maturation upon forced expression of recombined insulin promoter factor 1, also called pancreatic duodenal homeobox gene 1 (PDX-1) (16,17). PDX-1 plays crucial roles both for pancreatic development and insulin gene transcription (13). The insulin as well as islet amyloid polypeptide genes are selectively activated upon stable PDX-1 transfection (AN 1.1.10). In contrast, a transfected control clone (AN 1.2.9) devoid of insulin promoter factor 1 expression remains glucagon positive.

Experimental design

Islets. There were 150 islets/300 μ l medium precultured for 24 h with or without 20–250 ng/ml CNTF, 0.1–10 ng/ml IL-6, or 5–50 ng/ml β -NGF in CM + 0.5% normal human serum. These concentrations were chosen from the literature because IL-6 has previously been tested on rat islets (8,9,18) and NGF has been tested on an insulin-producing cell line (10). CNTF has not previously been tested on insulin-producing cells, and concentrations were chosen from reported experiments on nonendocrine cell lines (5,19). The islets were washed twice in CM + 0.5% nor-

mal human serum followed by a 24-h culture period with 0–150 pg/ml IL-1 β with or without 20–250 ng/ml CNTF, 0.1–10 ng/ml IL-6, or 5–50 ng/ml β -NGF. Medium was sampled for insulin and nitrite measurements; and 2 \times 25 islets were incubated for 1 h in 1.67 mmol/l glucose in Krebs-Ringer bicarbonate buffer with 0.2% human serum albumin. After 1 h, the medium was sampled for insulin, and the islets were exposed to a 2-h 16.7 mmol/l glucose challenge followed by another sample of medium for insulin. The remaining islets were snap-frozen on dry ice and used for quantitative polymerase chain reaction (PCR) analysis (see below).

RIN-5AH-T₂B cells. All experiments were made in triplicate. One day after plating the cells, the medium was changed to RPMI medium with or without 20–250 ng/ml CNTF. After 1 day, the medium was replaced with RPMI medium with or without 20–250 ng/ml CNTF or 150 pg/ml IL-1 β . One day later, the medium was sampled for nitrite and insulin release.

Unstimulated RIN cells and MSL cells were used for PCR analysis of CNTF mRNA expression.

Insulin was measured by radioimmunoassay (20). The detection limit was 35 fmol/ml. Intra- and interassay coefficients of variation between three known controls were: A: 6.6, 10.5%; B: 4.7, 8.5%; C: 4.3, 7.3%.

NO was determined by measurement of nitrite by the Griess reagent method (21). Absorbance was measured at 550 nm on an immunoreader (NIPPON InterMed. K.K., Tokyo, Japan). The detection limit was 1 μ mol/l corresponding to 2 pmol \cdot islet⁻¹ \cdot 24 h⁻¹. Intra- and interassay coefficients of variation calculated from three points on the standard curve were: 1 μ mol/l: 1.4, 6.5%; 10 μ mol/l: 0.7, 4.6%; 25 μ mol/l: 1.3, 4.9%.

Reverse transcription PCR. Total RNA was extracted by a modification of the 8 mol/l guanidine method (22). cDNA was prepared using oligo(dT) as primer according to the manufacturer's description. Each reverse transcription (RT)-PCR reaction was performed using dCTP as the ³²P-labeled nucleotide and a fixed volume (5 μ l) of cDNA. Each analysis was performed with a set of islet inducible NO-synthase (iNOS) primers (23) in combination with a set of primers for the house-keeping gene rat Sp-1, a transcription factor (24), as internal standard. Rat Sp-1 was chosen because the expression and amplification levels are comparable to those of the iNOS mRNA. Quantitation of iNOS is therefore based on a correlation to the internal standard included in each PCR. The PCR products were separated on a 6% acrylamide gel. After drying the amplified products were visualized following exposure to X-ray films. The RT-PCR products were quantified on a Molecular Dynamics PhosphorImager, Image Quant, Version 3.3 (Molecular Dynamics, Kent, U.K.).

To ensure that the number of RT-PCR cycles chosen for analysis was within the linear amplification interval, analysis was performed on samples from five RT-PCR cycles (cycles 25–29). Cycles 26 or 27 were both within the linear amplification interval and were used for analysis of the snap-frozen islets. In addition, the expression of Sp-1 was compared with Tata-binding protein, and IL-1 or IL-1 + CNTF did not change the expression of Sp-1.

CNTF PCR was performed as above with primers 5'-GAGTATGTATTGCCT-GATGGA and 3'-TCTATCTTGCCGATGTCTCCA, producing a 291 base pair sequence. Cycles 31 were used for analysis of the snap-frozen islets and the cell lines.

Statistical analysis. Results are presented as means \pm SE, and Wilcoxon's matched-pairs test and a two-way analysis of variance (ANOVA) were used for statistical analysis. $P < 0.05$ was chosen as the level of significance.

RESULTS

Effects of CNTF on islet insulin and NO release. The islets were precultured for 24 h with CNTF, washed twice, and cultured for 24 h with CNTF in the absence of IL-1 β . CNTF in concentrations from 20 to 250 ng/ml had no significant effects on islet accumulated insulin release or NO synthesis. However, high concentrations of CNTF inhibited insulin release during a 2-h 16.7 mmol/l glucose challenge associated with a slight but nonsignificant increase in islet NO synthesis (Table 1). Media were also sampled after the 24-h preculture period with CNTF. CNTF exposure for this time period had no effect on accumulated insulin release or NO synthesis (data not shown).

Potentiating effects of CNTF on IL-1 β modulation of insulin release and NO synthesis. Five pg/ml of IL-1 β increased accumulated insulin release ($P < 0.02$, Fig. 1). Higher IL-1 β concentrations dose-dependently decreased accumulated insulin release (two-way ANOVA $P < 0.0001$, Fig. 1, $P < 0.05$ at 50 pg/ml IL-1 β , and $P < 0.01$ at 150 pg/ml IL-1 β). Preculture for 24 h with 20 ng/ml CNTF followed by co-

TABLE 1

Accumulated insulin release, insulin response to a 2-h glucose challenge, and NO synthesis in isolated rat islets precultured in different concentrations of CNTF for 24 h followed by 24 h culture with CNTF without IL-1 β

24-h preculture with CNTF (ng/ml)	Accumulated insulin release (ng · islet ⁻¹ · 24 h ⁻¹)	Insulin release during 2-h 16.7 mmol/l glucose challenge (ng · islet ⁻¹ · 2 h ⁻¹)	Nitrite production (pmol · islet ⁻¹ · 24 h ⁻¹)
Control	14.17 ± 1.70	3.06 ± 0.43	2.43 ± 0.43
20	12.10 ± 1.42	2.38 ± 0.56	2.82 ± 0.90
50	14.17 ± 1.91	1.76 ± 0.37*	4.56 ± 1.73
250	10.58 ± 1.30	1.47 ± 0.12*	3.20 ± 1.09

Data are means ± SE. *n* = 6. **P* < 0.05 compared with control islet cultures.

culture for 24 h with 20 ng/ml CNTF and IL-1 β abolished IL-1 β -mediated stimulation at 5 pg/ml and potentiated IL-1 β -mediated inhibition of accumulated insulin release by 34–49% (two-way ANOVA *P* < 0.0043, *P* < 0.01 at 10 and *P* < 0.02 at 50 pg/ml IL-1 β).

By itself, 20 ng/ml CNTF inhibited slightly, but not significantly (*n* = 11), the insulin response to a 2-h 16.7 mmol/l glucose challenge (Fig. 2). IL-1 β dose-dependently inhibited this response (two-way ANOVA *P* < 0.0001, *P* < 0.05 at 10 pg/ml IL-1 β , and *P* < 0.01 at 15, 50, and 150 pg/ml IL-1 β). CNTF in combination with IL-1 β potentiated the inhibitory effect of IL-1 β by 31–36% (two-way ANOVA *P* < 0.0008, *P* < 0.01 at 5 and 10, and *P* < 0.05 at 50 pg/ml of IL-1 β).

CNTF at 20 ng/ml in itself had no effect on NO synthesis (Fig. 3). IL-1 β caused a dose-dependent increase in NO synthesis (two-way ANOVA *P* < 0.0001, *P* < 0.01 at 10, 15, 50, and 150 pg/ml IL-1 β). CNTF (20 ng/ml) potentiated the IL-1 β -mediated NO synthesis by 31–116% (two-way ANOVA *P* < 0.0004, *P* < 0.01 at 5, and *P* < 0.05 at 10 and 150 pg/ml of IL-1 β).

To determine the optimal pre-incubation period for CNTF potentiation of IL-1 β action, islets were pre-incubated for 0–24 h with 20 ng/ml CNTF before IL-1 β was added. Co-incu-

bation of CNTF had little or no potentiating effect, whereas increasing periods (2–12 h) of pre-incubation with CNTF before adding IL-1 β time-dependently potentiated IL-1 β -mediated NO synthesis and inhibition of accumulated insulin release (Fig. 4).

Effects of CNTF on IL-1 β action on RIN-5AH-T2B cells. The effect of CNTF in combination with IL-1 β was tested on an insulin-secreting β -cell line to examine if the action of CNTF in intact islets could be reproduced in clonal β -cells. CNTF (20 ng/ml) stimulated the accumulated insulin release by 16% (*P* < 0.05, Table 2). IL-1 β (150 pg/ml) had no significant effect on the accumulated insulin release. However, 20 ng/ml of CNTF plus 150 pg/ml of IL-1 β inhibited the accumulated insulin release by 31% (*P* < 0.05).

CNTF (20–250 ng/ml) had no effect on NO synthesis (Table 2 and data not shown). IL-1 β (150 pg/ml) stimulated NO synthesis ninefold (*P* < 0.05). CNTF (20 ng/ml) potentiated IL-1 β (150 pg/ml)-mediated NO synthesis by 83% (*P* < 0.05).

Effect of IL-6 on IL-1 β action on islet insulin and NO release. To investigate whether the potentiating action of CNTF on the effects of IL-1 β was shared by other cytokines signaling via gp130, dose-finding experiments were carried out

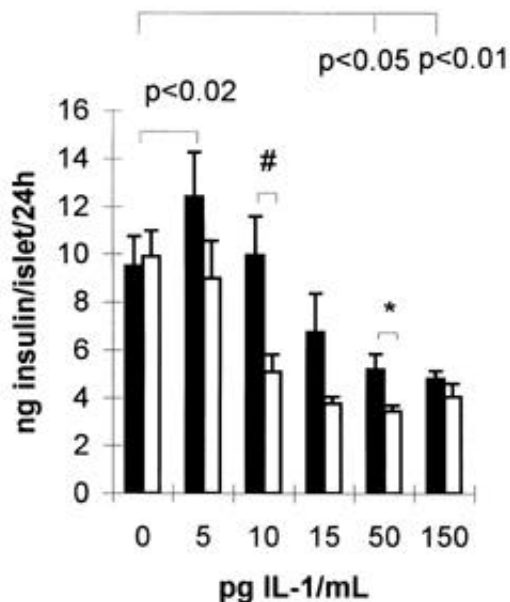


FIG. 1. Accumulated insulin release over 24 h from isolated rat islets incubated with 0–150 pg/ml of IL-1 β (■) or preincubated for 24 h with 20 ng/ml of CNTF and then coincubated with 20 ng/ml of CNTF and 0–150 pg/ml of IL-1 β (□). *n* = 8–11. Data are means ± SE. #*P* < 0.01, **P* < 0.02.

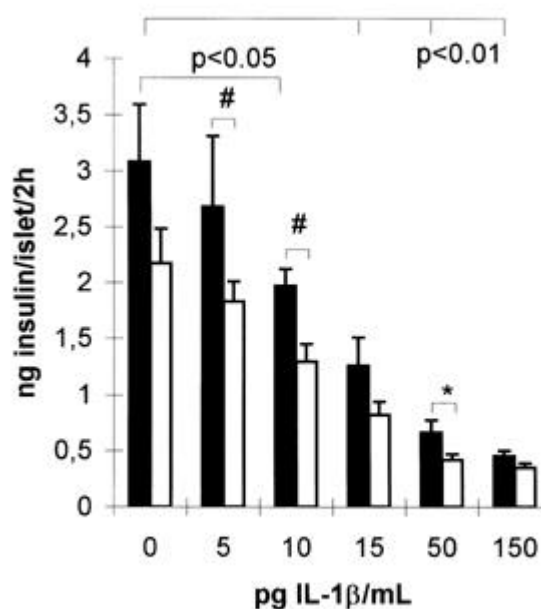


FIG. 2. Insulin response to a 2-h 16.7 mmol/l glucose challenge from isolated rat islets incubated with 0–150 pg/ml of IL-1 β (■) or preincubated for 24 h with 20 ng/ml of CNTF and then coincubated with 20 ng/ml of CNTF and 0–150 pg/ml of IL-1 β (□). *n* = 8–11. Data are means ± SE. #*P* < 0.01, **P* < 0.05.

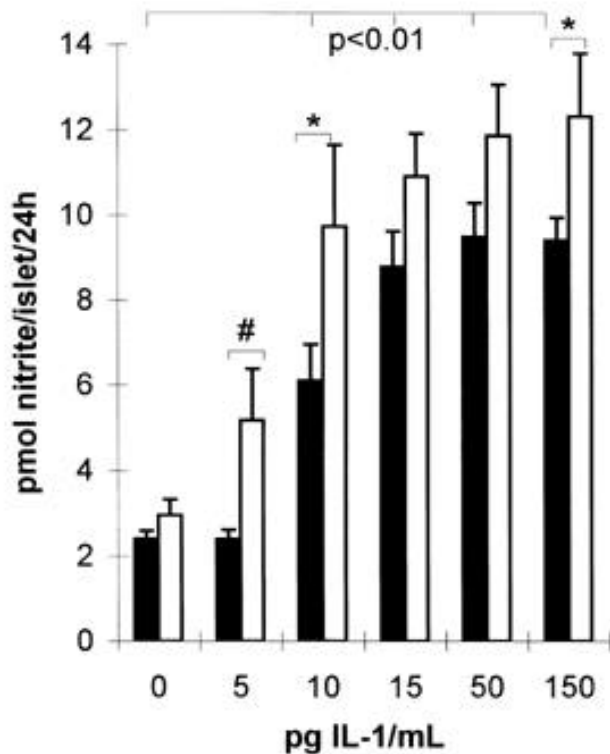


FIG. 3. Nitrite release over 24 h from isolated rat islets incubated with 0–150 pg/ml of IL-1 β (■) or preincubated for 24 h with 20 ng/ml of CNTF and then coincubated with 20 ng/ml of CNTF and 0–150 pg/ml of IL-1 β (□). $n = 8–11$. Data are means \pm SE. # $P < 0.05$, * $P < 0.05$.

with IL-6 concentrations between 0.1 and 10 ng/ml. The most marked potentiating effect of IL-6 on IL-1 β action was found to be 10 ng/ml (data not shown). This concentration of IL-6 was used for further experiments.

In itself, IL-6 inhibited the insulin secretion during a glucose challenge by 33% ($P < 0.01$), but it did not alter the accumulated insulin release or NO production (Table 3). Ten ng/ml of IL-6 potentiated 15 pg/ml IL-1 β -mediated inhibition of accumulated insulin secretion by 48% ($P < 0.02$) and inhibition of insulin response to a 2-h 16.7 mmol/l glucose challenge by 46% ($P < 0.02$). Further, IL-6 potentiated the IL-1 β -mediated NO synthesis by 64% ($P < 0.01$, Table 3).

Effects of β -NGF on IL-1 β action on islet insulin release and NO synthesis. The effect of β -NGF on islets was tested to examine if other neurotrophic factors had effects similar to CNTF on pancreatic islet insulin or NO synthesis. Neither 5 nor 50 ng/ml of β -NGF had effects by itself or on IL-1 β action (Table 4).

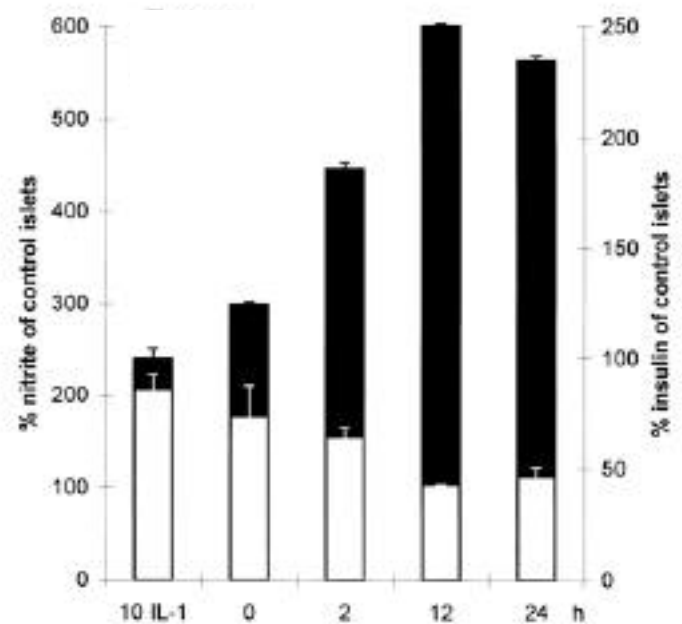


FIG. 4. Accumulated insulin (□) and nitrite (■) release over 24 h from isolated rat islets preincubated for 0–24 h with 20 ng/ml CNTF before the islets were washed and 10 pg/ml IL-1 β and 20 ng/ml CNTF were added. 10 IL-1: islets incubated 24 h with 10 pg/ml IL-1 β in the absence of CNTF. $n = 2–4$. Data are means \pm SE.

Quantitative PCR analysis for iNOS mRNA in rat islets.

Twenty ng/ml of CNTF had no effect on the expression of iNOS. Five and 10 pg/ml of IL-1 β induced iNOS expression. CNTF had a fourfold potentiating effect on 5 pg/ml IL-1 β -induced iNOS expression ($P < 0.02$, Table 5), but it did not significantly affect 10 pg/ml IL-1 β -induced iNOS expression.

PCR analysis of CNTF mRNA expression in rat islets and α - and β -cell lines. mRNA for CNTF was demonstrated in 4- to 7-day-old rat islets cultured for 1 week and in RIN-5AH cells (Fig. 5A). mRNA for CNTF was also found in AN 1.1.10, AN 1.2.9, NHI-6F ins, and in NHI-6F glu (Fig. 5B). mRNA analysis of rat islets and α - and β -cell lines exposed to 150 pg/ml IL-1 indicated that IL-1 does not modify CNTF expression (data not shown).

DISCUSSION

The data from this study show that pancreatic β -cells constitutively express CNTF and that CNTF markedly potentiates IL-1 β -mediated inhibition of accumulated and acute insulin release in response to glucose. CNTF also stimulated NO synthesis in association with potentiation of IL-1 β -induced

TABLE 2

Accumulated insulin release and NO synthesis from RIN-5AH cells treated with IL-1 β with or without CNTF

IL-1 β (pg/ml)	CNTF (ng/ml)	Insulin [$\mu\text{g} \cdot \text{ml}^{-1} \cdot (10^5 \text{ cells})^{-1} \cdot (24 \text{ h})^{-1}$]	Nitrite production [$\mu\text{mol} \cdot (10^5 \text{ cells})^{-1} \cdot (24 \text{ h})^{-1}$]
0	0	16.1 \pm 2.5	1.11 \pm 0.10
0	20	18.6 \pm 2.2*	1.18 \pm 0.12
150	0	13.8 \pm 2.2	10.63 \pm 1.36*
150	20	9.5 \pm 1.8†	19.49 \pm 2.23†

Data are means \pm SE. $n = 6$. * $P < 0.05$ compared with control cultures, † $P < 0.05$ compared with cells exposed to IL-1 β .

TABLE 3

Accumulated insulin release, insulin response to a 2-h glucose challenge, and NO synthesis in isolated rat islets exposed to IL-6 with or without IL-1 β

IL-6 (ng/ml)	IL-1 β (pg/ml)	Accumulated insulin release (ng · islet ⁻¹ · 24 h ⁻¹)	Insulin release during 2-h 16.7 mmol/l glucose challenge (ng · islet ⁻¹ · 2 h ⁻¹)	Nitrite production (pmol · islet ⁻¹ · 24 h ⁻¹)
0	0	12.67 ± 1.82	3.33 ± 0.29	2.32 ± 0.15
10	0	12.20 ± 1.47	2.23 ± 0.24*	2.64 ± 0.31
0	15	11.71 ± 1.61	1.52 ± 0.21*	6.49 ± 0.86*
10	15	6.11 ± 0.98†	0.83 ± 0.11†	10.62 ± 0.84††

Data are means ± SE. *n* = 8. **P* < 0.01 compared with control islets, †*P* < 0.02 and ‡*P* < 0.01 compared with islets treated with IL-1 β .

iNOS expression. These effects were mimicked by IL-6, which also signals via gp130, but not by β -NGF, a member of the family of neurotrophins acting via the *trk* family of receptor tyrosine kinases, which also binds to the p75 TNF receptor. Because IL-1 β , either alone or in synergy with TNF- α and IFN- γ , has been suggested to act as an immune effector molecule, causing β -cell destruction and IDDM, we hypothesize that CNTF liberated from damaged β -cells to the islet microenvironment accelerates cytokine-mediated β -cell destruction.

The potentiating effect of CNTF on the IL-1 β -induced increase in iNOS mRNA can be due either to increased mRNA stability or to increased iNOS gene expression. We have not performed nuclear run-off assays to test the first possibility. Because a 2-h or longer pre-incubation period with CNTF was needed to potentiate IL-1 β -mediated effects, we suggest that the priming effect of CNTF is exerted at the transcriptional level.

The promoter region of the mouse iNOS gene contains several transcription factor binding motifs, including binding sites for INF- γ response elements, interferon response factor 1 (IRF-1), γ -activated site, nuclear factor κ B (NF κ B), IFN- α -stimulated response element, activator protein 1 (AP-1), TNF response element, and nuclear factor IL-6 (NF-IL6) (25). IL-1 β and the gp130 activating family of cytokines induce NF κ B, NF-IL6, AP-1, and IRF-1 (1,7,26–34). The potentiating effect of the gp130 activating family of cytokines on IL-1 β

mediated NO production and iNOS expression may consequently be explained as follows: gp130 activation leads to generation of NF-IL6, junB, and IRF-1, by itself not capable of inducing NO production. However, when priming of the cells with cytokines that signal via gp130 is followed by signaling via the IL-1RT1, which induces NF κ B, AP-1, and NF-IL6 in addition to IRF-1, it is possible that preexisting NF-IL6, junB, and IRF-1 will potentiate iNOS expression.

In this study, IL-6 and CNTF did not alter the accumulated insulin release from rat islets (Tables 1 and 3), whereas CNTF stimulated the accumulated insulin release from RIN-5AH cells (Table 2). IL-6 and high concentrations of CNTF inhibited the insulin release during an acute glucose challenge from rat islets (Tables 1 and 3), in accordance with previous studies (8,9). High concentrations of CNTF may inhibit the acute insulin release through cytotoxic effects caused by a slight increase in NO. Previous findings of a stimulating effect of IL-6 on the accumulated insulin release (9,18) or failure of IL-6 to potentiate the inhibitory action of IL-1 β (9,18) may be explained by the lack of pre-incubation with IL-6 before IL-1 β was added, because we found CNTF to be ineffective when co-incubated with IL-1 β .

Functional NGF receptors have been detected in pancreatic β -cell lines and in fetal rat islets (35). This discovery made it interesting to examine if a neurotrophic factor other

TABLE 4

Accumulated insulin release, insulin response to a glucose challenge, and NO synthesis in isolated rat islets exposed to different concentrations of β -NGF in the absence or presence of IL-1 β

IL-1 β (pg/ml)	β -NGF (ng/ml)	Accumulated insulin release (ng · islet ⁻¹ · 24 h ⁻¹)	Insulin release during 2-h 16.7 mmol/l glucose challenge (ng · islet ⁻¹ · 2 h ⁻¹)	Nitrite production (pmol · islet ⁻¹ · 24 h ⁻¹)
0	0	14.00 ± 0.53	3.69 ± 0.40	3.77 ± 1.61
8	0	16.83 ± 1.19	2.63 ± 0.24	4.12 ± 1.40
15	0	12.00 ± 1.43	1.44 ± 0.19	7.29 ± 1.04
150	0	5.67 ± 0.38	0.43 ± 0.03	10.95 ± 0.97
0	5	13.17 ± 0.86	3.19 ± 0.29	3.20 ± 1.10
8	5	16.17 ± 1.38	2.53 ± 0.22	3.21 ± 0.38
15	5	11.17 ± 1.01	1.45 ± 0.15	7.66 ± 0.80
150	5	5.50 ± 0.77	0.42 ± 0.03	9.65 ± 0.73
0	50	12.83 ± 0.93	3.26 ± 0.19	2.13 ± 0.12
8	50	14.83 ± 1.09	2.40 ± 0.30	4.30 ± 1.17
15	50	9.83 ± 1.01	1.44 ± 0.08	6.25 ± 1.07
150	50	6.27 ± 1.18	0.41 ± 0.03	9.06 ± 0.95

Data are means ± SE. *n* = 6.

TABLE 5
PCR analysis of iNOS expression on snap-frozen rat islets incubated with CNTF and IL-1 β

IL-1 β (pg/ml)	0	5	10	0	5	10
CNTF (mg/ml)	0	0	0	20	20	20
<i>n</i>	11	12	9	8	11	9
Mean (iNOS/rat Sp1)	0.00 \pm 0.00	0.08 \pm 0.02 (<0.002)	0.57 \pm 0.08 (<0.01)	0.08 \pm 0.07	0.31 \pm 0.13* (<0.002)	0.43 \pm 0.09 (<0.01)

Data are means of densitometric values \pm SE (*P*). *P* values are compared with control islets. **P* < 0.02 compared with islets incubated with 5 pg/ml IL-1 β without CNTF.

than CNTF, e.g., β -NGF, had an effect by itself or modulated the inhibitory effect of IL-1 β on islets. Both the low-affinity TNF p75 and the high-affinity *trkA* receptors for β -NGF are expressed in β -cell lines (35). *trkA* activates the Ras-MAPK, and p75 activates the sphingomyelinase pathways (36). However, in this study, β -NGF had no effect on islets by itself or on IL-1 β action, indicating that the modulating effect of CNTF depends on gp130 signaling and is not shared by other neurotrophic factors. This finding also indicates that the well-known synergistic effect of TNF on IL-1 action on the β -cell is mediated via the p55 TNF receptor (37).

In conclusion, CNTF is expressed in rat islets of Langerhans and rat insulinoma cells. It potentiates the β -cell inhibitory effect of IL-1 β associated with increased NO synthesis, an effect shared by IL-6 but not by β -NGF. CNTF also increased IL-1 β -mediated iNOS expression. These findings indicate that signal transduction via gp130 influences islet NO synthesis associated with iNOS expression. We hypothesize that CNTF released from destroyed β -cells during the inflammatory islet lesion leading to IDDM may potentiate IL-1 action on the β -cells. CNTF has not yet been tested in an animal model of IDDM, but anti-IL-6 antibody has been shown to protect against NOD diabetes, supporting this hypothesis (38).

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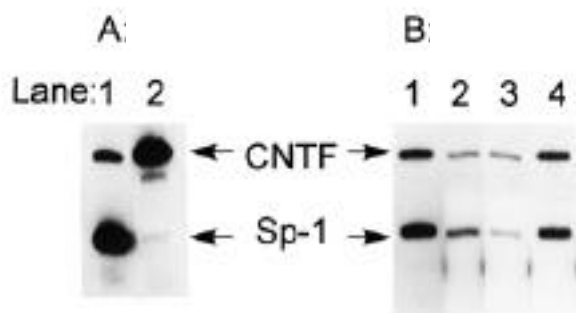


FIG. 5. **A:** mRNA for CNTF detected by PCR analysis of snap-frozen rat islets from 4- to 8-day-old Wistar rats cultured for 1 week (lane 1) or of RIN5AH cells (lane 2). **B:** mRNA for CNTF detected by PCR analysis of AN 1.1.10 (lane 1), AN 1.2.9 (lane 2), NHI-6F glu (lane 3), and NHI-6F ins (lane 4). Sp-1, housekeeping gene.

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Author Queries (please see Q in margin and underlined text)

Q1: <<Au: In the sentence beginning “CNTF (20 ng/ml) potentiated ...”: do you mean that CNTF potentiated the inhibition of accumulated insulin release and the inhibition of insulin response (as it is written here), or that CNTF inhibited accumulated insulin release?>

Q2: <<Au: For the sentence beginning “There were 150 islets...”, I changed \pm to “with or without” to make the sentence clearer. Is this what you meant by \pm ?>

Q2a: In Figure 1, the “ $p < 0.05$ ” is in the figure but is not mentioned in the figure legend. Please advise.

Q3: <<Au: Edits in separating the sentence beginning “The data from this study ...” into two shorter sentences OK?>

Q4: Edits in sentence beginning “These effects were mimicked OK?>

<<Au: Please add the closing page number for ref. 32 or indicate if it is an abstract, letter, or one-page article.>