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Nitric oxide mediates down regulation of lipoprotein lipase activity induced by tumor necrosis factor-α in brown adipocytes

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Abstract

We previously reported that tumor necrosis factor-α (TNF-α)/cachectin suppresses lipoprotein lipase activity and its gene expression in brown adipocytes differentiated in culture. Recent evidence suggests that the effect of TNF-α over various cells is related to the enhanced production of nitric oxide (NO). The present study examined whether the suppressive effect of TNF-α on lipoprotein lipase activity is mediated by production of NO in the brown adipocytes. A reverse transcription-polymerase chain reaction (RT-PCR) assay revealed that TNF-α caused a concentration- and time-dependent expression of inducible NO synthase in brown adipocytes. Increasing concentrations of TNF-α (0.5–50 ng/ml) for 24 h resulted in a concentration-dependent decrease in lipoprotein lipase activity with reciprocal increase in nitrite production in the medium. The suppressive effect of TNF-α on lipoprotein lipase activity was significantly prevented by NO synthase inhibitors, N^G-nitro-L-arginine methyl ester (L-NAME) and aminoguanidine, but not by D-NAME, an inactive isomer. Furthermore, 8-bromoguanosine 3′,5′-cyclic monophosphate, cell permeant cGMP, suppressed lipoprotein lipase activity and 1*H*-[1,2,4] oxadiazolo[4,3-*a*]quinoxalin-1-one, a selective inhibitor for soluble guanylate cyclase, restored the TNF-α-suppressed lipoprotein lipase activity. These results suggest that TNF-α stimulates brown adipocytes to express inducible NO synthase, followed by production of NO, which in turn mediates the suppressive effect of TNF-α on lipoprotein lipase activity. The effect of NO is mediated, at least partly, through production of cGMP. © 1997 Elsevier Science B.V.

Keywords: Brown adipocyte; TNF-α (tumor necrosis factor-α); Nitric oxide (NO) synthase, inducible; Lipoprotein lipase; Guanosine 3′,5′-cyclic monophosphate

1. Introduction

Brown adipose tissue (BAT) has a specific uncoupling protein thermogenin in its mitochondrial membrane (Nedergaard and Cannon, 1992) and plays a pivotal role in the regulation of energy balance (Nicholls and Locke, 1984; Cannon and Nedergaard, 1985). Reduction of its thermogenic activity is assumed to be involved in the development of obesity (Trayhurn, 1986; Himms-Hagen, 1989), while the role of BAT in the opposite state such as cachexia has been scarcely investigated so far. TNF-α/cachectin is well documented to play an important role in wasting syndrome (cachexia) in infection or malignancy, which is associated with diverse abnormalities in carbohydrate, protein, and lipid metabolism (Evans et al., 1989). TNF-α decreases the activity of lipoprotein lipase in white adipose tissue both in vivo (Semb et al., 1987; Evans and Williamson, 1988) and in vitro (Price et al., 1986; Kawakami et al., 1987). It suppresses lipoprotein lipase activity by decreasing gene transcription in 3T3-L1 adipocytes (Cornelius et al., 1988; Zechner et al., 1988). In addition, we have previously demonstrated that TNF-α suppresses lipoprotein lipase gene expression in primary culture of brown adipocytes (Uchida et al., 1994).

Nitric oxide (NO) has emerged as an important intra- and intercellular messenger controlling many physiological processes (Ignarro, 1990; Moncada et al., 1991; Lowenstein and Snyder, 1992). NO is synthesized from L-arginine by constitutive and inducible NO synthases found in a variety of cell types (Förstermann et al., 1991). TNF-α increases inducible NO synthase in various types of cells including macrophages (Moncada et al., 1991) and some

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TNF actions in osteoblast (Hukkanen et al., 1995), thyrocytes (Kasai et al., 1995), endothelial cells (Estrada et al., 1992) and skeletal muscle (Buck and Chojkier, 1996) are mediated by NO. However, the presence or induction of NO synthase has been little described in brown adipocytes as yet.

In this communication, to assess whether NO mediates the suppressive effect of TNF-α on lipoprotein lipase activity, we examined the effects of TNF-α on lipoprotein lipase activity and nitrite production using NO synthase inhibitors and confirmed expression of NO synthase mRNA in primary culture of brown adipocytes. Furthermore, as one of the well-characterized effects of NO is the activation of soluble guanylate cyclase, leading to increased levels of intracellular guanosine 3′,5′-cyclic monophosphate (cGMP) (Garbers, 1992), we investigated mediation by cGMP on the suppressive effect of TNF-α on lipoprotein lipase, using a cell permeant analog of cGMP, and a selective inhibitor of soluble guanylate cyclase.

2. Materials and methods

2.1. Cell isolation, culture and TNF-α treatment

Brown fat precursor cells were isolated from interscapular, cervical and axillary brown adipose tissue of 4-week-old male ICR mice as previously described by Rehnmark et al. (1990). Briefly, the minced tissue was incubated in a Hepes-buffered solution (pH 7.4) containing 0.2% collagenase type II for 30 min at 37°C with vortexing every 5 min. After incubation, the tissue remnants were filtered through a 250 μm nylon screen into plastic test tubes. The tubes were placed on ice for at least 30 min to allow the mature brown fat cells and lipid droplets to float on the surface of the cell suspension. The infranatant (stromal–vascular fraction) was collected and then filtered through a 25 μm nylon screen to remove cell aggregates. The stromal–vascular fraction containing precursor cells was pelleted by centrifugation for 10 min at $700 \times g$, and then resuspended in the culture medium.

The cells were plated in six-well multiplates and cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 4 nM insulin, 25 mg/ml sodium ascorbate, 10 mM Hepes, 4 mM glutamine, 50 IU/ml penicillin and 50 mg/ml streptomycin. The cells were grown at 37°C in an atmosphere of 5% CO_2 in air and characterized on day 9 after inoculation. At this stage, cells were converted to mature brown adipocytes which were verified by the presence of typical multilocular lipid droplets and by norepinephrine-induced expression of uncoupling protein mRNA detected by Northern analysis (Rehnmark et al., 1990).

TNF-α (rhTNF-α: 2.55×10^6 JRU/mg of protein) at concentrations of 0.5, 1, 5 and 50 ng/ml was included in the medium for the final 3, 6, 16, 24 and 48 h. NO synthase inhibitors including N^G-nitro-L-arginine methyl ester (L-NAME) (1, 10, 100 μM), aminoguanidine (10, 100, 1000 μM) or an inactive isomer D-NAME (10 μM), 8-bromoguanosine 3′:5′-cyclic monophosphate (8-Br-cGMP:cell permeant cGMP) (1, 10, 100, 1000 μM), and a selective inhibitor of soluble guanylate cyclase (1*H*-[1,2,4]oxadiazolol[4,3-*a*]quinoxalin-1-one (ODQ) (0.1, 1, 10 μM, freshly dissolved in dimethyl sulfoxide (DMSO) as 10 mM solution and diluted with distilled water), were added to the culture medium 25 h prior to the harvest of cells.

2.2. Measurement of lipoprotein lipase activity

At the end of the incubation with TNF-α and/or NO synthase inhibitors, the medium was replaced with fresh medium without TNF-α or NO synthase inhibitors and the cells were exposed to 10 IU/ml heparin for 1 h at 37°C. Then, activity of lipoprotein lipase released by heparin into the culture medium (heparin-releasable lipoprotein lipase activity) was determined as described previously (Uchida et al., 1995) using a stable, radioactive substrate emulsion containing [^{3}H]triolein (Nilsson-Ehle and Schotz, 1976). Radioactivity was quantified in aqueous counting scintillant II (Amersham) by a liquid scintillation counter (Aloka LSC-700, Tokyo). Lipoprotein lipase activity was expressed as nanomoles of FFA released per mg of protein/min. Protein contents of the cells were determined as follows. Cells were rinsed twice with ice-cold phosphate-buffered saline and scraped from dishes with rubber policemen into 50 mM NH_3/NH_4Cl buffer (pH 8.1) and were sonicated for 15 s. After precipitation with 10% trichloroacetic acid, cell protein was determined according to the method of Lowry et al. (1951) using bovine serum albumin as a standard.

2.3. Nitrite determination

Nitric oxide generation was estimated by a measurement of nitrite in the culture medium as follows. After 8 or 9 days of culture, the medium was replaced with 500 μl of fresh medium without phenol red, and fetal bovine serum and TNF-α were added at concentrations of 0.5, 1, 5 and 50 ng/ml. NO synthase inhibitors such as L-NAME, aminoguanidine or inactive isomer D-NAME were added 1 h prior to the addition of TNF-α. Nitrite production was determined colorimetrically using Griess reaction (Green et al., 1982). Briefly, a 300 μl aliquot of culture medium was removed at the end of the incubation and mixed with an equal volume of Griess reagent (1% sulfanylamide and 0.1% naphthylenediamine dihydrochloride in 2% phosphoric acid). The mixture was incubated for 30 min at room temperature and the absorbance was measured at 540 nm using a Shimadzu UV-2100 spectrophotometer (Kyoto).

2.4. Detection of inducible NO synthase mRNA by reverse transcription and polymerase chain reaction (RT-PCR)

Total RNA was isolated from cells on day 9 and brain of 4-week-old ICR male mice (for a positive control) by using Isogen (Nippongene, Toyama, Japan) and was subjected to RT-PCR. Isolated total RNA were reverse transcribed to first strand cDNA using the Superscript preamplification system (Gibco-BRL, Gaithersburg, MD, USA) under the conditions recommended by the supplier. Oligonucleotide primers for the PCR reaction used to amplify inducible NO synthase (Lyons et al., 1992), neuronal constitutive NO synthase (Ogura et al., 1993) and β-actin (Alonso et al., 1986) cDNAs were constructed according to the available sequence data (Table 1). PCRs were performed with an aliquot of first strand cDNA, 1 μM of each primer, dNTPs (ATP, GTP, CTP and TTP, 200 μM each), PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% w/v gelatin) and 0.5 U/ml of *Taq* polymerase (AmpliTaq DNA Polymerase, Perkin Elmer-Cetus, Branchburg, NJ, USA) in a final volume of 20 μl. After an initial denaturation at 94°C for 5 min, 30 cycles of 1 min denaturation at 94°C, 1 min annealing at 56°C for inducible NO synthase or at 54°C for neuronal NO synthase and 1 min extention at 72°C were performed. For the β-actin mRNA which served as an internal standard, 24 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min were carried out and the consistency of mRNA was confirmed. For all PCRs, a final 10 min elongation at 72°C followed. Under these conditions, the PCR products were proportional to the amount of cDNA subjected to PCR amplification. To exclude false positive signals caused by contamination with genomic DNA, nonreverse transcribed RNA (RT-) was amplified under identical conditions. The amplified product was analyzed by electrophoresis in a 2% agarose gel and stained with ethidium bromide.

To confirm the identity of the PCR product as mouse inducible NO synthase, neuronal constitutive NO synthase and β-actin cDNAs, the PCR products were subcloned into pT7Blue(R) T-Vector (Novagen, Madison, WI, USA) and sequenced by the dideoxynucleotide-chain terminater method (Sanger et al., 1977) using the HITACHI SQ-5500 sequencer (Tokyo, Japan).

Table 1
Oligonucleotide primers used in this study

Gene		Primer Sequence	Nucleotides of cDNA
iNOS	sense	5'-TGGGAGCCACAGCAATATAG-3'	2276-2295
	antisense	5'-CGTAATGTCCAGGAAGTAGG-3'	2652-2671
ncNOS	sense	5'-ACAAGGTCCGATTCAACAGC-3'	2620-2639
	antisense	5'-GTACTGCAACTCCTGATTCC-3'	3162-3181
β-actin	sense	5'-ACCGTGAAAAGATGACCCAG-3'	266-285
	antisense	5'-TACGGATGTCAACGTCACAC-3'	774-793

Positions in cDNAs for these genes correspond to sequences for murine inducible NO synthase (Lyons et al., 1992), neuronal constitutive NO synthase (Ogura et al., 1993) and β-actin (Alonso et al., 1986).

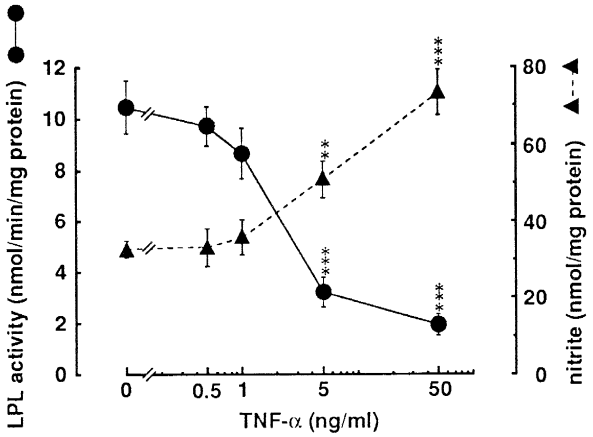

Fig. 1. Effect of increasing doses of TNF-α on lipoprotein lipase activity (LPL) (closed circle) and nitrite production (closed triangle) in brown adipocytes. Cells were treated with TNF-α for 24 h followed by heparin treatment for 1 h, then harvested. Nitrite production was assessed in a separate series of experiments with cells treated with TNF-α. Lipoprotein lipase activity and nitrite production in the absence of TNF-α (0 ng/ml) were 10.5 ± 1.0 nmol/min per mg protein and 32.7 ± 2.2 nmol/mg protein, respectively. Values are means ± S.E.M. (bars) obtained from 5–6 experiments. ** $P < 0.01$, *** $P < 0.001$ vs. 0 ng/ml (absence of TNF-α).

2.5. Materials

Dulbecco's modified Eagle's medium (DMEM), L-glutamine, streptomycin, penicillin, Hepes buffer and fetal bovine serum were obtained from ICN Biomedical (Costa Mesa, CA, USA). Insulin (Novolin) was from Novo Nordisk A/S (Copenhagen, Denmark). Collagenase type II, bovine serum albumin fraction V, L-α-phosphatidylcholine, N^G-nitro-L-arginine methyl ester, aminoguanidine hemisulfate and 8-bromoguanosine 3′:5′-cyclic monophosphate sodium salt were obtained from Sigma (St. Louis, MO, USA). N^G-nitro-D-arginine methyl ester was obtained from Nova Biochem (Läufelfingen, Germany). ODQ (1*H*-[1,2,4]oxadiazolol[4,3-*a*]quinoxalin-1-one) was obtained from Tockris Cookson (St. Louis, MO, USA). Heparin sodium salt and triolein were obtained from Nakalai Chemicals (Tokyo, Japan). [^{3}H]Triolein was purchased from New England Nuclear (Boston, MA, USA). ACS II was obtained from Amersham (Amersham, UK). rhTNF-α (2.55×10^6 JRU/mg of protein) was generously provided by Dainippon Pharmaceutical (Osaka, Japan).

2.6. Statistical analysis

The results are expressed as the mean ± S.E.M. The Dunnett multiple-range test (Dunnett, 1964) was used to determine the significance of differences between experimental groups.

3. Results

The effect of various concentrations of TNF-α on heparin-releasable fraction of lipoprotein lipase activity was determined in brown adipocytes. In the pilot study, the addition of 10 IU/ml heparin for 1 h produced a prompt release of lipoprotein lipase into the medium. Heparin-releasable lipoprotein lipase activity (10.5 ± 1.0 nmol/min per mg protein, $n = 6$) was approximately sevenfold higher than basal activity (1.40 ± 0.06 nmol/min per mg protein, $n = 6$) which represents the enzyme secreted spontaneously into the medium. As shown in Fig. 1, exposure to increasing concentrations of TNF-α (0.5–50 ng/ml) for 24 h resulted in a concentration-dependent decrease in the release of lipoprotein lipase, while there was a reciprocal increase in nitrite concentration in the medium. (Fig. 1). Significant changes in both lipoprotein lipase activity and nitrite concentration were found at the TNF-α concentrations greater than 5 ng/ml. The effect of duration of TNF-α exposure on lipoprotein lipase activity and nitrite concentration are shown in Fig. 2. A reduction of lipoprotein lipase activity was apparent as early as 3 h after addition of 50 ng/ml TNF-α. Approximately 70% of the enzyme activity was lost in the first 6 h and the suppression sustained up to 48 h. During the course of incubation, there was a low but steady accumulation of nitrite in the medium in brown adipocytes without TNF-α. Addition of TNF-α (50 ng/ml) markedly enhanced the accumulation of nitrite in the medium at 16–48 h.

The role of NO in TNF-α-induced suppression of lipoprotein lipase activity was examined using NO synthase inhibitors, namely, L-NAME and aminoguanidine. As shown in Fig. 3A, a decrease in lipoprotein lipase activity after the exposure to 50 ng/ml TNF-α for 24 h was prevented by preincubation with increasing concentrations of L-NAME (1–100 μM). Although not shown in the figure, the suppressive effect with higher concentrations (500 and 1000 μM) of L-NAME was not different from that of 100 μM L-NAME. Incubation with L-NAME without TNF-α treatment caused no changes in lipoprotein lipase activity. D-NAME, an inactive enantiomer of L-NAME, showed no effect in both TNF-α-stimulated and -nonstimulated conditions. In concert with prevention of TNF-α-induced suppression of lipoprotein lipase activity by L-NAME, nitrite accumulation was suppressed in L-NAME-treated cells (Fig. 3B). Again, D-NAME showed

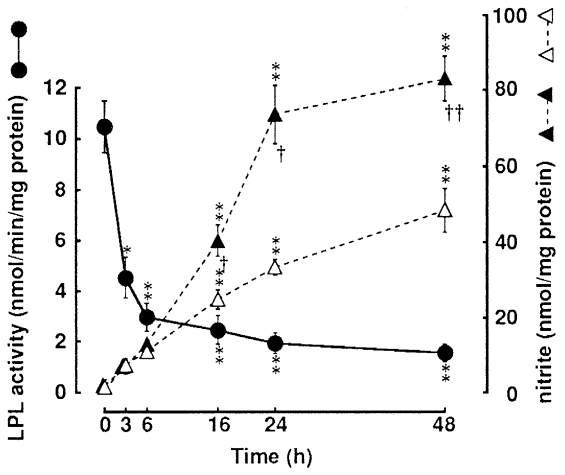

Fig. 2. Time-course of the effect of 50 ng/ml TNF-α on lipoprotein lipase activity (LPL) (closed circle) and nitrite production (triangle) in brown adipocytes. Cells were treated with 50 ng/ml TNF-α for the indicated times followed by heparin treatment for 1 h, then harvested. Nitrite production was assessed in a separate series of experiments with cells treated with 50 ng/ml TNF-α (closed triangle) or without TNF-α (open triangle). Values are means ± S.E.M. (bars) obtained from 5–6 experiments. * $P < 0.05$, ** $P < 0.01$ vs. 0 time. † $P < 0.05$, †† $P < 0.01$ vs. no addition control.

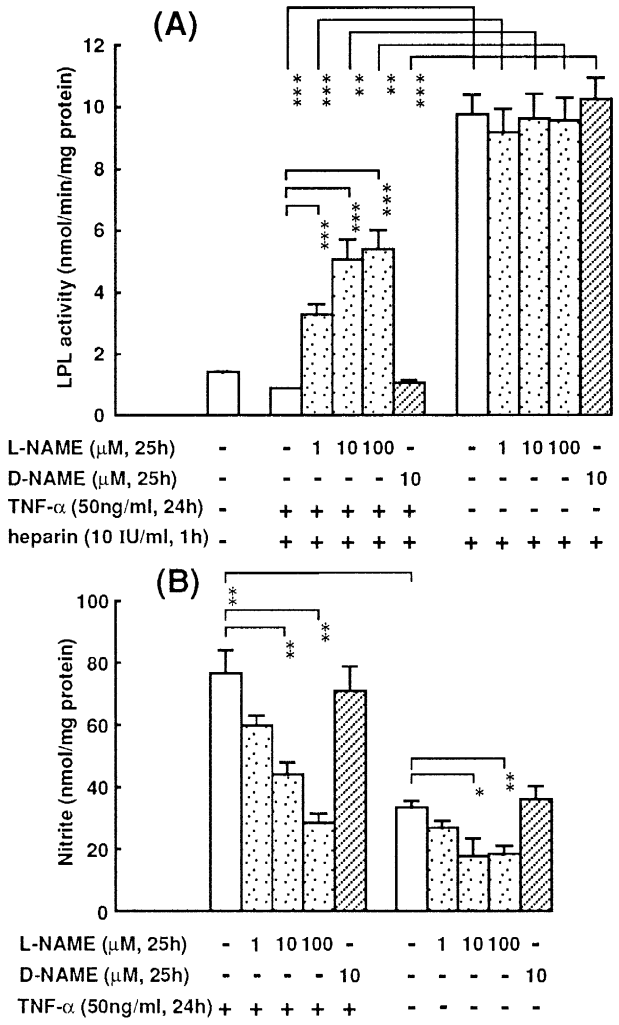

Fig. 3. Prevention of TNF-α-induced reduction of lipoprotein lipase (LPL) activity (A) and elevation of nitrite production (B) by N^{G}-nitro-L-arginine methyl ester (L-NAME). Brown adipocytes on day 8 were treated with L-NAME or D-NAME for 25 h with or without TNF-α (50 ng/ml). Values are means ± S.E.M.(bars) obtained from 5–6 experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ determined by the Dunnet multiple-range test.

no effect. Moreover, aminoguanidine, a putative selective inducible NO synthase inhibitor, blocked TNF-α effects on both lipoprotein lipase activity (Fig. 4A) and nitrite production dose-dependently (Fig. 4B). Aminoguanidine was different from L-NAME in that the effective concentrations were 10 times more than that of L-NAME and that aminoguanidine did not decrease the basal nitrite production.

We then investigated whether TNF-α actually increases inducible NO synthase mRNA abundance in brown adipocytes by RT-PCR assay. Incubation with increasing doses of TNF-α for 24 h induced a concentration-dependent increase in inducible NO synthase mRNA in brown adipocytes, whereas the mRNA was undetectable in unstimulated cells (Fig. 5). In the time-course study, the inducible NO synthase mRNA was weakly detected 3 h after stimulation by TNF-α and became maximum at 24 h, lasting up to 48 h (Fig. 6). In contrast, neuronal constitutive NO synthase mRNA was not detected in both control and TNF-α-treated brown adipocyte in the condition in which the mRNA was clearly amplified in mouse brain (Figs. 5 and 6). The sequence of PCR product for inducible NO synthase from mouse brown adipocytes revealed a 99.4% identity with mouse macrophage.

Finally, we investigated the role of cGMP in the suppressive effect of TNF-α on lipoprotein lipase. 8-bromo-cGMP partially decreased lipoprotein lipase activity in concentrations greater than 100 μM (Fig. 7A). A selective inhibitor of soluble guanylate cyclase, ODQ, restored partially the suppressive effect of TNF-α, while ODQ by

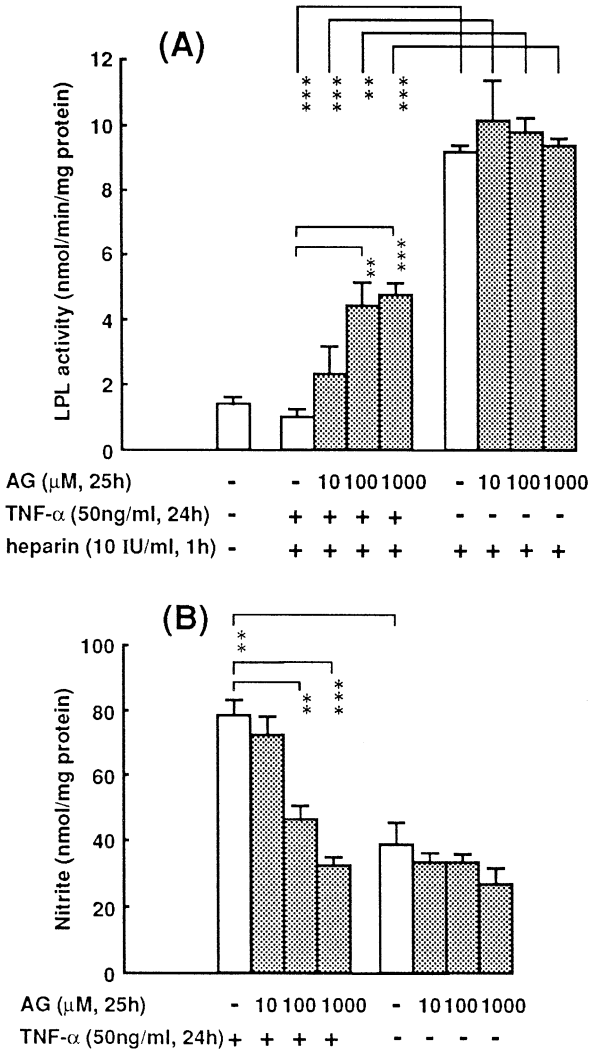

Fig. 4. Prevention of the TNF-α-induced reduction of lipoprotein lipase (LPL) activity (A) and elevation of nitrite production (B) by aminoguanidine (AG). Brown adipocytes on day 8 were treated with the indicated concentrations of aminoguanidine for 25 h with or without TNF-α (50 ng/ml). Values are means $\pm$ S.E.M. (bars) obtained from 4–6 experiments. ** $P < 0.01$, *** $P < 0.001$ determined by the Dunnet multiple-range test.

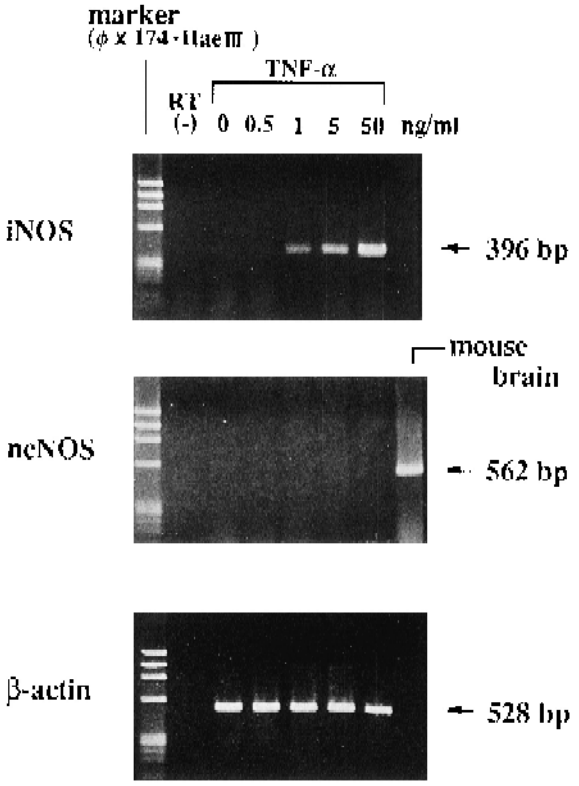

Fig. 5. Effect of increasing doses of TNF-α on NO synthase mRNA abundance in brown adipocytes. Cells on day 8 were treated with the indicated concentrations of TNF-α for 24 h and harvested on day 9. The abundance of inducible NO synthase mRNA, neuronal constitutive NO synthase mRNA and β-actin mRNA in the total RNA was detected by RT-PCR. PCR products amplified with primer pairs described in Table 1 from first strand cDNA or RNA(RT-: no reverse transcription) were analyzed by agarose gel and visualized by staining with ethidium bromide. DNA size markers in the left lanes correspond to: 1357, 1078, 872, 603 and 310 bp. Arrows indicate the predicted size of PCR products.

itself caused no effect on lipoprotein lipase activity (Fig. 7B).

4. Discussion

Early experiments with tumor-bearing mice showed that cachexia is associated with an increased sympathetic stimulation of BAT (Brooks et al., 1981). Cachexia is well documented to be mediated by tumor necrosis factor-α (TNF-α)/cachectin (Kawakami et al., 1982; Beutler et al., 1985). Intravenous injection of TNF-α stimulates sympathetic outflow to BAT in rats and subsequently elevates BAT thermogenic activity assessed by mitochondrial guanosine diphosphate (GDP)-binding (Coombes et al., 1987). Moreover, an intracerebroventricular injection of TNF-α elicits a direct central action to modulate the sympathetic efferent outflow to BAT (Rothwell, 1988; Holt et al., 1989). However, there was little in vitro study investigating direct actions of TNF-α to brown adipocytes. We have previously shown that TNF-α potently suppresses lipoprotein lipase activity by inhibiting the gene expression in brown adipocytes (Uchida et al., 1994).

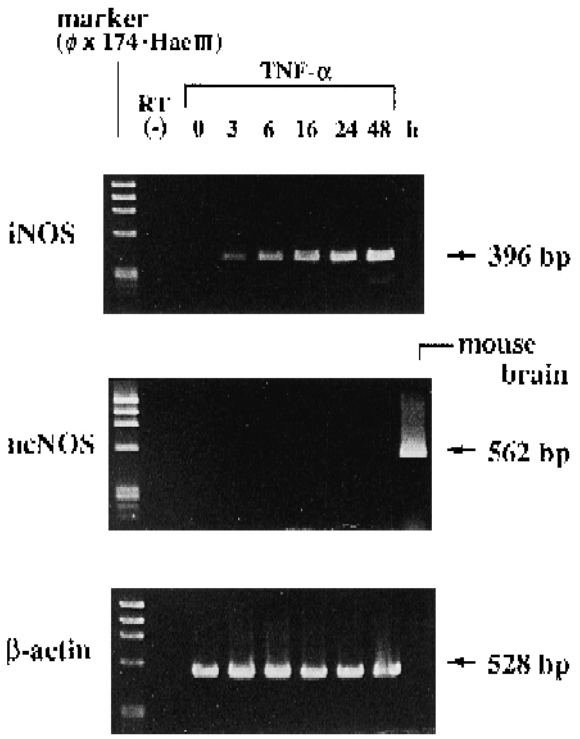

Fig. 6. Time-course of the effect of TNF-α (50 ng/ml) on NO synthase mRNA abundance in brown adipocytes. Cells on day 8 or 9 were treated with 50 ng/ml of TNF-α for the indicated times and harvested. The abundance of inducible NO synthase mRNA, neuronal constitutive NO synthase mRNA and β-actin mRNA in the total RNA was detected by RT-PCR. PCR products amplified with primer pairs described in Table 1 from first strand cDNA or RNA(RT-: no reverse transcription) were analyzed by agarose gel and visualized by staining with ethidium bromide. DNA size markers in the left lanes correspond to: 1357, 1078, 872, 603 and 310 bp. Arrows indicate the predicted size of PCR products.

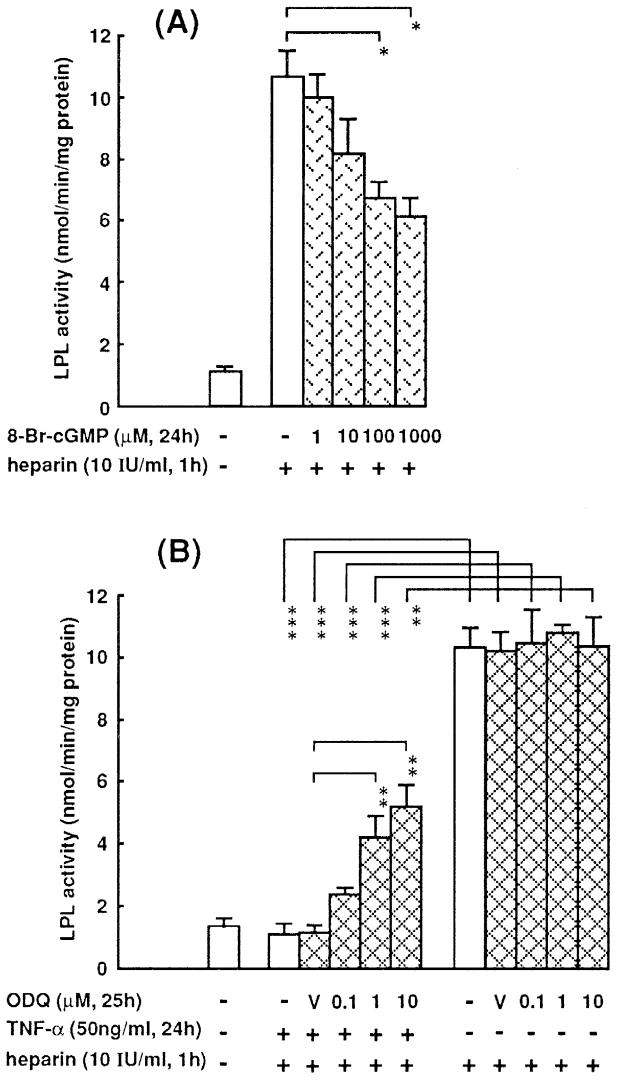

Fig. 7. Effect of increasing concentrations of 8-bromoguanosine 3′:5′-cyclic monophosphate (8-Br-cGMP) on lipoprotein lipase (LPL) activity (A) and reversal by a selective guanylate cyclase inhibitor, ODQ of the TNF-α-induced reduction of lipoprotein lipase activity (B). Brown adipocytes were treated with the indicated concentrations of 8-Br-cGMP for 24 h or with ODQ for 25 h in the presence or absence of TNF-α (50 ng/ml) for 24 h and harvested. Values are means ± S.E.M. (bars) obtained from 4–5 experiments. V indicated vehicle control, DMSO. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ determined by the Dunnet multiple-range test.

In the present study, the mechanism of TNF-α-induced down regulation of lipoprotein lipase was investigated. It is known that TNF-α exerts its effect through production of NO in a variety of cells (Estrada et al., 1992; Hukkanen et al., 1995; Kasai et al., 1995; Buck and Chojkier, 1996). Therefore, we examined whether NO production is linked to TNF-α-induced down regulation of lipoprotein lipase activity. We found that mouse brown adipocytes differentiated in culture produce NO following stimulation with

TNF-α in association with the induction of inducible NO synthase. The inverse relationship between lipoprotein lipase activity and accumulation of nitrite, a stable end product of the L-arginine/NO generating pathway, after addition of TNF-α suggests that NO generation in the cells is related to the suppression of lipoprotein lipase activity.

In order to further clarify whether NO production constitutes the mechanism for the suppressive effect of TNF-α on lipoprotein lipase, we investigated the effect of NO synthase inhibitors. L-NAME and aminoguanidine, but not D-NAME, significantly prevented the suppressive effect of TNF-α on lipoprotein lipase activity, indicating an involvement of NO in the effect of TNF-α. Recently, Schoonjans et al. (1996) demonstrated that mouse lipoprotein lipase gene contains functional peroxisome proliferator response elements (PPREs) in its promoter region. Zhang et al. (1996) further reported that TNF-α negatively regulates the PPARγ gene expression. Therefore, we can speculate that NO produced by TNF-α may modulate lipoprotein lipase expression by inhibiting PPARγ. Alternatively, Tanuma et al. (1995) showed a silencer element in the promoter region of the human lipoprotein lipase gene. NO may suppress lipoprotein lipase gene expression by activating the silencer element. Additionally, there still remains a possibility that the suppressive effect of TNF-α on lipoprotein lipase is mediated by signals other than NO, because the suppression of TNF-α-mediated reduction of lipoprotein lipase activity by NO synthase inhibitors was partial despite the complete inhibition of nitrite accumulation.

We observed the expression of TNF-α-induced inducible NO synthase, but not neuronal constitutive NO synthase, by RT-PCR assay. A low-level production of nitrite by the unstimulated cells was inhibited more effectively by L-NAME than by aminoguanidine in this study. This may suggest NO production by constitutive types of NO synthase in brown adipocytes, although we can not exclude the possibility that the low level of inducible NO synthase induced by some factors in the fetal bovine serum added to the culture medium may be responsible for the basal increase in nitrite levels. Other possibilities include the presence of phenol red in the medium. Phenol red has an estrogenic activity (Berthois et al., 1986) which has been demonstrated to stimulate constitutive NO synthase in other tissues (Hayashi et al., 1995; Ceccatelli et al., 1996). Besides, Ribiere et al. (1996) reported that rat white adipose tissue expresses both inducible NO synthase and endothelial constitutive NO synthase and generates NO which plays an important role in adipose tissue metabolism. The presence of endothelial NO synthase mRNA, another form of constitutive NO synthase in murine brown adipocyte, remains to be studied.

The majority of NO effects under physiological conditions appear to be mediated primarily by the activation of the intracellular NO receptor guanylate cyclase (Garbers, 1992), followed by cGMP production. Therefore, we investigated whether the cGMP is involved in the suppressive effect of TNF-α on lipoprotein lipase activity. A cell-permeant analogue of cGMP, 8-Br-cGMP was capable of partly inhibiting lipoprotein lipase activity and ODQ, which has been shown to potently and selectively prevent activation of soluble guanylate cyclase (Brunner et al., 1995; Garthwaite et al., 1995), significantly prevented the TNF-α-induced suppression of lipoprotein lipase activity. These results suggest that the NO/cGMP signal transduction system is a major mechanism for TNF-α-induced suppression of enzyme activity.

The mechanism of TNF-α in inducing NO synthase in the brown adipocyte is not clear. Recently, Nisoli et al. (1997) reported that norepinephrine induces the NO synthase in brown adipocytes through β-adrenoceptors. However, it is not known whether the mechanism of NO synthase induction by TNF-α is the same as that of norepinephrine. There is increasing evidence that induction of many TNF-α responsive genes is mediated, at least in part, through activation of a nuclear transcription factor, NF-κB (Verma et al., 1995). The 5′-flanking region of the murine inducible NO synthase gene contains NF-κB binding motifs (Lowenstein et al., 1993; Xie et al., 1993). Therefore, there is a possibility that NF-κB may be involved in the TNF-α-induced inducible NO synthase expression.

Collectively, we conclude that brown adipocytes express inducible NO synthase upon stimulation by TNF-α, followed by production of NO, which in turn down regulates lipoprotein lipase activity. The suppression of the enzyme by NO is at least in part mediated by the production of cGMP. Such a mechanism of down regulation of lipoprotein lipase may constitute pathophysiological conditions for cachexia which is promoted by TNF-α.

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