• BASIC RESEARCH •

Effect of cholecystokinin octapeptide on tumor necrosis factor a transcription and nuclear factor-kB activity induced by lipopolysaccharide in rat pulmonary interstitial macrophages

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Abstract

AIM: **To elucidate the anti-inflammatory mechanism of an intestinal neuropeptide, sulfated cholecystokinin octapeptide (sCCK-8), the effects of sCCK-8 on lipopolysaccharide (LPS)-induced tumor necrosis factor a (TNF-a) mRNA expression and NF-kB activity in pulmonary interstitial macrophages (PIMs) were studied.**

METHODS: **PIMs from rat were stimulated with LPS (1mg • L^{-1}) in the presence or absence of sCCK-8 (10^{-8}-10^{-6}mol • L^{-1}) or/and CCK receptor antagonist proglumide (2 mg • L^{-1}). The expression of TNF-a mRNA was assayed by reverse transcription polymerase chain reaction (RT-PCR) at 3h of the stimulation, and nuclear factor-kB (NF-kB) binding activity was analyzed by electrophoretic mobility shift assay (EMSA) at 1 h of stimulation. The IkB-a protein level in the cytoplasma at 30 min of the stimulation was detected by Western blot.**

RESULTS: **sCCK-8, at concentrations from 10^{-8} mol • L^{-1} to 10^{-6} mol • L^{-1} obviously inhibited LPS-induced TNF-a mRNA expression and NF-kB binding activity in a dose-dependent manner, $P<0.05$, $P<0.01$. Stimulation PIMs with LPS resulted in a reduction of IkB-a protein level, $P<0.01$, which was elevated by sCCK-8, $P<0.05$. The effects of sCCK-8 on NF-kB activity and IkB protein level were attenuated by CCK receptor antagonist proglumide, $P<0.01$.**

CONCLUSION: **sCCK-8 inhibits LPS-induced TNF-a mRNA expression by regulating NF-kB activity in rat PIMs, which is mediated through CCK receptors and inhibiting IkB-a degradation. This represents one of the anti-inflammatory mechanisms of sCCK-8.**

INTRODUCTION

Interactions between the central nervous system(CNS) and the immune system, particularly through neuropeptides, are of growing interest. Due to the abundant autonomic innervation and extensive lymphoid compartment localized in the gastrointestinal tract, a number of neuropeptides with observed and potential immunomodulatory effects have been isolated from gut tissues[1].These intestinal neuropeptides with immunomodulatory effects include growth hormone[2], somatostatin[3], vasoactive intestinal peptide(VIP)[4,5], calcitonin gene-related peptide(CGRP)[6], neuropeptide Y[7,8], neurotensin[9], and cholecystokinin(CCK)[10].

CCK is discovered initially in the gut with the function of contracting gallbladder, and subsequently localized in the central and peripheral nervous system[10]. CCK is identified as several different size of the peptide including 4, 8, 33, 39, and 58 amino acid forms. The sulfated carboxy-terminal octapeptide(sCCK-8) is the biologically predominant active form localized in the small intestine, blood and CNS[10]. In the last decade few studies have investigated the relation between CCK and immune cells. Okahata *et al*[11] demonstrated CCK immunoreactivity in peripheral blood leukocytes. Data from several labs suggest that CCK mediates an increase in intracellular calcium in human peripheral blood mononuclear cells and T lymphocyte cell line[12,13]. sCCK-8 can inhibit the mobility capacity and the mitogen-induced lymphocytic proliferation, but can increase the adherence and the spontaneous proliferation of lymphocytes[14,15]. In addition, sCCK-8 is a negative modulator of several functions of murine peritoneal macrophages and human neutrophils including the production of superoxide anion, phagocytosis and mobility[16-18]. Interestingly, sCCK-8 causes an *in vitro* inhibition of lipopolysaccharide(LPS)-induced TNF-α production, sCD14 release and mCD14 expression in rat pulmonary interstitial macrophages (PIMs) [19]. Consistently, the production of proinflammatory cytokines including TNF-α, IL-1α, and IL-6 in endotoxin shock(ES) rat was also inhibited by sCCK-8 *in vivo*[20,21]. These results suggested that sCCK-8 has anti-inflammatory effect to some extent, a new field about the biological action of CCK, which was also confirmed by a

morphological observation that sCCK-8 clearly lessened the inflammatory lesions in lung, spleen and liver tissues in ES rat[22].

It is well known that transcriptional factor NF-κB plays a pivotal role in LPS-induced TNF-α gene expression and involved in the inflammatory response[23]. To elucidate the anti-inflammatory mechanism of sCCK-8, we investigated the effects of sCCK-8 on TNF-α mRNA expression and NF-κB activity in the present study.

MATERIALS AND METHODS

Materials
Collagenase IA, LPS (*E. Coli* 0111: B4), sCCK-8 and proglumide were obtained from Sigma (St. Louis, MO). RPMI-1 640 culture medium and TRIzol reagents were obtained from Gibco BRL (Gercy-Pontoise, France). Avian myeloblastosis virus reverse transcriptase and Gel shift assay system were purchased from Promega (Madison, WI). Taq DNA polymerase was obtained from Sangon (Shanghai, China). Coomassie brilliant blue G250 assay kit was purchased from JianCheng Biotechnology (Nanjing, China). An anti-IκBα polyclonal antibody(C-21) was purchased from Santa Cruz (Santa Cruz, CA.) and horseradish peroxidase (HRP)-conjugated IgG was from Zhongshan Biotechnology(Beijing, China). Female, specific pathogen-free Sprague-Dawley rats (weighting 180- 220 g BW) were obtained from Experimental Animal Center of Hebei Province.

Preparation of rat PIMs
PIMs were isolated from perfused rat lungs with a collagenase digestion technique, modified as Wizemann *et al*[24] described. Alveoli were lavaged 12 to 14 times with 4 ℃ phosphate-buffered saline (PBS) containing 0.6 mmol • L^{-1} EDTA (PBS-EDTA) to remove alveolar macrophages. Lung vessles were perfused with 100 ml PBS-EDTA to remove the monocytes and other blood cells. Then the lung tissues were cut into 500 μm slices followed by digestion in 175 U • ml^{-1} collagenase IA containing 0.1 g • L^{-1} DNase and 100 ml • L^{-1} fetal bovine serum (FBS) in a shaking 37 ℃ water bath for 60 min. The suspension was then filtered through 30 μm mesh. After washing 2 times (400×g, 4℃, 10 min) with PBS, the cells were resuspended in RPMI-1640 medium containing 15 ml • L^{-1} FBS, 100 U • ml^{-1} penicillin, and 100 μg • ml^{-1} streptomycin and incubated for 2 h at the conditions of 37℃ and 50 ml • L^{-1} CO$_2$. Nonadherent cells were removed by gentle washing with warm medium. Remaining adherent cells contained more than 93 % PIMs and the contamination by polymorphonuclear leukocytes and alveolar macrophages was less than 7 %. PIMs viability was greater than 95 %, determined by trypan blue exclusion assay. PIMs (1×10^6) were plated on to culture dishes. After washing 3 times with serum-free medium, PIMs were stimulated with LPS (1 mg • L^{-1}), in the presence or absence of sCCK-8 (10^{-8}-10^{-6} mol • L^{-1}) or/and the CCK receptor antagonist proglumide (2 mg • L^{-11}) for indicated time.

Semi-quantitative RT-PCR for the detection of TNF-a
To determine whether sCCK-8 affect TNF-α transcription, PIMs were stimulated with LPS in the presence or absence of 10^{-8} -10^{-6} mol • L^{-1} sCCK-8 for 3 h. Total cellular RNA was prepared with TRIzol reagents. cDNA was synthesized from 4 μg of the total RNA by extension of random primers with avian myeloblastosis virus reverse transcriptase. PCR of the cDNA was performed in a final volume of 25 μl containing 2 mmol • L^{-1} MgCl$_2$, 4 units Taq DNA polymerase, and 25 pmol specific primers. Amplification of the house-keeping enzyme

glyceraldehydes-3-phosphate dehydrogenase (GADPH) was always involved to serve as control of reaction efficacy. The PCR was performed at the conditions of denaturation for 45 s at 94 ℃, annealing for 45 s at 48 ℃ for TNF-α and 55 ℃ for GADPH, extension for 45 s at 72 ℃, and at the end of 35 cycles, further extension for 5 min at 72 ℃.The primers were TNF-α sense, 5' -CCAACAAGGAGGAGAAGT-3' ; TNF-α antisense, 5' -GTATGAAGTGGCAAATCG-3' (323bp). The synthesized PCR products were separated by electrophoresis on a 20 g • L^{-1} agarose gel and analyzed by Gel-Pro analyzer version 3.1 software (Media Cybernetics). The ratio of arbitrary unit (AU, D_{area} • $D_{density}$) of target genes over GADPH was used for expressing the relative level of mRNA expression.

Electrophoretic Mobility Shift Assay (EMSA) for NF-κB activity
PIMs were stimulated with LPS in the presence or absence of sCCK-8 for 1 h. Nuclear extracts were prepared essentially as described by Liu *et al*[25]. Cells were washed with PBS, lysed with five-pellet volume of buffer A containing 10 mmol • L^{-1} HEPES (pH 7.9), 10 mmol • L^{-1} KCl, 1.5 mmol • L^{-1} MgCl$_2$, 0.5 mmol • L^{-1} DTT, 0.5 mmol • L^{-1} PMSF, and 5 ml • L^{-1} NP-40 and centrifuged for 10 min at 1 850× *g*. Then the pellets were resuspended with three-pellet volume of buffer B (same as buffer A, but without NP-40) and incubated on ice for 10 min. The cells were centrifuged for 15 min at 3 000× *g* to pellet the nuclei.Nuclear pellets were extracted by gently resuspending the nuclei in two thirth-nuclei pellet volume of buffer C (20 mmol • L^{-1} HEPES (pH 7.9), 10 mmol • L^{-1} KCl, 1.5 mmol • L^{-1} MgCl$_2$, 200 ml • L^{-1} glycerol, 0.2 mmol • L^{-1} EDTA, 0.5 mmol • L^{-1} DTT, and 0.5 mmol • L^{-1} PMSF) and one thirth-nuclei pellet volume of buffer D (same as buffer C but with 400 mmol • L^{-1} KCl). After the nuclei were shaken in buffer C plus buffer D for 60 min on ice, the supernatants were collected by centrifugation at 15 000× *g* for 30 min at 4 ℃. Protein concentration was determined using Coomassie brilliant blue G250 assay kit. Double-stranded deoxyoligonucleotides containing the NF-κB consensus recognition site (5'-AGTTGAGGGGACTTTCCCAGG-3') were end labeled with [γ -^{32}P]ATP using T4 polynucleotide kinase and purified with ethanol precipitation. The nuclear proteins (10 μg per lane) were incubated with the radiolabeled probe DNA (3.5pmol, 10 μCi) in the presence of 1mmol • L^{-1} MgCl$_2$, 0.5 mmol • L^{-1} EDTA, 0.5 mmol • L^{-1} DTT, 50 mmol • L^{-1} NaCl, 10 mmol • L^{-1} Tris-HCl (pH 7.5), 0.05 μg poly(dI-dC), and 40 ml • L^{-1} glycerol in a final volume of 10 μl. Binding reactions were then incubated at room temperature for 30 min. The DNA-protein complexes were separated on a 6 % nondenaturing polyacrylamide gel. The gel was then dried and visualized by autoradiography at -80 ℃.

Western blot analysis for IκB**a** protein level
PIMs were stimulated with LPS in the presence or absence of sCCK-8 for 30 min. Cytoplasmic protein was extracted using lytic buffer containing 100 mmol • L^{-1} HEPES, 10 ml • L^{-1} Triton X-100, 1 mmol • L^{-1} EDTA, 10 mmol • L^{-1} DTT, and 1 mmol • L^{-1} PMSF. The lysate was centrifuged 20 000×g for 15 min at 4 ℃ after ice bath for 15min. The supernatant was collected and the protein concentration was determined using Coomassie brilliant blue G250 assay kit. Each sample of 100 mg protein was separated on a SDS-10 % polyacrylamide gel and transferred to nitrocellulose membrane. The membrane was sequentially blocked in TBS containing 20 g • L^{-1} milk

and then incubated with 2 μg • ml^{-1} I κ Bα antibody C-21 (Santa Cruz) overnight, washed, and further incubated with a goat anti-rabbit IgG horseradish peroxidase-conjugated secondary antibody. Blocking and secondary antibody incubation each lasted 1 h at 37 ℃. After several washes, the membrane was developed with DAB. Semi-quantitative analysis of immunoreactivity were measured by Gel-Pro analyzer software, and the results were expressed as AU (D_{area} • $D_{density}$).

Statistical analysis

Data were presented as $x \pm s$ and compared with ANOVA and least significant difference test using SPSS statistical program. A level of $P<0.05$ was considered statistically significant.

RESULTS

sCCK-8 inhibited LPS-induced TNF-α mRNA expression

Increase in TNF-α mRNA was observed 3 h after stimulating PIMs with 1 mg • L^{-1} LPS ($P<0.01$), and the increase was about 3.1 fold. However in the PIMs co-incubated by LPS and sCCK-8, TNF-α mRNA expression level was obviously lowered in comparison with LPS group ($P<0.05$, $P<0.01$). The inhibitory rate of 10^{-8}, 10^{-7} and 10^{-6} mol • L^{-1} sCCK-8 was 19 %, 41 % and 33 % respectively. TNF-α mRNA expression level was not affected by sCCK-8 alone ($P>0.05$) (Figure 1).
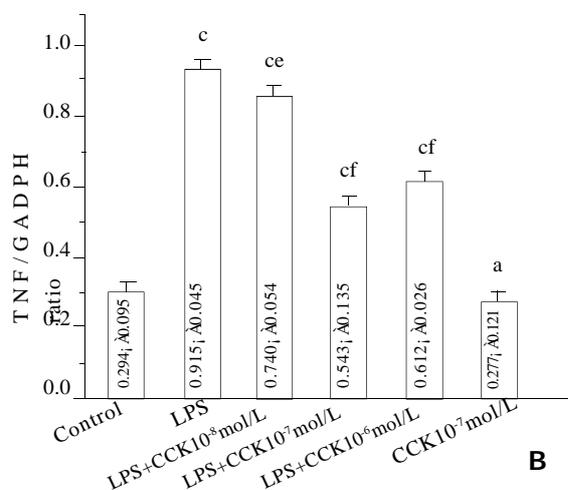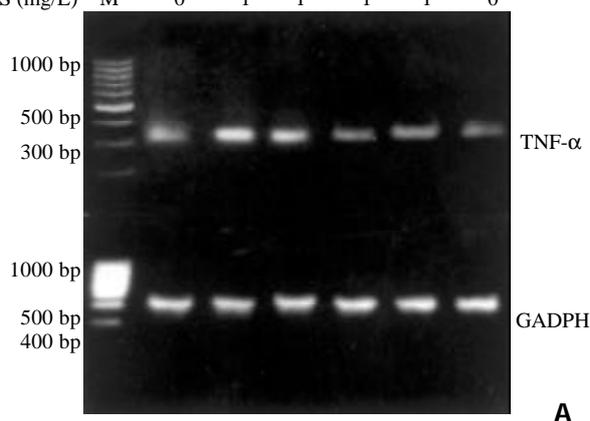

Figure 1 sCCK-8 inhibited LPS-induced TNF-α mRNA expression in PIMs in a dose-dependent manner. A: Representative pictures of three experiments of RT-PCR. B: Relative level of TNF-α mRNA expression. $n=3$, $\overline{x} \pm s$, $^a P>0.05$, $^c P<0.01$ vs control, $^e P<0.05$, $^f P<0.01$ vs LPS.

sCCK-8 inhibited LPS-induced NF-κB binding activity

The NF-κB binding activity was significantly higher in PIMs stimulated with 1 mg • L^{-1} LPS in comparison with unstimulated cells ($P<0.01$), and additional treatment with sCCK-8 markedly reduced the binding activity in a dose-dependent manner. sCCK-8 at the concentrations of 10^{-8}, 10^{-7}, and 10^{-6} mol • L^{-1} inhibited LPS-induced NF-κB binding activity by 34 %, 93 % and 100 % respectively ($P<0.05$, $P<0.01$). The effect of sCCK-8 was abrogated by proglumide ($P<0.01$). sCCK-8 alone had no effect on the NF-κB binding activity ($P>0.05$). The binding specificity was confirmed by using homologous (NF-κB) and nonhomologous (AP-2) oligonucleotides as competitors (Figure 2).
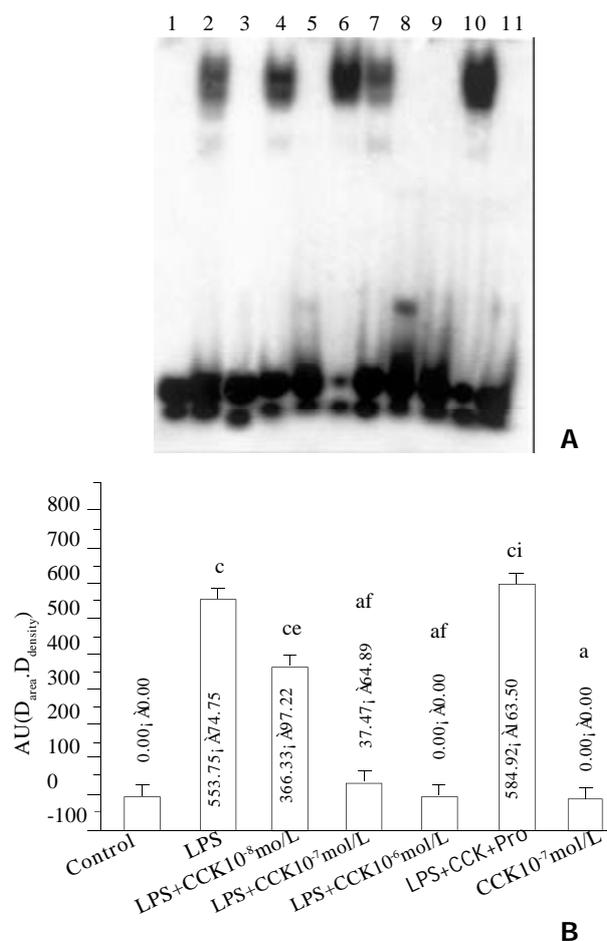

Figure 2 sCCK-8 inhibited LPS-induced NF-κB binding in PIMs in a dose-dependent manner through CCK receptor. A: Representative autoradiography picture of three experiments of EMSA. Lane 1, negative control; lane 2, positive control; lane 3, ^{32}P-labeled NF-κB Oligo plus unlabeled NF-κB Oligo (competitor); lane 4, ^{32}P-labeled NF-κB Oligo plus unlabeled AP-2 Oligo (noncompetitor); lane 5, control; lane 6, LPS; lane 7, LPS+CCK10^{-8} mol • L^{-1}; lane 8, LPS+CCK10^{-7} mol • L^{-1}; lane 9, LPS+CCK10^{-6} mol • L^{-1}; lane 10, LPS+CCK+proglumide; lane 11, CCK10^{-7} mol • L^{-1}. B: Densitometry of EMSA showing decreased NF-κB binding in PIMs treated with sCCK-8. $n=3$, $\overline{x} \pm s$, $^a P>0.05$, $^c P<0.01$ vs control; $^e P<0.05$, $^f P<0.01$ vs LPS; $^i P<0.01$ vs LPS+CCK10^{-7} mol • L^{-1}.

sCCK-8 increased I κ Bα protein level

The I κ Bα protein level in PIMs markedly decreased at 30 min of LPS incubation ($P<0.01$), sCCK-8 at concentrations of 10^{-8}-10^{-6} mol • L^{-1} clearly increased I κ Bα protein level in

PIMs exposure to LPS($P<0.05$). The effect was dose dependent and was attenuated by proglumide($P<0.01$). sCCK-8 alone had no effect on the κBα protein level($P>0.05$) (Figure 3).

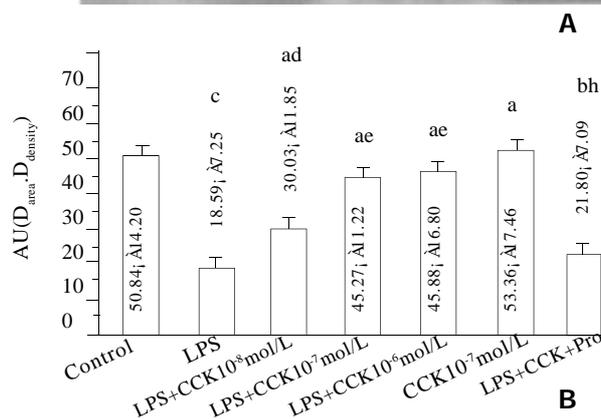| Proglumide | 0 | 0 | 0 | 0 | 0 | 0 | 2 mg/L |
| CCK(mol/L) | 0 | 0 | 10^{-8} | 10^{-7} | 10^{-6} | 10^{-7} | 0 |
| LPS(mg/L) | 0 | 1 | 1 | 1 | 0 | 0 | 0 |

Figure 3 sCCK-8 increased IκBα protein level at 30 min after LPS stimulation in a dose-dependent manner in PIMs. A: Representative Western blot of three experiments. B: Normalized total protein of IκBα immunoreactivity. $n=3$, $\bar{x}\pm s$, [a]$P>0.05$, [b]$P<0.05$, [c]$P<0.01$ *vs* control; [d]$P>0.05$, [e]$P<0.05$, [f]$P<0.01$ *vs* LPS; [h]$P<0.05$ *vs* LPS+CCK10^{-7} mol • L^{-1}.

DISCUSSION

As an intestinal neuropeptide, sCCK-8 not only protects gastric mucosa against alcohol-induced injury[26-28], but also is a potent protective agent against acute lung injury by LPS[22,29]. It obviously reduced the pulmonary artery hypertention and lessened the inflammatory lesion in lung tissues of ES rats[22,29]. We investigated the effect of sCCK-8 on PIMs since these cells play an important role in the inflammatory response to LPS in the lung. Recently, it was reported that treating rats with LPS resulted in a significant increase in production of reactive oxygen intermediates by PIMs, but not by alveolar macrophages(AM). This treatment also markedly enhanced phagocytosis only in PIMs and caused a significant increase in chemotaxis of PIMs towards C5a. These data demonstrated that PIMs play a role in the inflammatory response of the lung to acute endotoxemia[24]. In this study we showed that incubation of rat PIMs with sCCK-8 caused a decrease in LPS-induced TNF-α mRNA expression level in a dose-dependent manner, which suggested that the inhibitory effect of sCCK-8 on TNF-α production occured at a transcriptional level.

It is well known that transcriptional factor NF-κB plays a pivotal role in LPS-induced TNF-α gene expression[23,30]. The present study indicated that sCCK-8 inhibited NF-κB binding activity in PIMs response to LPS in a dose-dependent manner, which was consistent with our results in the previous *in vivo* study that sCCK-8 inhibited NF-κB activity in ES rat lung tissues[31]. These results demonstrated that NF-κB might be the upstream mechanism of the inhibitory effect of sCCK-8 on LPS-induced TNF-α mRNA expressions. Because NF-κB activation can lead to enhanced expression of

proinflammatory cytokines, chemokines, inflammaroty enzymes such as inducible NO synthase (iNOS) and cyclooysgenase (COX-2), adhension molecules and inflammatory receptors[23,30,32,33], modulation of NF-κB activation may provide a direct way of inhibiting inflammatory mediators[34]. Directing drug discovery efforts towards NF-κB activation rather than towards any one of its many target genes could produce a much greater therapeutic benefit by inhibiting expression of the constellation of NF-κB-induced pro-inflammatory genes[35]. Several anti-inflammatory drugs, including corticosteroids, the non-steroidal cyclooxygenase inhibitor sulindac, the benzophenanthridine alkaloid sanguinarine, aspirin and salicylate have all been shown to inhibit NF-κB activation at different stages[35]. Corticosteroids inhibit NF-κB by upregulating the expression of its inhibitor IκB, which displaces DNA-bound NF-κB and restores the latent cytoplasmic IκB-NF-κB complex[35,36]. Sulindac[37], aspirin and salicylate[38] specifically inhibit IκKα kinase activity, and it has also been demonstrated that sanguinarine inhibits NF-κB activation at the level of IκB phosphorylation[39]. In this study, we first demonstrated that the inhibitory effect of sCCK-8 on NF-κB activity was an important signal transduction mechanism of its anti-inflammatory effect. This can also explain the anti-ES role of sCCK-8[22], because NF-κB acts as an important transcriptional factor in the pathogenesis of ES.

Activation of NF-κB is triggered by phosphorylation of an inhibitory subunit, IκB. In unstimulated cells, NF-κB is sequestered in the cytoplasm through interaction with IκBα and IκBα inhibitory proteins. In response to proinflammatory stimuli(eg LPS, cytokines, viruses), IκB is first phosphorylated in its N-terminal domain by a large multikinase complex, then polyubiquitinylayted, and finally degraded by the proteasome. The released NF-κB complex translocates into the nucleus where it will initiate gene transcription upon binding its cognate DNA motifs in regulatory segments of TNF-α gene and other target genes involved in inflammatory and immune process[40]. To elucidate the upstream mechanism of sCCK-8 inhibiting NF-κB activity, we further detected whether sCCK-8 affected IκBα protein level with method of Western blot. And the results demonstrated that the decreased IκBα protein level in LPS-stimulated PIMs was elevated by sCCK-8 in a dose-dependent manner. The increase in IκBα protein level could be the result of decreased degradation, increased synthesis, or a combination of these two mechanisms. Because we detected the elevation of IκBα protein level at 30 min of LPS and sCCK-8 incubation, it was not likely that there could be *de novo* protein synthesis in such short time. So the increase of IκBα protein level was caused by its diminished degradation, suggesting that sCCK-8 inhibited the degradation of IκBα, and subsequently abrogated the translocation to nucleus of NF-κB. However, recently few studies showed that in pancreatic cells, supraphysiological concentrations of CCK(10^{-7} mol • L^{-1}) induced chemokine expression through the activation of NF-κB[41,42]. CCK(10^{-7} mol • L^{-1}) also induced the degradation of IκBα in pancreatic cells[41]. So treatment rats with the supraphysiological concentrations of CCK analogue caerulein led to an acute inflammatory response resembling aspects of clinically important disease acute pancteatitis and it had been used to duplicate the experimental model of acute pancreatitis[41,42]. In our study, the same concentrations of CCK-8(10^{-7} mol • L^{-1}) showed significant inhibitory effect on NF-κB activation and IκBα degradation in LPS-stimulated PIMs, but had no effect on them in the unstimulated PIMs. This discrepancy may be

due to the specificity of the role of CCK in different cells. It is reported that stimulation murine peritoneal macrophages and human neutrophils with CCK led to an inhibition of protein kinase C(PKC) activity[16,18], yet it caused an activation of PKC in rat pancreatic cells[41,42].

Recent data demonstrated the presence of CCK receptors on human monocytes[43] and lymphocytes[44,45]. Our laboratory reported that the pulmonary macrophages expressed CCK receptor detected by *in situ* hybridization and *in situ* RT-PCR[46]. In this study, we found that a CCK receptor antagonist proglumide blocked the effects of sCCK-8 on NF-κB binding activity and IκB protein level, indicating that the roles of sCCK-8 were mediated through CCK receptor. Yet the signal transduction mechanisms between CCK receptor and IκB remain to be further determined.

In this study, the concentration of sCCK-8 that showed a distinct effect on LPS-induced TNF-α mRNA expression and NF-κB activity was from 10^{-8} to 10^{-6} mol • L^{-1}. However, sCCK-8 alone, at a concentration of 10^{-7} mol • L^{-1} had no effect on the above functions of the resting PIMs. Similarly, De la Fuente *et al*[16] reported that several functions of resting mouse peritoneal macrophages were inhibited by sCCK-8 at the lower concentrations of 10^{-10}-10^{-8} mol • L^{-1} but not the higher concentration of 10^{-6} mol • L^{-1}. They considered that the lower response to higher concentrations of sCCK-8 might be ascribed to a process of cell desensitization, which is characteristic of sequestration and/or down-regulation of CCK receptor. We reported that LPS up-regulated the expression of CCK receptor[47], so LPS activating PIMs rather than resting PIMs could response to higher concentration of sCCK-8.

In conclusion, our results show that sCCK-8 reduces LPS-induced TNF-α production at transcriptional level by inhibiting NF-κB activity, and the inhibitory effect of NF-κB activity is mediated through the decrease of IκBα degradation, which represents one of the anti-inflammatory mechanisms of sCCK-8.

REFERENCES

1   **Jonsdottir IH**. Special feature for the Olympics: effects of exercise on the immune system: neuropeptides and their interaction with exercise and immune function. *Immunol Cell Biol* 2000;**78**:562-570

2   **Heemskerk VH**, Daemen MA, Buurman WA. Insulin-like growth factor-1 (IGF-1) and growth hormone (GH) in immunity and inflammation. *Cyto Gro Fac Rev* 1999;**10**:5-14

3   **Corsi MM**, Fulgenzi A, Tiengo M, Pravettoni G, Gaja G, Ferrero ME. Effect of somatostatin on beta-endorphin release in rat experimental chronic inflammation *Life sci* 1999;**64**:2247-2254

4   **Leceta J**, Gomariz RP, Carmen M, Abad C, Ganea D, Delgado M. Receptors and transcriptional factors involved in the anti-inflammatory activity of VIP and PACAP. *Ann N Y Acad Sci* 2000; **921**:92-102

5   **Ganea D**, Delgado M. Neuropeptides as modulators of macrophage functions. Regulation of cytokine production and antigen presentation by VIP and PACAP. *Arch Immunol Ther Exp* 2001;**49**:101-110

6   **Cadieux A**, Monast NP, Pomerleau F, Fournier A, Lanoue C. Bronchoprotector properties of calcitonin gene-related peptide in guinea pig and human airways. Effect of pulmonary inflammation *Am J Res Cri Care Med* 1999;**159**:235-243

7   **Dela Fuente M**, Del Rio M, Medina S. Changes with aging in the modulation by neuropeptide Y of murine peritoneal macrophage functions. *J Neuroimmunol* 2001;**116**:156-167

8   **Elenkov IJ**, Wilder RL, Chrousos GP, Vizi ES. The sym-
pathetic nerve--an integrative interface between two supersystems: the brain and the immune system. *Pharmacol Rev* 2000;**52**:595-638

9   **Feldberg RS**, Cochrane DE, Carraway RE, Brown E, Sawyer R, Hartunian M, Wentworth D. Evidence for a neurotensin receptor in rat serosal mast cells. *Inflammat Res* 1998;**47**:245-250

10 **Crawley JN**, Corwin RL. Biological actions of cholecystokinin. *Peptides* 1994; **15**:731-755

11 **Okahata H**, Nishi Y, Muraki K, Sumii K, Yukitaka M, Tomofusa U. Gastrin/cholecystokinin-like immunoreactivity in human blood cells. *Life Sci* 1985; **36**:369-373

12 **Sacerdote P**, Breda M, Barcellini W, Meroni PL, Panerai AE. Age-related changes of beta-endorphin and cholecystokinin in human and rat mononuclear cells. *Peptides* 1991; **12**:1353-1356

13 **Akiyoshi J**, Yamauchi C, Furuta M, Katsuragi S, Kohno Y, Yamamoto Y, Miyamoto M, Tsutsumi T, Isogawa K, Fujii I. Relationship between SCL-90, Maudsley Personality Inventory and CCK4-induced intracellular calcium response in T cells. *Psychiatry Res* 1998;**81**:381-386

14 **Medina S**, Rio MD, Cuadra BD, Guayerbas N, Fuente MD. Age-related changes in the modulatory action of gastrin-releasing peptide, neuropeptide Y and sulfated cholecystokinin octapeptide in the proliferation of murine lymphocytes. *Neuropeptides* 1999;**33**:173-179

15 **Dela Fuente M**, Carrasco M, Del Rio M, Hernanz A. Modulation of murine lymphocyte functions by sulfated cholecystokinin octapeptide. *Neuropeptides* 1998;**32**:225-233

16 **Dela Fuente M**, Campos M, Del Rio M, Hernanz A. Inhibition of murine peritoneal macrophage functions by sulfated cholecystokinin octapeptide. *Regul Pept* 1995;**55**:47-56

17 **Dela Fuente M**, Medina S, Del Rio M, Ferrandez MD, Hernanz A. Effect of aging on the modulation of macrophage functions by neuropeptides. *Life Sci* 2000;**67**:2125-2135

18 **Carrasco M**, Del Rio M, Hernanz A, De la Fuente M. Inhibition of human neutrophil functions by sulfated and nonsulfated cholecystokinin octapeptides. *Peptides* 1997; **18**:415-422

19 **Li SJ**, Cong B, Yan YL, Yao YX, Ma CL, Ling YL. Cholecystokinin octapeptide inhibits the in vitro expression of CD14 in rat pulmonary interstitial macrophage induced by lipopolysaccharide. *Chin Med J* 2002; **115**:276-279

20 **Ling YL**, Meng AH, Zhao XY, Shan BE, Zhang JL, Zhang XP. Effect of cholecystokinin on cytokines during endotoxic shock in rats. *World J of Gastroenterol* 2001;**7**:667-671

21 **Meng AH**, Ling YL, Zhang XP, Zhao XY, Zhang JL. CCK-8 inhibits expression of TNF-[alpha] in the spleen of endotoxic shock rats and sigual transduction mechanism of p38 MAPK. *World J Gastroenterol* 2002;**8**:139-143

22 **Ling YL**, Huang SS, Wang LF, Zhang JL, Wan M, Hao RL. Cholecystokinin octapeptide reverses experimental endotoxin shock. *Shengli Xuebao* 1996; **48**: 390-394

23 **Christman JW**, Lancaster LH, Blackwell TS. Nuclear factor kappa B: a pivotal role in the systemic inflammatory response syndrome and new target for therapy. *Intensive Care Med* 1998;**24**:1131-1138

24 **Wizemann TM**, Laskin DL. Enhanced phagocytosis, chemotaxis, and production of reactive oxygen intermediates by interstitial macrophages following acute endotoxemia. *Am J Respir Cell Mol Biol* 1994; **11**: 358-365

25 **Liu S**, Khemlani LS, Shapiro RA, Johnson ML, Liu K, Geller DA, Watkins SC, Goyert SM, Billiar TR. Expression of CD14 by hepatocytes: upregulation by cytokines during endotoxemia. *Infect Immun* 1998; **66**: 5089-5098

26 **Mercer DW**, Cross JM, Smith GS, Miller TA. Protective action of gastrin-17 against alcohol-induced gastric injury in the rat: role in mucosal defense. *Am J Physiol* 1997;**273**: G365-G373

27 **Mercer DW**, Cross JM, Chang L, Lichtenberger LM. Bombesin prevents gastric injury in the rat: role of gastrin.

Dig Dis Sci 1998;**43**: 826-833

28 **Mercer DW**, Smith GS, Miller TA. Cyclooxygenase inhibition attenuates cholecystokinin-induced gastroprotection. *Dig Dis Sci* 1998;**43**:468-475

29 **Ling YL**, Cong B, Gu ZY, Li SJ, Zhou XH. Inhibitory effect of cholecystokinin octapeptide on pulmonary arterial hypertension during endotoxic shock. *Zhongguo Xueshu Qikan Wenzhai* 2000; **6**:890-892

30 **Baeuerle PA**, Baltimore D. NF-κB: ten years after. *Cell* 1996;**87**:13-20

31 **Cong B**, Ling YL, Gu ZY. Effect of cholecystokinin octapeptide on lipopolysaccharide- induced increase of NF-κB activity in rat lung tissues (Abstract). *Zhongguo Bingli Shengli Zazhi* 2000;**16**:991-991

32 **Goldring CE**, Reveneau S, Pinard D, Jeannin JF. Hyporesponsiveness to lipopolysaccharide alters the composition of NF-κB binding to the regulatory regions of inducible nitric oxide synthase gene. *Eur J Immunol* 1998; 28:2960-2970

33 **Barnes PJ**, Adcock IM. NF-κB: a pivotal role on asthma and a new target for therapy. *Trends in Phamacol Sci* 1997; **18**:46-50

34 **Zhang GL**, Ghosh S. Toll-like receptor - mediated NF-κB activation: a phylogenetically conserved paradigm in innate immunity. *J Clin Invest* 2001; **107**: 13-19

35 **Emery JG**, Ohlstein EH, Jaye M. Therapeutic modulation of transcription factor activity. *Trends in Phamacol Sci* 2001; **22**:233-240

36 **Barnes PJ**. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clin Sci (Lond)* 1998;**94**:557-572

37 **Yamamoto Y**, Yin MJ, Lin KM, Gaynor RB. Sulindac inhibits activation of the NF-κB pathway. *J Biol Chem* 1999; **274**:27307-27314

38 **Yin MJ**, Yamamoto Y, Gaynor RB. The anti-inflammatory agents aspirin and salicylate inhibit the activity of Iκ B kinase-β . *Nature* 1998;**396**:77-80

39 **Chaturvedi MM**, Kumar A, Darnay BG, Chainy GB, Agarwal S,Aggarwal BB.Sanguinarine(pseudochelerythrine) is a potent inhibitor of NF-κB activation, α phosphorylation, and degradation. *J Biol Chem* 1997; **272**: 30129-30134

40 **Baeuerle PA**. Iκ B- NF-κ B structures: at the interface of inflammation control. *Cell* 1998;**95**:729-731

41 **Tando Y**, Algul H, Wagner M, Weidenbach H, Adler G, Schmid RM. Caerulein-induced NF-κB/Rel activation requires both Ca²⁺ and protein kinase Cas messengers. *Am J Physiol* 1999;**277**:G678-686

42 **Han B**, Logsdon CD. CCK stimulates mob-1 expression and NF-kappaB activation via protein kinase C and intracellular Ca²⁺. *Am J Physiol Cell Physiol* 2000;**278**:C344-351

43 **Schmitz F**, Schrader H, Otte J, Schmitz H, Stuber E, Herzig K, Schmidt WE. Identification of CCK-B/gastrin receptor splice variants in human peripheral blood mononuclear cells. *Regul Pept* 2001; **101**:25-33

44 **Cuq P**, Gross A, Terraza A, Fourmy D, Clerc P, Dornand J, Magous R. mRNAs encoding CCKB but not CCKA receptors are expressed in human T lymphocytes and Jurkat lymphoblastoid cells. *Life Sci* 1997; **61**:543-555

45 **Dornand J**, Serge R, Francoise M, Jean-Pierre B, Suzanne C, Jean F, Richard M. Gastrin-CCK-B type receptors on human T lymphoblastoid Jurkat cells. *Am J Physiol* 1995; **268**:G522-529

46 **Cong B**, Ling YL, Zuo M. In situ RT-PCR detection of cholecystokinin receptor gene expression in rat lung tissues (Abstract). *Zhongguo Bingli Shengli Zazhi* 1998; **14**:335-335

47 **Cong B**, Ling YL, Gu ZY. The expression of cholecystokinin receptor A and B gene in rat lung tissues (Abstract). *Zhongguo Bingli Shengli Zazhi* 1997;**13**:55-55

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