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Endogenous αCGRP protects against concanavalin A-induced hepatitis in mice

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Abstract

To evaluate hepatoprotective effect of α-calcitonin gene-related peptide (αCGRP), we compared the susceptibilities of αCGRP−/− and wild-type mice to concanavalin A (Con A)-induced hepatitis. Twelve hours after Con A administration, serum transaminases were markedly higher in αCGRP−/− than wild-type mice, and much more extensive TUNEL-positive lesions and DNA fragmentation were detected in the livers of αCGRP−/− mice. Notably, expression of IL-6 was selectively diminished in αCGRP−/− mice, suggesting that induction of IL-6 during acute inflammatory responses is blunted in αCGRP−/− mice. In addition, primary cultured αCGRP−/− hepatocytes were more susceptible to IFN-γ-induced cell death than hepatocytes from wild-type mice. Administration of exogenous αCGRP reduced the incidence of apoptosis among hepatocytes and endothelial cells. It thus appears that αCGRP exerts a hepatoprotective effect by modulating cytokine expression and preventing apoptosis.

Keywords: CGRP; Knockout mice; Liver; Hepatitis; Apoptosis; IL-6

Hepatitis is known to be caused by a variety of agents, including viruses, bacteria, alcohol, and various chemicals. It is currently treated using glycyrrhizin, ursodeoxycholic acid, and steroids, but all of these are merely palliative and there continues to be a strong need to find novel hepatoprotective substances.

α-Calcitonin gene-related peptide (αCGRP) is a 37-amino acid peptide produced by alternative splicing of the primary transcript of the calcitonin/αCGRP gene [1]. αCGRP is ubiquitously expressed, but particularly in the central and peripheral nervous systems. Nonadrenergic, noncholinergic (NANC) neurons containing αCGRP are widely distributed in the vasculature, where αCGRP exerts a potent vasodilating effect [2]. Indeed, several investigators have suggested that αCGRP plays a key role in regulating peripheral vascular tone and regional blood flow under both physiological and pathophysiological conditions [3,4]. Apart from its vasodilatory effect, αCGRP also has pleiotropic functions that have been implicated in the regulation of cell proliferation [5], apoptosis [6,7], and differentiation [8]. Expression of αCGRP also has been detected in the normal liver, and it is synthesized by primary hepatocytes in culture [9]. What's more, hepatocytes express functional receptors for αCGRP [10,11], suggesting a paracrine αCGRP system could be involved in hepatic function, although the precise actions of αCGRP in liver remain unknown.

Recently, αCGRP was reported to exert a protective effect against organ damage in several disease models [12,7,13], which prompted us to speculate that αCGRP might also exert protective effects against hepatic injury. To investigate that possibility, we established a strain of αCGRP knockout mice (αCGRP−/−) by deleting αCGRP-specific exon 5 from the calcitonin/αCGRP gene. These mice express calcitonin normally, but they completely

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lack αCGRP and show somewhat elevated blood pressure and heart rates as the result of increased sympathetic nervous activity [14].

In the present study, we induced hepatic injury in αCGRP−/− mice and their wild-type littermates by administering them concanavalin A (Con A). Con A-induced hepatitis is considered to be a useful model of the immune-mediated liver damage that occurs in humans with viral hepatitis [15]. Using this model, we evaluated the hepatoprotective effects of endogenous αCGRP.

Materials and methods

Con A-induced hepatitis model. Male αCGRP−/− mice and their wild-type littermates were studied at 10 weeks of age. The genetic background of them is 129/Sv × C57BL/6 hybrid. The mice were maintained under specific pathogen-free conditions in an environmentally controlled clean room at the Division of Laboratory Animal Research, Department of Life Science, Research Center for Human and Environmental Sciences, Shinshu University. Con A (Seikagaku, Japan) was dissolved in phosphate-buffered saline (PBS) to a concentration of 2 mg/ml and then administered to mice at a dosage of 20 μg/g-body weight via the tail vein. PBS was intravenously administered to control mice. Blood samples were collected 12 h after the injections, and the activities of serum transaminases (ALT and AST) were measured using a Model 7150 Automatic Analyzer (Hitachi, Japan). All animal experiments were conducted in accordance with the ethical guidelines of Shinshu University.

Histological examination. Livers were fixed in 10% formalin neutral buffer solution, embedded in paraffin, and cut into 5 μm sections, which were stained with hematoxylin and eosin (H&E) for histological examination. Apoptosis was detected using the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-digoxigenin nick end labeling (TUNEL) method with an Apoptosis In Situ Detection Kit (Chemicon, Temecula, CA). Cultured primary mouse hepatocytes were fixed in 1% paraformaldehyde in PBS (pH 7.4), post-fixed in ethanol/acetic acid (2:1), and then stained using the same methods used for tissue sections.

Analysis of DNA fragmentation. Livers were incubated for 12 h at 55 °C in lysis buffer containing 1 mg/ml proteinase K, 0.5% SDS, 100 mM EDTA, and 50 mM Tris–HCl (pH 8.0), after which chromosomal DNA was obtained using phenol–chloroform extraction and ethanol precipitation. Samples of the DNA in Tris–EDTA buffer were then incubated with 10 μg/ml RNase A for 30 min at 37 °C and electrophoresed on a 1.5% agarose gel in Tris–acetate–EDTA buffer containing 0.1 μg/ml ethidium bromide.

RNA extraction and semiquantitative RT-PCR. Total RNA was extracted from mouse livers using Trizol Reagent (Invitrogen, Carlsbad, CA), after which it was treated with DNA-Free (Ambion, Austin, TX) to remove contaminating DNA, and 2 μg samples were subjected to reverse transcription using Superscript III first-strand synthesis system with oligo(dT) primer (Invitrogen). Semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) was then carried out using Ex *Taq* DNA polymerase (Takara, Japan). The annealing temperature was 65 °C. The forward and reverse primers and product sizes were as follows: IL1-β, 5′-TCTCACAGCAGCACATCAAC-3′ and 5′-TCGTTGCTTGGTTCTCCTTG -3′ (product size, 361 bp); IL-6, 5′-GACTTCACAGAGGATACCAC-3′ and 5′-ATCTCTCTGAAGGACTCTGG-3′ (product size, 336 bp); TNF-α, 5′-TCAGCCTCTTCTCATTCCTG-3′ and 5′-AGAGGAGGTTGACTTTCTCC-3′ (product size, 420 bp); IFN-γ, 5′-CACACTGCATCTTGGCTTTG-3′ and 5′-GCTGTTGCTGAAGAAGGTAG-3′ (product size, 308 bp); Fas, 5′-CAAGGAGGCCCATTTTGCTG-3′ and 5′-GTTGCTGTGCACGGCTCAAG-3′ (product size, 320 bp); hypoxanthine phosphoribosyltransferase (hprt), 5′-GTTGGATACAGGCCAGACTTTGTTG-3′ and 5′-GAGGGTAGGCTGGCCTATAGGCT-3′ (product size, 269 bp). Hprt was amplified with 25 cycles to normalize the level of each PCR product. IL1-β, TNF-α, IL-6, IFN-γ, and Fas were amplified with 38, 38, 40, 40, and 30 cycles, respectively.

Quantitative real-time RT-PCR analysis of IL-6 expression. RNA extraction and reverse transcription were performed using the same methods used for RT-PCR. The primers used were IL-6 forward: 5′-CCCAATTTCCAATGCTCTCC-3′ and reverse: 5′-TGAATTGGATGGTCTTGGTCC-3′. Quantitative real-time RT-PCR analysis was performed on ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) using SYBR Green (Toyobo, Japan) and normalized to GAPDH (Applied Biosystems).

Cell culture. Primary adult mice hepatocytes were isolated using a two-step collagenase perfusion, followed by centrifugation through a 50% Percoll (MP Biomedicals, Inc, Solon, OH) gradient. The isolated hepatocytes were then cultured in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen) supplemented with 10% fetal bovine serum (FBS) (Equitech-Bio Inc, Kerrville, TX) and a mixture of 100 U/ml penicillin-100 μg/ml streptomycin (Invitrogen). The cells were seeded to a density of 3×10^5 cells/well in 6-well plates or to a density of 10^4 cells/well in 96-well plates coated with gelatin and incubated at 37 °C under a 5% CO_2 atmosphere. Human umbilical vein endothelial cells (HUVECs) (Cell Applications Inc, San Diego, CA) were cultured in endothelial cell basal medium-2 (EBM-2) (Cambrex, Walkersville, MD).

Cytotoxicity assay. Hepatocytes were stimulated with recombinant mouse interferon-γ (IFN-γ; 100 U/ml) (Chemicon) for 48 h, while HUVECs were stimulated with recombinant human TNF-α (40 ng/ml) (Wako, Japan) for 24 h. In some instances, human αCGRP (Peptide Institute, Japan) was added to a concentration of $10^{-10} - 10^{-7}$ M 3 h before IFN-γ or TNF-α. Lactose dehydrogenase (LDH) in the supernatants of the treated cells was measured using a CytoTox 96 Nonradioactive Cytotoxicity Assay Kit (Promega, Madison, WI).

Cell viability assay. The viability of the hepatocytes was assessed using a WST-8 kit (Dojindo, Japan) according to the manufacturer's instructions. Hepatocytes were stimulated with recombinant mouse IFN-γ (100 U/ml) for 48 h, then incubated for 1 h with WST-8 assay solution, after which the formazan formed was measured using a spectrophotometer as a function of the OD_{450}.

Statistical analysis. Values are expressed as means ± SE. Student's *t* tests were used to compare groups. Values of $P < 0.05$ were considered significant.

Results

Increased liver damage and apoptosis in αCGRP−/− mice

We initially evaluated the susceptibility of αCGRP−/− mice to Con A-induced hepatic injury. Basal levels of serum ALT and AST were not different between αCGRP−/− and

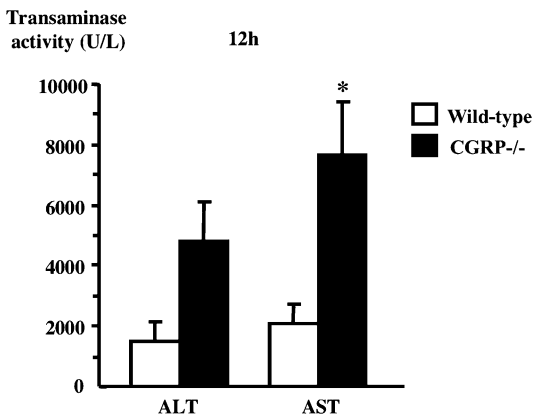

Fig. 1. Serum levels of ALT and AST in αCGRP−/− and wild-type mice 12 h after Con A administration. Serum AST was significantly higher in αCGRP−/− than wild-type mice. Bars are means ± SE; $n = 7$ and 10 for wild-type and αCGRP−/− mice, respectively; *$P < 0.05$ vs. wild-type mice.

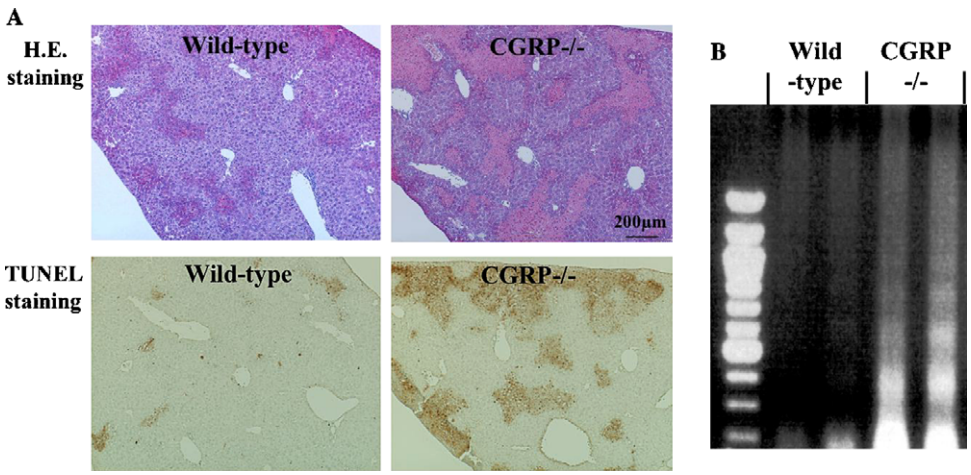

Fig. 2. (A) Histological evaluation of liver specimens obtained 24 h after Con A administration to wild-type and αCGRP−/− mice. Eosin-positive patchy lesions in H&E stained sections and TUNEL-positive apoptotic lesions were both more extensive in the livers of αCGRP−/− mice. We examined six sections in each animal. Figures show the representative histology from six individuals of each genotype. (B) DNA fragmentation characteristic of apoptosis was readily detected in liver tissue from αCGRP−/− mice after Con A administration. Figures show the representative electrophoresis photograph from six individuals of each genotype.

wild-type mice (data not shown), but 12 h after administration of Con A, serum ALT and AST levels were much higher in αCGRP−/− mice (Fig. 1). Consistent with this finding, histological examination of the livers showed severe tissue damage in αCGRP−/− mice; eosin-positive patchy lesions were much more extensive in the livers of αCGRP−/− mice than wild-type mice (Fig. 2A, upper panels). In addition, extensive TUNEL-positive lesions (Fig. 2A, lower panels) and DNA fragmentation (Fig. 2B) were seen in liver samples from αCGRP−/− mice. Thus, Con A caused much more severe liver damage and apoptosis in αCGRP−/− mice than wild-type mice.

Altered expression of inflammatory cytokines in αCGRP−/− mice

We next analyzed the expression of inflammatory cytokines and Fas in liver samples showing Con A-induced hepatitis. Expression of IL-1β, TNF-α, IL-6, and Fas was assessed 4 h after Con A administration, while expression of IFN-γ was assessed after 24 h. We found that expression of IL-1β, TNF-α, IFN-γ, and Fas did not differ between αCGRP−/− and wild-type mice. On the other hand, expression of IL-6 at 4 h after Con A administration was selectively diminished in αCGRP−/− mice (Fig. 3). Expression of IL-6 was also reduced in the spleen samples from αCGRP−/− mice at 4 h after Con A administration (data not shown). We then analyzed the time-course and relative gene expression level of IL-6 (Fig. 4). The expression of IL-6 was low and not different between wild-type and αCGRP−/− mice at basal level. The expression was increased at 4 h after Con A administration in both wild-type and αCGRP−/− mice, however, the expression level was significantly lower in αCGRP−/− mice. At 24 h, the expression was reduced in wild-type mice, but still high in αCGRP−/− mice. These results suggest that, in

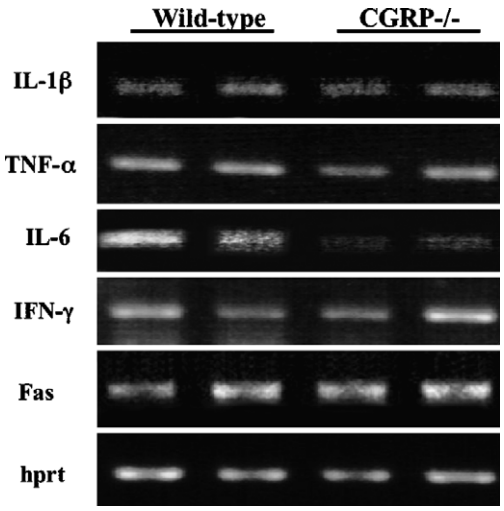

Fig. 3. RT-PCR analysis of cytokine expression in livers exhibiting Con A-induced hepatitis. Expression of IL-1β, TNF-α, IL-6 and Fas was assessed 4 h after Con A administration, while expression of IFN-γ was assessed after 24 h. Expression of hprt served as an internal control. Expression of IL-6 was selectively diminished in αCGRP−/− mice. Figures show the representative electrophoresis photograph from four individuals from each genotype.

αCGRP−/− mice, induction of IL-6 expression is blunted at earlier phase of inflammation, whereas it continues to be higher level at later phase of inflammation.

Lower viability of αCGRP−/− hepatocytes

To test whether the increased susceptibility of αCGRP−/− mice to liver injury reflected the increased sympathetic nervous system activity seen in these mice [15], we next analyzed the features of hepatocytes in primary culture, so that any sympathetic nervous system effects should be eliminated. We found that, after IFN-γ treatment, αCGRP−/− hepatocytes released significantly

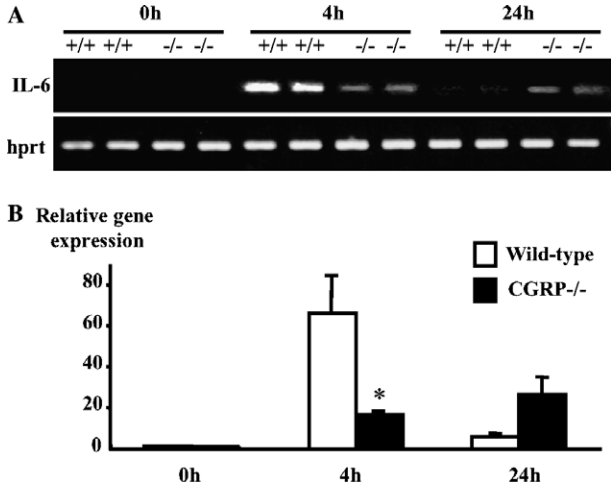

Fig. 4. Time-course and relative expression of IL-6 in livers exhibiting Con A-induced hepatitis. (A) Representative RT-PCR electrophoresis photograph from four individuals from each genotype. (B) Quantitative real-time RT-PCR analysis of IL-6 expression. The expression of IL-6 was normalized to that of GAPDH. The normalized expression was then further normalized with respect to that obtained with samples from wild-type mice at 0 h. $*P < 0.05$ vs. wild-type mice.

greater amounts of LDH into the supernatant than the wild-type cells did, which is indicative of greater cytotoxicity (Fig. 5A). Correspondingly, cell viability was significantly lower among αCGRP−/− hepatocytes (Fig. 5B). In addition, TUNEL-positivity (Fig. 6) was more frequently observed among αCGRP−/− than wild-type hepatocytes. That αCGRP−/− hepatocytes in culture exhibited changes similar to those seen in the liver indicates the changes were unrelated to an alteration in sympathetic nervous system activity.

αCGRP reduces apoptosis among hepatocytes and endothelial cells

Based on the findings presented thus far, we hypothesized that αCGRP exerts an anti-apoptotic effect on the cells of the liver. To test that idea, we evaluated the anti-apoptotic effect of αCGRP on IFN-γ-treated hepatocytes from adult mice. As we expected, αCGRP (10^{-9} M) treatment significantly reduced the incidence of apoptosis of hepatocytes induced by IFN-γ treatment (Fig. 7A). Next, we analyzed the effects of αCGRP on endothelial cells. We found that αCGRP also significantly reduced the incidence of TNF-α-induced apoptosis in HUVECs (Fig. 7B).

Discussion

Using αCGRP knockout mice, we have been able to show that endogenous αCGRP exerts a protective effect against hepatic injury. We previously showed that αCGRP−/− mice are apparently normal, though they have slightly elevated blood pressures and heart rates [14]. This appears to be the result of increased sympathetic tone, as endogenous αCGRP contributes to the inhibitory modulation of sympathetic nervous activity. Despite these physiological changes, however, major organs, including the liver, showed no histological abnormalities. In the present study, we found that Con A-induced liver damage and release of liver enzymes into the circulation were far more pronounced in αCGRP−/− than wild-type mice. In addition, TUNEL staining and DNA laddering showed an increased incidence of apoptosis in the livers of αCGRP−/− mice. Taken together, these findings suggest that αCGRP exerts a hepatoprotective effect under specific pathological conditions, but that it is not essential for the normal liver development and function.

Protective effects of αCGRP also have been reported in other organ systems. For instance, αCGRP deficiency makes the heart and kidneys more vulnerable to hypertension-induced organ damage [12], and αCGRP plays an important role in mediating the protective effects observed with ischemic preconditioning in the heart [7]. With respect to the liver, Yoneda et al. [16] reported that intravenous infusion of an αCGRP receptor antagonist completely inhibited the protective effect of thyrotropin-releasing

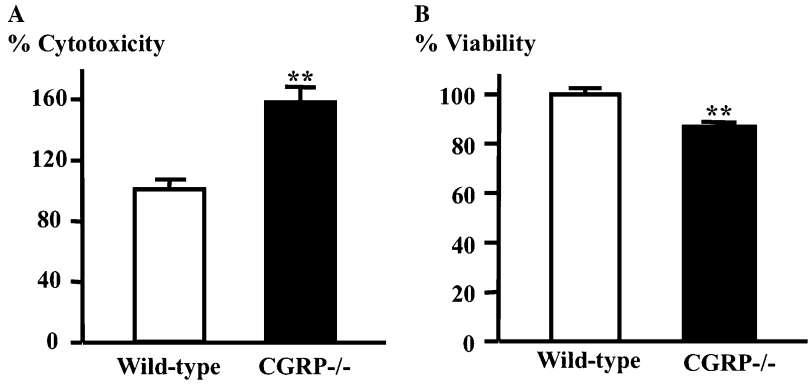

Fig. 5. Cytotoxicity (A) and cell viability (B) assays of primary hepatocytes from αCGRP−/− mice and wild-type mice after IFN-γ treatment. LDH release was used as an index of cytotoxicity. Cytotoxicity and cell viability were expressed as a percentage that seen in wild-type mice. LDH release was significantly increased (A) and cell viability was significantly reduced (B) in αCGRP−/− hepatocytes. Bars are means ± SE; $n = 12$ in each group; $**P < 0.01$ vs. wild-type mice. The experiments were repeated four times with identical outcome.

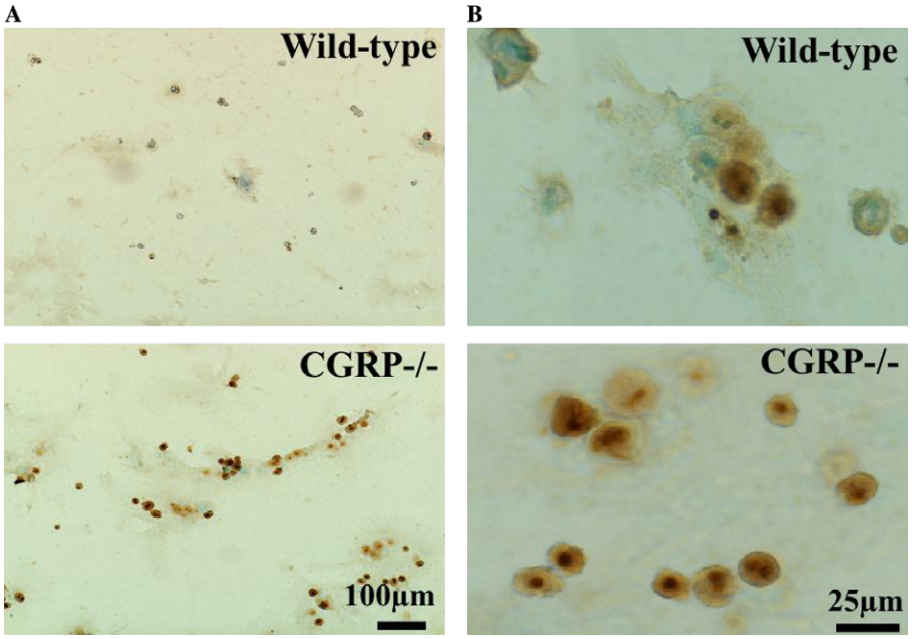

Fig. 6. Lower (A) and higher (B) magnification of TUNEL-staining of wild-type and αCGRP−/− mice hepatocytes following treatment with IFN-γ. TUNEL-positive apoptotic cells were observed much more frequently among αCGRP−/− hepatocytes.

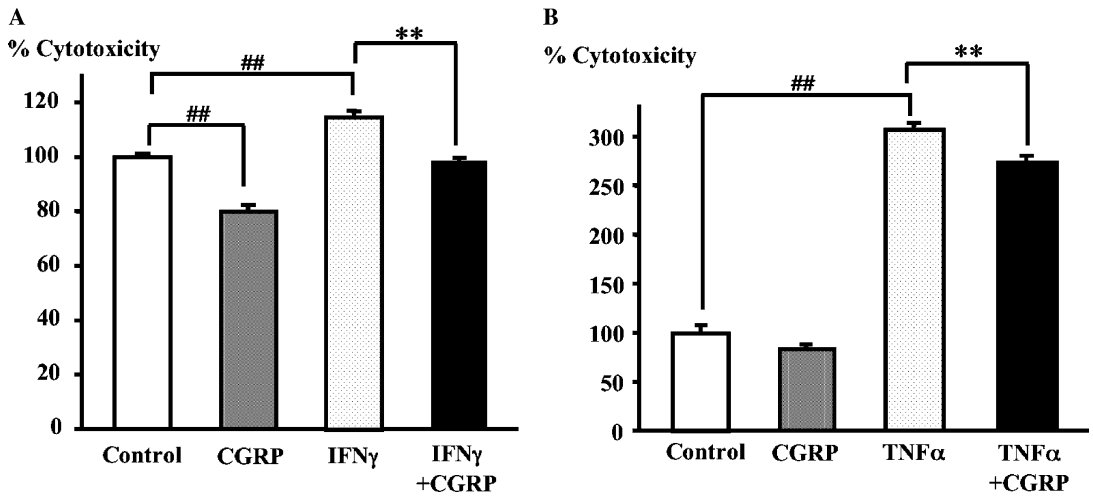

Fig. 7. Effect of exogenous αCGRP on cytotoxicity among primary cultured hepatocytes from C57BL/6J mice (A) and HUVECs (B). LDH release was used as an index of cytotoxicity, which was expressed as a percentage that seen in control. Incubating hepatocytes for 48 h in the presence of 100 U/ml IFN-γ had a significant cytotoxic effect that was significantly attenuated by treatment with 10^{-9} M αCGRP. Incubating HUVECs for 24 h in the presence of 40 ng/ml TNF-α had a significant cytotoxic effect that was also significantly attenuated by treatment with 10^{-9} M αCGRP. Bars are means ± SE; $n = 12$ in each group; $^{\#\#}P < 0.01$ vs. control cells, $^{**}P < 0.01$ vs. IFN-γ or TNF-α-treated cells. The experiments were repeated four times with identical outcome.

hormone (TRH) against acute liver injury caused by carbon tetrachloride (CCl_4), which suggests that peripheral αCGRP is involved in the protective effect induced by central TRH.

We also found that the expression of IL-6 is selectively reduced in the livers of αCGRP−/− mice with Con A-induced hepatitis and that αCGRP administration significantly reduced apoptosis among cultured hepatocytes and endothelial cells. It has been reported that αCGRP potentiates IL-6 release via activation of cAMP pathway in various cell types [17–19]; that both αCGRP and IL-6 are secreted at sites of local inflammation; and that plasma levels of αCGRP and IL-6 are significantly elevated following injection of endotoxin and in septic shock patients [20,21]. Thus, αCGRP might exert immunomodulatory effects via induction of IL-6.

It has been suggested that IL-6 has bimodal role in the hepatitis model; IL-6 induced in early phase after Con A injection triggers hepatoprotective pathways, while continuation of IL-6 production beyond this early phase is harmful to hepatocytes. Actually, we have shown that IL-6-deficient mice are more susceptible to Con A-induced

hepatitis than wild-type mice [22]. In this study, we found that IL-6 expression was diminished in αCGRP−/− mice compared with wild-type mice during the acute phase of inflammation. In contrast, at later phase of inflammation, IL-6 expression was higher in αCGRP−/− mice. This might explain in part their vulnerability to hepatic injury and suggests that αCGRP might exert its hepatoprotective effect via induction of IL-6 expression at earlier phase of inflammation.

We also found that αCGRP exerts an anti-apoptotic effect in the liver. Primary cultured hepatocytes from αCGRP−/− mice subjected to Con A-induced hepatitis showed a greater incidence of apoptosis and lower viability indexes than cells from wild-type mice. In addition, αCGRP exerted a significant anti-apoptotic effect on cultured hepatocytes treated with IFN-γ. Furthermore, pretreatment with αCGRP significantly inhibited TNF-α-induced apoptosis among cultured endothelial cells. Shichiri et al. [6] reported that αCGRP upregulates the expression of Max, a dimerization partner of c-Myc that functions as an anti-apoptotic factor. That up-regulation of Max prevents apoptosis triggered by serum deprivation in endothelial cells suggests that the hepatoprotective effect of αCGRP is mediated in part by its anti-apoptotic effects on endothelial cells.

This study is the first to show the possible protective effects of endogenous αCGRP against liver injury. Further analysis of the effects of αCGRP and their mechanisms in other models of liver injury, including ischemic–reperfusion injury and the chronic hepatitis model, should shed light on whether αCGRP could be useful for the treatment and prevention of liver disease.

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References

[1] M.G. Rosenfeld, J.J. Mermod, S.G. Amara, L.W. Swanson, P.E. Sawchenko, J. Rivier, W.W. Vale, R.M. Evans, Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing, Nature 304 (1983) 129–135.

[2] P. Holzer, Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides, Neuroscience 24 (1988) 739–768.

[3] S.J. Wimalawansa, Calcitonin gene-related peptide and its receptors: molecular genetics, physiology, pathophysiology, and therapeutic potentials, Endocr. Rev. 17 (1996) 533–585.

[4] D.J. DiPette, K. Schwarzenberger, N. Kerr, O.B. Holland, Dose-dependent systemic and regional hemodynamic effects of calcitonin gene-related peptide, Am. J. Med. Sci. 297 (1989) 65–70.

[5] N.N. Chattergoon, F.M. D'Souza, W. Deng, H. Chen, A.L. Hyman, P.J. Kadowitz, J.R. Jeter Jr., Antiproliferative effects of calcitonin gene-related peptide in aortic and pulmonary artery smooth muscle cells, Am. J. Physiol. Lung Cell. Mol. Physiol. 288 (2005) L202–L211.

[6] M. Shichiri, H. Kato, M. Doi, F. Marumo, Y. Hirata, Induction of max by adrenomedullin and calcitonin gene-related peptide antagonizes endothelial apoptosis, Mol. Endocrinol. 13 (1999) 1353–1363.

[7] S. Sueur, M. Pesant, L. Rochette, J.L. Connat, Antiapoptotic effect of calcitonin gene-related peptide on oxidative stress-induced injury in H9c2 cardiomyocytes via the RAMP1/CRLR complex, J. Mol. Cell. Cardiol. (2005).

[8] A. Thievent, S. Sena, A. Parlakian, G. Breuzard, A. Beley, L. Rochette, J.L. Connat, Potential role of the neuropeptide CGRP in the induction of differentiation of rat hepatic portal vein wall, Peptides 26 (2005) 1567–1572.

[9] S. Bracq, B. Clement, E. Pidoux, M.S. Moukhtar, A. Jullienne, CGRP is expressed in primary cultures of human hepatocytes and in normal liver, FEBS Lett. 351 (1994) 63–66.

[10] M.D. Houslay, N.J. Morris, A. Savage, A. Marker, M. Bushfield, Regulation of hepatocyte adenylate cyclase by amylin and CGRP: a single receptor displaying apparent negative cooperatively towards CGRP and simple saturation kinetics for amylin, a requirement for phosphodiesterase inhibition to observe elevated hepatocyte cyclic AMP levels and the phosphorylation of Gi-2, J. Cell. Biochem. 55 (Suppl.) (1994) 66–82.

[11] M. Bushfield, A. Savage, N.J. Morris, M.D. Houslay, A mnemonical or negative-co-operativity model for the activation of adenylate cyclase by a common G-protein-coupled calcitonin-gene-related neuropeptide (CGRP)/amylin receptor, Biochem. J. 293 (Pt. 1) (1993) 229–236.

[12] S.C. Supowit, A. Rao, M.C. Bowers, H. Zhao, G. Fink, B. Steficek, P. Patel, K.A. Katki, D.J. Dipette, Calcitonin gene-related peptide protects against hypertension-induced heart and kidney damage, Hypertension 45 (2005) 109–114.

[13] M. Yoneda, Y. Sato, K. Nakamura, S. Yokohama, T. Kono, H. Watanobe, A. Terano, Involvement of calcitonin gene-related peptide and capsaicin-sensitive afferents in central thyrotropin-releasing hormone-induced hepatic cytoprotection, Eur. J. Pharmacol. 478 (2003) 173–177.

[14] Y. Oh-hashi, T. Shindo, Y. Kurihara, T. Imai, Y. Wang, H. Morita, Y. Imai, Y. Kayaba, H. Nishimatsu, Y. Suematsu, Y. Hirata, Y. Yazaki, R. Nagai, T. Kuwaki, H. Kurihara, Elevated sympathetic nervous activity in mice deficient in alphaCGRP, Circ. Res. 89 (2001) 983–990.

[15] G. Tiegs, J. Hentschel, A. Wendel, A T cell-dependent experimental liver injury in mice inducible by concanavalin A, J. Clin. Invest 90 (1992) 196–203.

[16] M. Yoneda, H. Watanobe, A. Terano, Central regulation of hepatic function by neuropeptides, J. Gastroenterol. 36 (2001) 361–367.

[17] Y. Tang, Y. Feng, X. Wang, Calcitonin gene-related peptide potentiates LPS-induced IL-6 release from mouse peritoneal macrophages, J. Neuroimmunol. 84 (1998) 207–212.

[18] I. Tatsuno, A. Somogyvari-Vigh, K. Mizuno, P.E. Gottschall, H. Hidaka, A. Arimura, Neuropeptide regulation of interleukin-6 production from the pituitary: stimulation by pituitary adenylate cyclase activating polypeptide and calcitonin gene-related peptide, Endocrinology 129 (1991) 1797–1804.

[19] Y. Sakagami, G. Girasole, X.P. Yu, H.S. Boswell, S.C. Manolagas, Stimulation of interleukin-6 production by either calcitonin gene-related peptide or parathyroid hormone in two phenotypically distinct bone marrow-derived murine stromal cell lines, J. Bone Miner. Res. 8 (1993) 811–816.

[20] H. Tilg, E. Trehu, M.B. Atkins, C.A. Dinarello, J.W. Mier, Interleukin-6 (IL-6) as an anti-inflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor p55, Blood 83 (1994) 113–118.
[21] X. Wang, S.B. Jones, Z. Zhou, C. Han, R.R. Fiscus, Calcitonin gene-related peptide (CGRP) and neuropeptide Y (NPY) levels are elevated in plasma and decreased in vena cava during endotoxin shock in the rat, Circ. Shock 36 (1992) 21–30.
[22] Y. Tagawa, P. Matthys, H. Heremans, C. Dillen, Z. Zaman, Y. Iwakura, A. Billiau, Bimodal role of endogenous interleukin-6 in concanavalin A-induced hepatitis in mice, J. Leukoc. Biol. 67 (2000) 90–96.