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Running title: Central urocortin and acute liver injury

Abbreviations: CRF, corticotropin-releasing factor; ALT, alanine aminotransferase; AST, aspartate aminotransferase

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Summary

Background & Aims: Central corticotropin-releasing factor (CRF) is known as a key chemical messenger in response to stress, and plays important roles in physiological regulation mediated through the autonomic nervous system. We have demonstrated that intracisternal injection of CRF exacerbates CCl_4-induced acute liver injury through the sympathetic nervous system. Recently CRF receptors, CRF1 and CRF2, have identified, and distribution or role of these receptors have reported. remains unclear. Urocortin, recently discovered peptide, is one of the CRF agonist family and has high affinity for CRF receptors, especially for CRF2-receptor. However, nothing is known about a role of urocortin, or the specific receptor of action for CRF and its agonis to elicit aggravation of CCl_4-induced acute liver injury. Purpose**:** To investigate the effect of central urocortin on CCl_4-induced acute liver injury in rats. Method: Male Wistar rats (280-320 g) were injected with CCl_4 (2 ml/kg) subcutaneously. Either urocortin (0.5-10 μg) or saline vehicle was injected intracisternally or intravenously just before and 6 h after CCl_4 injection. The liver tissues were removed 24 h after CCl_4 injection and the specimens were stained with H&E. Degeneration and necrosis areas were observed under a light microscope, and measured by a computerized image analyzer. The blood samples were obtained before and 24 h after CCl_4 injection and serum AST and ALT levels were determined. Hepatic sympathectomy (-3 days), hepatic branch vagotomy (-3 days), or respective sham operation was performed. Results**:** Administration of CCl_4 induced regeneration and necrosis in the hepatic tissue 24 h later. Intracisternal urocortin dose-dependently enlarged the regeneration and necrosis areas induced by CCl_4 (Mean ± SE, %: saline 5.5 ± 1.2; 0.5 μg 6.3 ± 3.0; 1 μg 10.5 ± 2.2; 3 μg 17.5 ± 1.9; 5 μg 24.3 ± 5.0; 10 μg 23.5 ± 2.6, n=5-6). Elevations of serum AST and ALT levels were also dose-dependently enhanced by intracisternal urocortin. Intravenous urocortin did not influence CCl_4-induced acute liver injury. The aggravating effect of central urocortin on CCl_4-induced acute liver injury was abolished by sympathectomy, but not by vagotomy. Conclusion**:** Urocortin acts in the brain to exacerbate acute liver injury through sympathetic nervous system and these results suggest the partialy involvement of CRF2 receptor in the exacerbating effect of central CRF and its agonists on CCl_4-induced acute liver injury.

Key words: sympathetic nerve, peptide, hepatic damage

Introduction

Abundant anatomical and physiological evidence has suggested a role for the central and autonomic nervous systems in the regulation of hepatic function (1-3). Neuropeptides have been recognized as neurotransmitters in the central and peripheral nervous systems (4-6), and centrally acting neuropeptides have been reported to regulate a variety of physiological functions including of digestive system (7-9) through autonomic nervous systems. We have paid attention to the relationshp between central nervous system and hepatobiliary system, and in previous study we have shown that central TRH enhances hepatic blood flow (10) and hepatic proliferation (11), and also central Neuropeptide Y increases bile secretion through the parasympathetic nervous systems (12,13). In the pathophygiological function of the liver, we have revealed that central TRH protects CCl_4-induced acute liver injury through vagal-cholinergic patheway (14), but central CRF exacerbates the experimental acute liver injury through the sympathetic-noradrenergic pathways in rats (15).

Corticotropin-releasing factor (CRF) is one of the central neuropeptides, and effect of central CRF on physiological, pharmacological, and pathophysiological regulations of the digestive system have been reported (15-22). Recently two G protein-coupled receptors have been identified that bind CRF and its agonist family (urocortin, savagin, urotensin I), and named CRF1 receptor and CRF2-receptor (23-28). Urocortin is a recently isolated 40 amino acid-containing neuropepetide and shares 45 % sequence homology with CRF (29). While urocortin and CRF both display a similar high affinity for the CRF1 receptor, the affinity of urocortin for the CRF2-receptor is more than 10-fold higher than that of CRF (30).

In this study, we investigate that the effect of urocortin on the CCl4-induced acute liver injury in rats and the involvement of CRF receptors in the exacerbating effect of the experimental liver injury.

Material and Methods

Animals

Male Wistar rats weighing 280-320 g (Charles River Japan Inc., Yokohama, Japan) were housed in group cages under condition of controlled temperature (22-24 oC) and illumination (12-h light cycle starting at 6 AM) for at least 7 days before experiments. Animals were maintained on laboratory chow and water. Experiments

were performed in rats deprived of foods for 12 h (starting at 6 PM), but given free access to water up to the beginning of the study. Protocols describing the use of rats were approved by the Animal Care Committee of Asahikawa Medical College, and in accordance with the National Institute of Health "Guide for the Care and Use of Laboratory Animals".

Drugs

The following substances were used: rat urocortin (Peptide Institute Inc., Osaka, Japan), carbon tetrachloride (CCl_4, Wako Pure Chemical Industries, Osaka, Japan), Phenol (Wako). Urocortin was dissolved in 0.9 % saline (pH 7.4) before the experiment and injected intracisternally in 10 µl using a 50-µl Hamilton microsyringe (Hamilton Co., Reno, NV).

Experimental design

After 12 h fasting, rats were anesthetized with ether and mounted on ear bars of a stereotaxic apparatus (Kopf model 900, David Kopf Instruments, Tujunga, CA) and injected with urocortin (0.5, 1, 3, 5, 10 µg) or saline vehicle intracisternally or intravenously just before and 6 h after CCl_4 administration. CCl_4 was mixed with an equal volume of olive oil and injected subcutaneously at a dose of 2 ml/kg. Rats were kept in individual cages and blood samples were obtained 24 h after CCl_4 administration from the jugular vein. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) were measured by commercially available kits (Wako). The liver tissues were removed from the median lobe 24 h after CCl_4 administration and fixed in 10% formalin solution. The specimens were stained with Hematoxylin & Eosin. Five fields at a 75x magnification per each slide were blindly evaluated under a light microscope. Percentage of the degeneration and necrosis areas surrounded by foamy cells were measured by a computerized image analyzer. To exclude the effect of intracisternal injection of urocortin on food intake, rats were pair-fed with vehicle-treated rats.

Effect of hepatic plexus denervation and hepatic branch vagotomy on urocortin-induced modulation of acute liver injury induced by CCl_4.

Either hepatic plexus denervation or vehicle treatment was performed 7 days

before the peptide injection. Denervation of hepatic plexus (anterior plexus and posterior plexus) was achieved rapidly (< 20 min) by phenol (85 %) applied to the region where the hepatic artery and the portal vein run in close apposition (1). Either hepatic branch vagotomy or sham operation was performed 72 h before the peptide injection. Hepatic branch vagotomy was achieved by selective section of the hepatic branch of the vagus nerve branching off from the anterior vagal trunk a few millimeters proximal to the cardia under a dissection microscope (31). To exclude the effect of denervation of hepatic plexus, and hepatic branch vagotomy on food intake, rats were pair-fed with respective vehicle treated- or sham operated-rats.

Statistical Analysis

All results were expressed as mean ± SE. Comparison between two independent groups was calculated by unpaired Student's t test. Multiple group comparisons were performed by analysis of variance (ANOVA) followed by Fisher's LSD. A P value < 0.05 was considered statistically significant.

Results

Effect of intracisternal urocortin on CCl_4-induced acute liver injury

Histological studies revealed dose-dependent aggravating effect of intracisternal urocortin injection on CCl_4-induced hepatic degeneration and necrosis area surrounded by foamy cells (Mean ± SE, %: saline 5.5 ± 1.2; 0.5 μg 6.3 ± 3.0; 1 μg 10.5 ± 2.2; 3 μg 17.5 ± 2.0; 5 μg 28.1 ± 4.0; 10 μg 23.5 ± 2.3; n=5-6) (Fig. 1, 2). Also intracisternal injection of urocortin (just before and 6 h after CCl_4 injection) dose-dependently enhanced the CCl_4-induced elevation of serum AST (Mean ± SE, IU/L: saline 110 ± 14; 0.5 μg 138 ± 32; 1 μg 166 ± 21; 3 μg 407 ± 111; 5 μg 866 ± 464; 10 μg 592 ± 245; n=5-6) and ALT levels (Mean ± SE, IU/L: saline 19 ± 2; 0.5 μg 20 ± 8; 1 μg 25 ± 3; 3 μg 44 ± 9; 5 μg 81 ± 25; 10 μg 79 ± 30; n=5-6) (Fig. 2). Intravenously administration of CRF (10 μg) did not influence the CCl_4-induced heparic regeneration and necrosis area, and elevation of serum transaminase level (Table 1).

Effect of hepatic plexus denervation and hepatic branch vagotomy on intracisternal urocortin-induced enhancement of acute liver injury by CCl_4.

Denervation of hepatic plexus by 85% phenol (7 days before) completely

abolished the aggravating effect of intracisternal urocortin (5 μg) on the CCl_4-induced hepatic degenertion and necrosis, and elevation of serum transaminase level and (Fig.3). On the other hand, hepatic branch vagotomy (3 days before) did not influence the aggravating effect of intracisternal injection of urocortin (5 μg) on the CCl_4-induced hepatic degeneration and necrosis, and elevation of serum transaminase level (Fig. 3).

Discussion

In the present study, we demonstrated that intracisternal injection of urocortin exacerbated CCl_4-induced acute liver injury in rats. We measured serum AST and ALT levels, and also examined histological changes of the liver. Intracisternal urocortin dose-dependently enlarged the CCl_4-induced hepatic degeneration and necrosis. Similary intracisternal urocortin dose-dependently enhanced the CCl_4-induced elevation of serum AST and ALT levels. The increase of serum transaminase levels and hepatic regeneration and necrosis areas by intracisternal injection of urocortin was dose-related in doses ranging from 0.5 to 5 μg. Administration of up to 10 μg of urocortin did not further enhance the CCl_4-induced increase of serum transaminase levels and hepatic regeneration and necrosis areas, indicating that maximal effect were achieved with 5 μg dose. In contrast, urocoritn injected intravenously at the maximal effective intracisternal dose did not influence CCl_4-induced acute liver injury. These results indicate that urocortin injected into the cisternal magna, acts in the central nervous system to aggravate CCl_4-induced acute liver injury and not through leakage into the peripheral circulation (32).

The pathways through which central administration of urocortin enhanced CCl_4-induced acute liver injury were investigated. In the present study, the enhancement of CCl_4-induced acute liver injury by intracisternal injection of urocortin was completely abolished by denervation of hepatic plexus by 85% phenol, whereas hepatic branch vagotomy had no effect. Since the treatment of hepatic plexus with phenol is known to dominantly denervate the hepatic sympathetic nerve (1), these results indicate that urocortin acts centrally to enhance CCl_4-induced acute liver injury in rats through sympathetic nervous system similar to the central CRF.

CRF is one of the central neuropeptides, and it affects peripheral organ through the autonomic nervous system (21). In regard to the digestive system previous reports have shown that central CRF inhibits gastric secretion and motility and exocrine

secretion of the pancreas through sympathetic-noradrenergic nervous system (16,17,20,22). Meanwhile, central CRF stimulates the colonic motility through parasympathetic nervous system (18,19). And also we have reported central CRF exacerbated CCl_4-induced acute liver injury in rats through sympathetic-noradrenergic nervous system (15).

Recently, two G protein-coupled receptors have been identified that bind CRF and its agonist family (urocortin, savagin, urotensin I) (23-28). they are named CRF1 receptor and CRF2-receptor, and have identified their role or distribution in central nervous system and peripheral organs (33). In regard to digestive system, it is reported that the different subtype of CRF receptors mediate motility of gastrointestinal tract (34,35).

Urocortin is a recently isolated 40 amino acid-containing neuropepetide and shares 45 % sequence homology with CRF (29). While urocortin and CRF both display a similar high affinity for the CRF1 receptor, the affinity of urocortin for the CRF2-receptor is more than 10-fold higher than that of CRF (29,30). Previously we have reported that intracisternal CRF achieved maximal aggragative effect to CCl_4-induced acute liver injury with 10 μg (2.00 nmol) dose. Intracisternal urocortin achives maximal effect with about half dose (5 μg=1.05 nmol) of intracisternal CRF in same experimental design. These results suggest the partialy involvement of CRF2 receptor in the exacerbating effect of central CRF and its agonists on CCl_4-induced acute liver injury.

The liver is known to be richly innervated (36-40) and there has been abundant evidence indicating important roles of central and autonomic nervous system in hepatic function (1-3, 41-45). Very little is known about the central neuropeptides involved in the modulation of hepatic function . In previous study we have shown that central TRH enhances hepatic blood flow (10) and hepatic proliferation (11) and central Neuropeptide Y increases bile secretion through the parasympathetic nervous systems (12,13). In the pathophygiological function of the liver, we have also revealed that central TRH protects CCl_4-induced acute liver injury through vagal-cholinergic patheway in rats (14), but central CRF exacerbates the experimental acute liver injury through the sympathetic-noradrenergic pathways (15). Addition to these result, this study suggests the involvement of several subtype of CRF receptors in the exacerbating effect of central CRF and its agonists on experimental liver injury. It is also of interest to

study a detailed role of subtypes of CRF receptor in experimental liver injury using selective antagonists of them.

In conclusion, the present data indicate that urocortin injected intracisternally acts in the brain to induce a potent enhancement of CCl_4-induced acute liver injury in rats. The peptide action is mediated through a sympathetic pathways. Central injection of urocortin provides a useful tool to further investigate brain sites that influence sympathetic regulation of liver injury.

References

1. Lautt, W.W., 1983. Afferent and efferent neural roles in liver function. Prog Neurobiol 21, 323-348.

2. Shimazu, T., Matsushita, H., Ishikawa, K., 1976. Cholinergic stimulation of the rat hypothalamus: effects of liver glycogen synthesis. Science 194, 535-536.

3. Jungermann, K., Gardemann, A., Beuers, U., Balle, C., Sannemann, J., Beckh, K., Hartmann H., 1987. Regulation of liver metabolism by the hepatic nerves. Adv Enzy Regul 26, 63-88.

4. Smith, J.R., La, H.T., Chesnut, R.M., Carino, M.A., Horita, A., 1977. Thyrotropin-releasing hormone: stimulation of colonic activity following intracerebroventricular administration. Science 196, 660-662.

5. Brown, M., Tache, Y., Fisher, D., 1979. Central nervous system action of bombesin: mechanism to induce hyperglycemia. Endocrinology 105, 660-665.

6. Brown, M., Rivier, J., Vale, W., 1979. Somatostatin: central nervous system actions on glucoregulation. Endocrinology 104, 1709-1715.

7. Tache, Y., Yang, H., 1990. Brain regulation of gastric acid secretion by peptides. Sites and mechanisms of action. Ann NY Acad Sci 597, 128-145.

8. Lenz, H.J., Druge, G., 1990. Neurohumoral pathways mediating stress-induced inhibition of gastric acid secretion in rats. Gastroenterology 98, 1490-1492.

9. Lenz, H.J., Raedler, A., Greten, H., Vale, W.W., Rivier, J.E., 1988. Stress-induced gastrointestinal secretory and motor responses in rats are mediated by endogenous corticotropin-releasing factor. Gastroenterology 95, 1510-1517.

10. Tamori, K., Yoneda, M., Nakamura, K., Makino, I., 1998. Effect of intracisternal thyrotropin-releasing hormone on hepatic blood flow in rats. Am J Physiol 274, G277-G282

11. Yoneda, M., Tamori, K., Sato, Y., Yokohama, S., Nakamura, K., Makino, I., 1997. Central thyrotropin-releasing hormone stimulates hepatic DNA synthesis in rats. Hepatology 26, 1203-1208.

12. Yoneda, M., Tamasawa, N., Takebe, K., Tamori, K., Yokohama, S., Sato, Y., Nakamura, K., Makino, I., Tache, Y., 1995. Central neuropeptide Y enhances

bile secretion through vagal and muscarinic but not nitric oxide pathways in rats. Peptides 16, 727-732.

13. Yoneda, M., Yokohama, S., Tamori, K., Sato, Y., Nakamura, K., Makino, I., 1997. Neuropeptide Y in the dorsal vagal complex stimulates bicarbonate-dependent bile secretion in rats. Gastroenterology 112, 1673-1680.

14. Sato, Y., Yoneda, M., Yokohama, S., Tamori, K., Nakamura, K., Makino, I., 1996. Protective effect of central thyrotropin-releasing hormone (TRH) on CCl_4-induced liver damage in rats. Gastroenterology 110, A1312.

15. Yokohama, S., Yoneda, M., Nakamura, K., Makino, I., 1999. Effect of central corticotropin-releasing factor on carbon tetrachloride-induced acute liver injury in rats. Am J Physiol 276, G622-G628.

16. Tache, Y., Goto, Y., Gunion, M.W., Vale, W., River, J., Brown, M., 1983. Inhibition of gastric acid secretion in rats by intracerebral injection of corticotropin-releasing factor. Science 222, 935-937.

17. Gunion, M.W., Tache, Y., 1987. Intrahypothalamic microinfusion of corticotropin-releasing factor inhibits gastric acid secretion but increases secretion volume in rats. Brain Res 411, 156-161.

18. Monnikes, H., Raybould, H.E., Schmidt, B., Tache, Y., 1993. CRF in the paraventricular nucleus of the hypothalamus stimulates colonic motor activity in fasted rats. Peptides 14, 743-747.

19. Monnikes, H., Schmidt, B.G., Tebbe, J., Bauer, C., Tache, Y., 1994. Microinfusion of corticotropin releasing factor into the locus coeruleus/subcoeruleus nuclei stimulates colonic motor function in rats. Brain Res 644, 101-108.

20. Barquist, E., Zinner, M., Rivier, J., Tache, Y., 1992. Abdominal surgery-induced delayed gastric emptying in rats: role of CRF and sensory neurons. Am J Physiol 262, G616-620.

21. Tache, Y., Monnikes, H., Bonaz, B., Rivier, J., 1993. Role of CRF in stress-related alterations of gastric and colonic motor function. Ann NY Acad Sci 697, 233-243.

22. Lenz, H.J., Messmer, B., Zimmerman, F.G., 1992. Noradrenergic inhibition of canine gallbladder contraction and murine pancreatic secretion during stress by corticotropin-releasing factor. J Clin Invest 89, 437-443.41. Balle, C., Beuers, U., Engelhardt, R., Jungermann, K., 1987. Intracellular mechanism of action of

sympathetic hepatic nerves on glucose and lactate balance in the perfused rat liver. Eur J Biochem 170, 193-199.

23. Chen, R., Lewis K.A., Perrin M.H., 1993. Expression cloning of a human corticotropin-releasing factor receptor. Proc Natl Acad Sci USA 90, 8967-8971.

24. Vita, N., Laurent, S., Lefort, S., 1993. Primary structure and functional expression of mouse pituitary and human brain corticotropin releasing factor receptors. FEBS Lett 335, 1-5.

25. Lovenberg, T.W., Chen, W.L., Grigoriadis, D.E., 1995. Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. Proc Natl Acad Sci USA 92, 836-840.

26. Perrin, M., Donaldson, C., Chen, R., 1995. Identification of a second corticotropin-releasing factor receptor gene and characterization of a cDNA expressed in heart. Proc Natl Acad Sci USA 92, 2969-2973.

27. Kishimoto, T., Pearse II, R.V., Lin, C.R., 1995. A sauvagine/corticotropin-releasing factor receptor expressed in heart and skeletal muscle. Proc Natl Acad Sci USA 92, 1108-1112.

28. Stenzel, P., Kesterson, R., Yeung, W., 1995. Identification of a novel murine receptor for corticotropin-releasing hormone expressed in the heart. Endocrinol 9, 637-645.

29. Vaughan, J., Donaldson, C., Bittencourt, J., 1995. Urocortin, a mammalian neuropeptide related to fish urotensin I and to cortiocotropin-releasing factor. Nature 378, 287-292.

30. Perrin M.H., Sutton, S.W., Cervini, L., 1999. Comparison of an agonist, urocortin, and an antagonist, astressin, as radioligands for characterization of CRF receptors. J Pharm Exp Ther 288, 729-734.

31. Tanaka, K., Ohkawa, S., Nishino, T., Niijima, A., Inoue, S., 1987. Role of the hepatic branch of the vagus nerve in liver regeneration in rats. Am J Physiol 253, G439-G444.

32. Passaro Jr, E., Debas, H., Oldendorf, W., Yamada, T., 1982. Rapid appearance of intraventricularly administrated neuropeptides in the peripheral circulation. Brain Research 241, 335-340.

33. Perrin, M.H., Vale, W.W., 1999. Corticotropin relesing factor receptors and their ligand family. Ann N Y Acad Sci 885, 312-328.

34. Maillot, C., Million, M., Wei, J.Y., Gauthier, A., Tache, Y., 2000. Peripheral corticoropin-releasing factor and stress-stimulated colonic motor activity involve type 1 receptor in rats. Gastroenterology 119, 1569-1579.

35. Martinez, V., Barquist, E., Rivier, J., Tache, Y., 1998. Central CRF inhibits gastric emptying of a nutrient solid meal in rats: the role of CRF2 receptors. Am J Physiol 274, G965-G970.

36. Skaaring, P., Bierring, F., 1976. On the intrinsic innervation of normal rat liver. Histochemical and scanning electron microscopical studies. Cell Tiss Res 171, 141-155.

37. Rogers, R.C., Hermann, G.E., 1983. Central connections of the hepatic branch of the vagus nerve: a horseradish peroxidase histochemical study. J Auton Nerv Syst 7, 165-174.

38. Hermann, G.E., Kohlerman, N.J., Rogers, R.C., 1983. Hepatic-vagal and gustatory afferent interactions in the brainstem of the rat. J Auton Nerv Syst 9, 477-495.

39. Burt, A.D., Tiniakos, D., MacSween, R.N., Griffiths, M.R., Wisse, E., Polak, J.M., 1989. Localization of adrenergic and neuropeptide tyrosine-containing nerves in the mammalian liver. Hepatology 9, 839-845.

40. El Salhy, M., Stenling, R., Grimelius, L., 1993. Peptidergic innervation and endocrine cells in the human liver. Scand J Gastroenterol 28, 809-815.42. Beckh, K., Arnold, R., 1991. Regulation of bile secretion by sympathetic nerves in perfused rat liver. Am J Physiol 261, G775-G780.

41. Balle C, Beuers U, Engelhardt R, Jungermann K. Intracellular mechanism of action of sympathetic hepatic nerves on glucose and lactate balance in the perfused rat liver. Eur J Biochem 1987;170:193-9.

42. Beckh K, Arnold R. Regulation of bile secretion by sympathetic nerves in perfused rat liver. Am J Physiol 1991;261:G775-80.43. Gardemann, A., Puschel, G., Jungermann, K., 1992. Nervous control of liver metabolism and hemodynamics. Eur J Biochem 207, 399-411.

44. Gardemann, A., Jungermann, K., 1986. Control of glucose balance in the perfused rat liver by the parasympathetic innervation. Biol Chem Hoppe-seyler 367, 559-566.

45. Hartmann, H., Beckh, K., Jungermann, K., 1982. Direct control of

glycogen metabolism in the perfused rat liver by the sympathetic innervation. Eur J Biochem 123, 521-526.

Figure legends

Fig. 1 The effect of intracisternal urocortin on CCl_4-induced hepatic histological changes. Saline (A) or urocortin (5 μg) (B) was injected intracisternally just before and 6 h after CCl_4 (2ml/kg) administtrasion, and the liver tissues were obtained 24 h after CCl_4 administration and the specimens were stained with Hematoxylin & Eosin (75 x).

Fig. 2 The dose response of intracisternal urocortin effect on CCl_4-induced hepatic histological change and elevation of serum transaminase levels. Saline or urocortin (0.5, 1, 3, 5 or 10 μg) was injected intracisternally and 6 h after CCl_4 (2 ml/kg) administration. Control animals were intracisternally injected with saline just before and 6 h after CCl_4 administration. Liver tissues and blood samples were collected 24 h after CCl_4 administration.Each column represents the mean ± SE of hepatic necrosis and regeneration areas and serum transaminase levels. *P < 0.05, **P < 0.01 compared with saline injection group.

Fig. 3 The effect of hepatic plexus denervation and hepatic branch vagotomy on the intracisternal urocotin-induced enhancement of the hepatic histological change and elevation of serum ALT levels by CCl_4. Hepatic plexus denervation by 85% phenol was performed 7 days before and hepatic branch vagotomy was performed 3 days before CCl_4 administration. Saline or urocortin (5 μg) was injected intracisternally just before and 6 h after CCl_4 (2ml/kg) administration. Each column represents the mean ± SE. *P < 0.05, **P < 0.01 compared with respective control group.