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Short communication

Central injection of astressin inhibits carbon tetrachloride-induced acute liver injury in rats

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Abstract

The effect of intracisternal astressin, a specific and potent corticotropin-releasing factor $(CRF)_1$ and CRF_2 receptor antagonist on carbon tetrachloride (CCl_4)-induced acute liver injury was investigated in rats. Intracisternal astressin inhibited the elevation of serum alanine aminotransferase level induced by CCl_4. Intracisternal astressin also reduced CCl_4-induced liver histological changes. The protective effect of central astressin on CCl_4-induced liver damage was abolished by sympathectomy but not by hepatic branch vagotomy. These findings demonstrate that astressin acts in the central nervous system to induce hepatic cytoprotection, possibly through the sympathetic pathways in rats. These results further establish a role of endogenous CRF in the brain in hepatic pathophysiological regulation.
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1. Introduction

Corticotropin-releasing factor (CRF), a neuropeptide, is known to act in the brain as a neurotransmitter to regulate physiological and pathophysiological functions through the autonomic nervous system. The effects of central CRF on physiological, pathophysiological and pharmacological regulation of the gastrointestinal tract have been reported (Martinez et al., 1997; Taché et al., 1987, 1993). With regard to the hepatobiliary system, we have recently found that CRF acts in the brain to control hepatic physiological functions and modulate experimental liver injury in animal models (Tamori et al., 1998, 1999; Yokohama et al., 1999, 2001; Yoneda et al., 1995, 1997a,b, 2001). In particular, intracisternal injection of CRF aggravates carbon tetrachloride (CCl_4)-induced acute liver injury (Yokohama et al., 1999).

To date, the development of CRF receptor antagonists has played an important role in the field of CRF research on the potential involvement of CRF receptors in stress (Radulovic et al., 1999). The most commonly used peptic CRF receptor antagonists are α-helical CRF-(9–41) and astressin, which were derived from a truncated and structurally constrained form of human/rat CRF. Astressin and α-helical CRF-(9–41) bind to both CRF_1 and CRF_2 receptors. However, in vitro, astressin was found to be 100 times more potent than α-helical CRF-(9–41) to inhibit adrenocorticotropic hormone secretion (Gulyas et al., 1995). Additionally, α-helical CRF-(9–41) acts as a partial agonist at the human CRF_1 receptor with an intrinsic activity of 33% of the value achieved with human/rat CRF (Smart et al., 1999), whereas astressin exhibits only weak agonistic potency (Miranda et al., 1997). We have recently shown that intracisternal administration of α-helical CRF-(9–41) protects against CCl_4-induced acute liver injury (Nakade et al., 2002).

In this study, the effect of astressin, a specific and potent CRF_1 and CRF_2 receptor antagonist on CCl_4-induced acute liver injury was investigated in rats to confirm the regulatory role of brain endogenous CRF of the brain in experimental liver injury.

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2. Materials and methods

The following substances were used: a CRF receptor antagonist, astressin (Sigma, St. Louis, MO), α-helical CRF-(9–41) (Sigma), carbon tetrachloride (CCl_4, Wako Pure Chemical Industries, Osaka, Japan), and phenol (Wako). Male Wistar rats weighing 200–240 g (Charles River Japan, Yokohama, Japan) were housed in group cages under conditions of controlled temperature (22–24 °C) and illumination (12-h light cycle starting at 06:00) for at least 7 days before experiments. After a 24-h fast, the 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 astressin (0.1, 0.25, 0.5, 1, or 3 μg) or saline vehicle intracisternally (10 μl) or intravenously (1 ml/kg weight) both immediately before and 6 h after CCl_4 administration. The time schedule for astressin injection was determined according to our previous study (Nakade et al., 2002). CCl_4 was mixed with an equal volume of olive oil and injected subcutaneously in a volume of 2 ml/kg. The rats were kept in individual cages and given free access to water and food, and blood samples were obtained from the jugular vein 24 h after CCl_4 administration. Serum alanine aminotransferase was determined with commercially available kits (Wako). Liver tissue was removed from the median lobe 24 h after CCl_4 administration and fixed in 10% formalin solution. These specimens were stained with hematoxylin and eosin. Ten fields at ×75 magnification per slide were evaluated "blind" under a light microscope. Percentage degeneration and necrotic areas surrounded by fatty degeneration were measured with a computerized image analyzer. To exclude the inhibitory effect of intracisternal injection of astressin on food intake, the rats were pair-fed with vehicle-treated rats. To compare the antagonistic potency of astressin to α-helical CRF-(9–41), α-helical CRF-(9–41) (0.1, 0.25, 0.5, 1, or 3 μg) was intracisternally injected instead of astressin.

Either hepatic plexus denervation or vehicle treatment was performed under pentobarbital anesthesia 7 days before the peptide injection. Denervation of the hepatic plexus (anterior plexus and posterior plexus) was achieved rapidly (<20 min) with phenol (85%) applied to the region where the hepatic artery and the portal vein run in close apposition (Lautt, 1983). Either hepatic branch vagotomy or sham operation was performed under pentobarbital anesthesia 72 h before the peptide injection.

Protocols describing the use of rats were approved by the Animal Care Committee of Asahikawa Medical College, and were in accordance with the National Institute of Health "Guide for the Care and Use of Laboratory Animals".

All results were expressed as mean ± S.E. Comparison of two independent groups was done using the Mann–Whitney *U*-test. The values between before and after CCl_4 were compared using a paired Student's *t*-test. Multiple group comparison were performed by analysis of variance (ANOVA) followed by Fisher's least significant difference test. A P value <0.05 was considered statistically significant.

3. Results

Twenty-four hours after administration of CCl_4 (2 ml/kg) the serum alanine aminotransferase level was significantly elevated from 6 ± 1 to 330 ± 21 IU/l ($P<0.01$). Intracisternal administration of astressin (0.1–1 μg) dose-dependently inhibited the CCl_4-induced elevation of serum alanine aminotransferase (Fig. 1A). The effect of intracisternal astressin on the CCl_4-induced elevation of the serum alanine aminotransferase level was more potent than that of α-helical CRF-(9–41) (Fig. 1B). Histological studies showed that intracisternal astressin (1 μg) injection decreased the necrotic areas surrounded by fatty degeneration (mean ± S.E., %: saline 55 ± 1; astressin 32 ± 2: $n=5$, $P<0.01$). Intravenous injection of astressin (1 μg) did not influence the CCl_4-induced elevation of serum alanine aminotransferase level (mean ± S.E., IU/l: saline 330 ± 22; astressin 340 ± 26). Denervation of the hepatic plexus with 85%

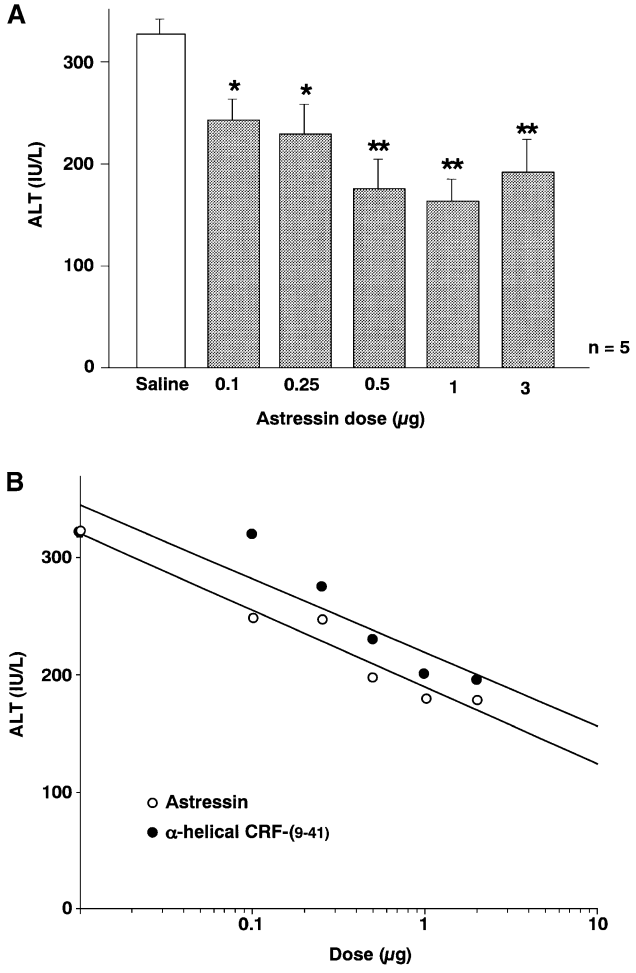

Fig. 1. The effect of intracisternal astressin and α-helical CRF-(9–41) on CCl_4-induced elevation of serum alanine aminotransferase level (mean ± S.E.). Saline, astressin or α-helical CRF-(9–41) (0.1, 0.25, 0.5, 1, or 3 μg) was injected intracisternally just before and 6 h after CCl_4 (2 ml/kg) administration. Blood samples were obtained 24 h after CCl_4 administration. Each column represents the mean ± S.E. *$P<0.05$, **$P<0.01$ compared with saline injection group.

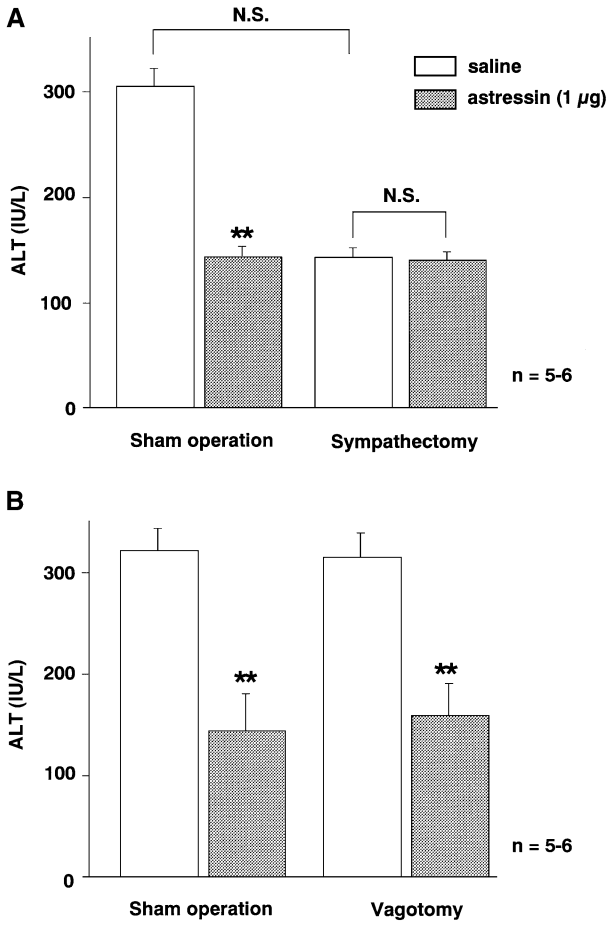

Fig. 2. Effect of hepatic plexus denervation (A), and hepatic branch vagotomy (B) on intracisternal astressin-induced inhibition of elevation of serum alanine aminotransferase level by CCl_4. Hepatic plexus denervation and hepatic branch vagotomy was performed 72 h before CCl_4 (2 ml/kg) administration. Saline or astressin (0.1, 0.25, 0.5, 1, or 3 μg) was injected intracisternally just before and 6 h after CCl_4 (2 ml/kg) administration. Each column represents the mean ± S.E. $^{**}P<0.01$ compared with respective control group.

phenol itself partially inhibited the elevation of serum alanine aminotransferase level 24 h after CCl_4 administration (53% inhibition), but the serum alanine aminotransferase level was still abnormally high in rats with these pretreatments (Fig. 2). Intracisternal injection of astressin did not induce any improvement of the elevated serum alanine aminotransferase level. On the other hand, hepatic branch vagotomy did not influence the protective effect of intracisternal injection of astressin (1 μg) on CCl_4-induced elevation of serum alanine aminotransferase level (Fig. 2).

4. Discussion

In the present study, we demonstrated that, in conscious rats, intracisternal injection of astressin partially inhibited CCl_4-induced acute liver injury assessed by measurement of serum alanine aminotransferase level and by liver histology. The reduction of the CCl_4-induced serum alanine aminotransferase level increase by intracisternal astressin was dose-related with doses ranging from 0.1 to 1 μg. Similarly, intracisternal astressin also reduced histological changes such as centrilobular necrosis and fatty degeneration. In contrast, when injected intravenously at a dose that was maximally effective when given intracisternally, astressin did not influence the CCl_4-induced liver injury. These results indicate that astressin injected into the cisterna magna acts in the central nervous system to induce hepatic cytoprotection against CCl_4 and not through leakage into the peripheral circulation.

The pathways through which central administration of astressin inhibited CCl_4-induced acute liver injury were investigated in this study. Treatment of the hepatic plexus with phenol is known to predominantly denervate the hepatic sympathetic nerve and this chemical sympathectomy itself incompletely inhibited the CCl_4-induced elevation of the serum alanine aminotransferase level in the present study, indicating that sympathetic nerve tone may play a role in aggravating CCl_4-induced acute liver injury. Although chemical sympathectomy lessened CCl_4-induced liver injury by about 50%, as assessed from the serum alanine aminotransferase level, this serum level 24 h after CCl_4 in rats with this pretreatment was still abnormally high compared with that after vehicle treatment. However, intracisternal administration of astressin did not induce any improvement of the elevated serum alanine aminotransferase level in these rats, indicating that the potency of the central astressin action was equivalent to sympathectomy. These findings suggest a possible mediation through the sympathetic nervous systems for the action of central astressin.

CRF is known to act in the brain as a neurotransmitter to regulate physiological and pathophysiological functions through the autonomic nervous system (Taché et al., 1993). To date, two generations of CRF analogues with antagonistic activity have been described, α-helical CRF-(9–41), and D-PHE CRF(12–41) (Gulyas et al., 1995; Hernandez et al., 1993). However, they display some limitations due to their poor solubility and persistence of intrinsic activity (Fisher et al., 1991; Gulyas et al., 1995). Astressin is a newly developed CRF receptor antagonist which has low intrinsic activity and high affinity to both CRF_1 and CRF_2 receptor subtypes (Gulyas et al., 1995; Perrin et al., 1995). An in vitro study showed that astressin is 100 times more potent than α-helical CRF-(9–41) to inhibit adrenocorticotropic hormone secretion (Gulyas et al., 1995). In the present study, astressin injected intracisternally exerted a more potent protective effect than did α-helical CRF-(9–41) at each dose (0.1–3 μg).

We have previously demonstrated that a CRF_2 receptor-selective agonist, urocortin, acts in the brain to aggravate acute liver injury through the sympathetic nervous system (Yokohama et al., 2001). Several specific receptor antagonists for CRF_1 and CRF_2 have been discovered and it would be of interest to investigate their receptor specificity, but

these antagonist cannot be injected intracisternally because of their poor solubility in saline (Higelin et al., 2001; Okuyama et al., 1999).

The pathophysiological effect of stressors on the liver has been reported. Some stressors exacerbate liver injury (Iwai et al., 1986), and increase CRF mRNA expression in the central nervous system (Kalin et al., 1994). In this study, we have investigated the role of endogenous CRF in hepatic pathophysiological regulation using a CRF receptor antagonist, astressin, and demonstrated that astressin acts in the central nervous system and lessens CCl_4-induced acute liver injury. These findings establish the pathophysiological role of endogenous CRF in the brain in experimental liver injury. Since the sick condition induced by CCl_4 liver injury itself can be a stress for animals and may stimulate brain CRF synthesis, resulting in sympathetic activation, it would be of interest to investigate CRF mRNA expression in the brain after CCl_4 administration.

In summary, the present study indicated that astressin injected intracisternally acts in the brain to induce hepatic cytoprotection against experimental liver injury, possibly through sympathetic pathways. These findings provide further evidence for a role of endogenous neuropeptides in the central nervous system in hepatic pathophysiological regulation. It would also be of interest to confirm the role of endogenous CRF in the brain in liver injury in other experimental models, such as ischemia-reperfusion liver injury and partial hepatectomy.

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