Involvement of endogenous corticotropin-releasing factor in carbon tetrachloride-induced acute liver injury in rats

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Running title: Central CRF and liver injury

Abbreviations: CRF, corticotropin-releasing factor; CCl₄, carbon tetrachloride; 6-OHDA, 6-hydroxydopamine; ALT, alanine aminotransferase; AST, aspartate aminotransferase

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Involvement of endogenous corticotropin-releasing factor in carbon tetrachloride-induced acute liver injury in rats. Am J Physiol Regulatory Integrative Comp Physiol- Central neuropeptides play important roles in many physiological and pathophysiological regulation mediated through the autonomic nervous system. In regard to the hepatobiliary system, several neuropeptides act in the brain to regulate bile secretion, hepatic blood flow and hepatic proliferation. Central injection of corticotropin-releasing factor (CRF) aggravates carbon tetrachloride (CCl₄)-induced acute liver injury through the sympathetic nervous pathway in rats. However, still nothing is known about a role of endogenous neuropeptides in the brain in hepatic pathophysiological regulations. Involvement of endogenous CRF in the brain in CCl₄-induced acute liver injury was investigated by centrally injecting CRF receptor antagonist in rats. Male fasted Wistar rats were injected with CRF receptor antagonist, α-helical CRF9-41 (0.125 - 5 µg), intracisternally just before and 6 h after CCl₄ (2 ml/kg) administration and blood samples were obtained before and 24 h after CCl₄ injection for measurement of hepatic enzymes. The liver sample was removed 24 h after CCl₄ injection and histological changes were examined. Intracisternal α-helical CRF9-41 dose-dependently (0.25 - 2 µg) reduced the elevation of alanine aminotransferase and aspartate aminotransferase levels induced by CCl₄. Intracisternal α-helical CRF9-41 reduced CCl₄-induced liver histological changes, such as centrilobular necrosis. The effect of central CRF receptor antagonist on CCl₄-induced liver injury was abolished by sympathectomy and 6-hydroxydopamine pretreatment but not by hepatic branch vagotomy or atropine pretreatment. These findings suggest the regulatory role of endogenous CRF in the brain in experimental liver injury in rats.

**Key Words:** endogenous CRF; hepatic sympathectomy; central nervous system; liver damage
Introduction

Convergent neuroanatomical, neuropharmacological evidences have suggested roles of the central and autonomic nervous systems in the regulation of hepatic function (16, 26). However little is known about neurotransmitter that may mediate these effects in the central nervous system. Neuropeptides have recently been recognized as neurotransmitter in the central and peripheral nervous system (2, 27), and centrally acting neuropeptides have been reported to regulate a variety of physiological functions (18, 30). In particular the effect of central corticotropin-releasing factor (CRF) on physiological, pharmacological, and pathophysiological regulations of gastrointestinal tract have been reported. With respect to the gastrointestinal tract, central injection of CRF inhibited gastric motility and enhanced colonic motility through the autonomic nervous system (21, 29). Physiological stressors are reported to increase CRF mRNA expression and CRF immunoreactivity in the hypothalamus and amygdala (8, 15), and stress-induced alterations of gastrointestinal functions are abolished by central administration of CRF receptor antagonist (1, 18, 20), suggesting involvement of endogenous CRF in these alterations of the gastrointestinal tract. In regard to the hepatobiliary system, the autonomic nervous system affects hepatic metabolism and hemodynamics (7, 16). It has been reported that some physiological stressors, electrical stimulation of hypothalamus, and continuous activation of sympathetic nerve enhance liver injury in animal models (6, 11, 12, 13). We have recently shown that central injection of CRF induces a marked aggravation of carbon tetrachloride-induced acute liver injury in rats (33). These facts led us to speculate that endogenous CRF may play a role in experimental liver injury through the autonomic nervous system. Therefore, in the present study, we aimed to investigate an involvement of endogenous CRF in CCl₄-induced liver injury in rats, by blocking an effect of endogenous CRF in the brain by central injection of CRF receptor antagonist.

Materials and Methods

Animals

Male Wistar rats weighing 200-240 g (Charles River Japan Inc., Yokohama, Japan) were housed in group cages under conditions of controlled temperature (22-24 °C) 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. Before the experiment, rats were deprived of food for 24 h 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 American Physiological Society
"Guiding Principles for Research involving Animals and Human Beings".

Chemicals

The following substances were used: a CRF receptor antagonist, α-helical CRF9-41, (Sigma, St. Louis, MO), CCl₄ (Wako Pure Chemicals, Osaka, Japan), phenol (Wako), atropine methyl nitrate (Sigma), 6-hydroxydopamine (6-OHDA; Aldrich, Milwaukee, WI). α-helical CRF9-41 was dissolved in 0.9% saline (pH 7.4) before the experiment and injected intracisternally in a 10 µl volume using a 50-µl Hamilton microsyringe (Hamilton, Reno, NV).

Experimental Design

After 24 h of 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 α-helical CRF9-41 (0.125 - 5 µg) or saline intracisternally or intravenously through the jugular vein just before and 6 h after CCl₄ administration. CCl₄ was mixed with an equal volume of olive oil and injected subcutaneously in a volume of 2 ml/kg. We chose the dose and administration method for CCl₄ by pilot experiments, because 2 ml/kg of mixed solution of CCl₄ and olive oil injected subcutaneously induced moderate and reproducible liver injury 24 h after CCl₄ in 24-h-fasted rats under our experimental conditions. Rats in the control group were injected with olive oil at a volume of 2 ml/kg. Rats were kept in individual cages and blood samples were obtained before and 24 h after CCl₄ administration from the jugular vein. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined by commercially available kits (Wako). The liver sample was removed from the hepatic median lobe 24 h after CCl₄ administration and fixed in 10% formalin solution. The specimens were stained with hematoxylin and eosin. Five fields per each slide at X75 magnification were blindly evaluated under a light microscope. Percentage of the necrotic areas surrounded by fatty degeneration (33) were measured by a computerized image analyzer. Microscopic findings were photographed with color print films (Super G 200, Fuji Film Co., Ltd., Tokyo, Japan), converted to digital signals by an image scanner (JX-330, Sharp Electric Co., Ltd., Tokyo, Japan), and analyzed by a computer (Power Macintosh 8100, Apple Computer, Inc., Cupertino, CA) equipped with National Institute of Health Image analyzer software. To exclude the effect of intracisternal injection of α-helical CRF9-41 on food intake, rats were pair fed with vehicle-treated rats.

To confirm an antagonistic effect of α-helical CRF9-14, the antagonist (2 µg) was injected intracisternally 5 min before intracisternal injection of CRF (10 µg) in CCl₄-administered rats. Rats were fasted for 12 h before CCl₄ (2 m/kg) injection to obtain mild liver injury, and CRF was intracisternally
injected just before and 6 h after CCl₄. Liver injury was assessed by serum ALT level 24 h after CCl₄.

Effect of hepatic plexus denervation, 6-hydroxydopamine, atropine and hepatic branch vagotomy on α-helical CRF9-41-induced modulation of acute liver injury by CCl₄

Either hepatic plexus denervation or vehicle treatment was performed under pentobarbital anesthesia (Abbott, North Chicago, IL; 50 mg/kg, ip) 7 days before the peptide injection, according to the method of Lautt (16). 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. 6-hydroxydopamine dissolved in saline was intraperitoneally injected (100 mg/kg on the first day, 80 mg/kg on the forth day), and intracisternal injection of α-helical CRF9-41 was performed on the seventh day (32). Atropine methyl nitrate (0.15 mg/kg) dissolved in saline was injected intraperitoneally 30 min before the peptide injection in a 1.0 ml/kg volume. Either hepatic branch vagotomy or sham operation was performed under pentobarbital anesthesia (50 mg/kg, ip) 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. To exclude the effect of hepatic plexus denervation, 6-OHDA 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 Mann-Whitney U test. Comparison of the values between before and after CCl₄ was calculated by paired Student's t test. Multiple group comparison were performed by analysis of variance followed by Fisher's protected least significant difference test. A P value < 0.05 was considered statistically significant.

Results

Effect of intracisternal CRF receptor antagonist, α-helical CRF9-41, on CCl₄-induced liver injury

Administration of CCl₄ (2 mg/kg) induced an elevation of serum ALT level from 6 ± 1 IU/l to 330 ± 21 IU/l 24 h after CCl₄ in 24-h-fasted rats (P < 0.01). Intracisternal administration of CRF receptor antagonist, α-helical CRF9-41 (2 µg) both just before and 6 h after CCl₄ injection reduced the elevation of serum ALT level induced by CCl₄, although either intracisternal single injection of α-helical CRF9-41 only just before or 6 h after CCl₄ did not influence serum ALT level (Fig. 1). Intracisternal administration of α-helical CRF9-41 (just before and 6 h after CCl₄ injection) dose-dependently reduced the
CCl4-induced elevation of serum ALT level in dose ranging from 0.25 µg to 2 µg (Mean ± SE, IU/l: saline, 330 ± 21; 0.125 µg, 342 ± 13; 0.25 µg, 246 ± 22; 0.5 µg, 223 ± 19; 1 µg, 181 ± 20; 2 µg, 137 ± 15; 5 µg, 139 ± 3; n=5-7; Fig. 2). Elevation of serum AST induced by CCl4 was also dose-dependently reduced by intracisternal α-helical CRF9-41 injection (Fig. 3). Histological studies showed marked centrilobular necrosis and fatty degeneration (steatotic hepatocytes) (Fig. 4). Intracisternal α-helical CRF9-41 (2 µg) injection decreased necrotic areas surrounded by fatty degeneration (Fig. 4 and Table 1). Intracisternal α-helical CRF9-41 (2 µg) injection alone did not influence serum ALT level when α-helical CRF9-41 was injected with olive oil vehicle (2 ml/kg, sc) instead of CCl4 (Table 2). Intravenous administration of α-helical CRF9-41 (2 µg) did not influence the CCl4-induced elevation of serum ALT level (Table 3).

Intracisternal injection of CRF (10 µg) aggravated CCl4-induced liver injury, and preinjection of α-helical CRF9-14 (2 µg) completely abolished these effect of CRF (Table 4).

**Effect of hepatic plexus denervation, 6-hydroxydopamine, atropine and hepatic branch vagotomy on serum ALT level 24 h after CCl4 administration in response to intracisternal α-helical CRF9-41**

Denervation of hepatic plexus by 85% phenol (7 days before) or denervation of noradrenergic fibers by 6-OHDA intraperitoneal injection (100 mg/kg ip, -7 days before and 80 mg/kg ip, -4 days before) by itself partially reduced the elevation of serum ALT level 24 h after CCl4 administration, but the serum ALT level was still abnormally high in rats with these pretreatment (Fig. 5, A and B). Intracisternal injection of α-helical CRF9-41 did not induce any improvement on the elevated serum ALT level in rats with hepatic plexus denervation or 6-OHDA pretreatment (Fig. 5, A and B). On the other hand, hepatic branch vagotomy (3 days before) or atropine methyl nitrate (0.15 mg/kg ip, 30 min before) did not influence the effect of intracisternal injection of α-helical CRF9-41 on the CCl4-induced elevation of serum ALT level (Fig. 5, C and D).

**Discussion**

In the present study, we demonstrate that the CRF receptor antagonist, α-helical CRF9-41 injected intracisternally lessened CCl4-induced acute liver injury in conscious rats assessed by serum ALT and AST levels, and by liver histology. The reduction of CCl4-induced serum ALT and AST level elevation by intracisternal α-helical CRF9-41 was dose-related in doses ranging from 0.25 µg to 2 µg. Administration of 5 µg of α-helical CRF9-41 did not further inhibit the CCl4-induced increase of serum
ALT and AST levels, indicating that the maximal effective dose of α-helical CRF9-41 injected intracisternally on CCl₄-induced liver injury is 2 µg and the maximal effect was 58% and 71% reduction on serum ALT and AST, respectively. In contrast, when injected intravenously at the dose that was maximally effective when given intracisternally, α-helical CRF9-41 did not influence CCl₄-induced liver injury. These results indicate that α-helical CRF9-41 injected into the cisterna magna, acts in the central nervous system to lessen CCl₄-induced acute liver injury and not through leakage into the peripheral circulation. Intracisternal administration of α-helical CRF9-41 (2 µg) alone just before and 6 h after olive oil vehicle administration instead of CCl₄ did not influence serum ALT level, suggesting that α-helical CRF9-41 does not have any ability to influence serum ALT level by itself. Although intracisternal injection of α-helical CRF9-41 (2 µg) both just before and 6 h after CCl₄ administration lessened CCl₄-induced acute liver injury, α-helical CRF9-41 injection only just before or 6 h after CCl₄ administration did not influence it. These results indicate that continuous or intermittent blocking of central CRF action by α-helical CRF9-41 is essential to lessen CCl₄-induced acute liver injury.

The pathways through which central administration of α-helical CRF9-41 lessened CCl₄-induced acute liver injury were investigated in this study. Previous reports have shown that central CRF affects peripheral organs in part through the autonomic nervous system (29). In regard to the digestive system, central CRF inhibits gastric secretion and motility and exocrine secretion of the pancreas through the sympathetic-noradrenergic nervous system and central CRF receptor antagonist partially reverses these effects (1, 17, 28). Meanwhile we have recently demonstrated that intracisternal injection of CRF aggravates CCl₄-induced liver injury through the sympathetic-noradrenergic nervous system (33). In the present study, the effect of intracisternal α-helical CRF9-41 was abolished by denervation of hepatic plexus by phenol and 6-OHDA pretreatment, whereas hepatic branch vagotomy or atropine methyl nitrate treatment had no effect. The treatment of hepatic plexus with phenol is known to dominantly denervate the hepatic sympathetic nerve and 6-OHDA treatment chemically depletes noradrenergic nerve fibers via biosynthetic adrenergic intermediates (16, 32). Chemical sympathectomy by 85% phenol or noradrenergic nerve denervation by 6-OHDA, by itself, incompletely reduced CCl₄-induced elevation of serum ALT level in the present study, indicating that sympathetic and noradrenergic nerves tone may play a role in aggravating CCl₄-induced acute liver injury. These findings are very consistent with a previous report which indicated that chemical sympathectomy improved CCl₄-induced liver injury in spontaneously hypertensive rats in which the sympathetic nerve tone is thought to be activated (12). Although chemical sympathectomy and noradrenergic nerve denervation lessened CCl₄-induced liver
injury by about 50% assessed by serum ALT level, these serum level 24 h after CCl4 in rats with these pretreatments were still abnormally high compared with vehicle treatment. However, intracisternal injection of α-helical CRF9-41 did not induce any improvement on the elevated serum ALT level in these rats, indicating that the partially reducing effect of central α-helical CRF9-41 on serum ALT was at least in part mediated through sympathetic-noradrenergic nervous systems. From these findings, it is suggested that during CCl4-induced liver injury the sympathetic tone is activated resulting in aggravation of the liver injury and endogenous CRF in the brain may play a role in the activation of the sympathetic tone.

The pathophysiological effect of stressors and the autonomic nervous system on the liver has been reported. Some stressors or enhancement on the sympathetic nervous activity exacerbate experimental liver injury (6, 11, 12, 13, 33). It has been shown that some physiological stressors increases CRF mRNA expression and CRF immunoreactivity in the hypothalamus and amygdala (8, 15) and endogenous CRF regulates stress-induced alternation of the gastrointestinal functions through the autonomic nervous system (1, 18, 20). In this study, we have investigated a role of endogenous CRF in hepatic pathophysiological regulations using CRF receptor antagonist, α-helical CRF9-41, and demonstrated that α-helical CRF9-41 acts in the central nervous system and lessens CCl4-induced acute liver injury at least partially through the sympathetic-noradrenergic nervous systems in rats. These findings establish the pathophysiological role of endogenous CRF in the brain in experimental liver injury. Since the sick condition induced by CCl4 liver injury can be stress for animals and may stimulate brain CRF synthesis resulting in sympathetic-noradrenergic activation, it is of interest to investigate CRF mRNA expression in the brain after CCl4 administration.

CRF nerve fibers and receptors are widely distributed in the central nervous systems (4) and the site of action for CRF on experimental liver injury remains investigated because microinjection of CRF into the specific brain nuclei was not performed. In the present study, the dose of α-helical CRF9-41 to induce a maximal effect is relatively low compared with previous studies (1, 18, 20), and we injected the antagonist into the cisterna magna which is close to the medulla. Therefore it can be suggested that the site of action for CRF antagonist is near the medulla, because CRF nerve terminals and receptors are located in the nuclei in this area (4).

CRF mediates its actions through activation of specific, seven transmembrane domain receptors, which are coupled to a guanine nucleotide stimulatory factor signaling protein resulting in increased intracellular cAMP levels (3). To date, two CRF receptor subtypes, designated CRF1 and CRF2 receptors, have been identified through molecular cloning from distinct genes in the rat and human (3, 19). CRF2
receptor is located on brain neurons, whereas CRF2 receptor is found in nonneuronal brain tissue and in the periphery (19, 25). We have found that intracisternal injection urocortin, endogenous CRF2 receptor agonist, aggravates CCl₄-induced liver injury, suggesting an involvement of CRF2 receptor in the brain (34).

CCl₄ is well known hepatotoxic chemical. The main cause of liver injury by CCl₄ is free radicals of its metabolites. Cleavage of the CCl₃-Cl bond by superoxide (O₂⁻) probably proceeds via the microsomal cytochrome P-450 reductase and NADPH-dependent reductive pathways. Formation of free radicals may cause lipid peroxidation and subsequent membrane injury (25). Decrease in hepatic blood flow is suggested as one of the important factors in aggravation of experimental liver injury induced by stimulation of hepatic sympathetic nerve (13). Since central injection of CRF decreases hepatic blood flow through the sympathetic nerve (22), it may be suggested that activation of sympathetic nerves by central endogenous CRF decreases hepatic blood flow and reduces oxygen supply to hepatocytes, resulting in aggravation of CCl₄-induced injury and intracisternal injection of α-helical CRF₉-₄₁ abolishes these events.

The liver injury induced by CCl₄ in this study was severe compared to that in our previous study (33). The difference of the study protocol between the present study and our previous study was the duration of fasting state. Since in the pilot study, we found that severity of liver injury induced by CCl₄ was partially depend on fasting time, we chose longer fasting duration (24 h) than that of previous study (12 h) to induce relatively severe liver injury in the present study.

Because some hepatototoxic agent has been reported to stimulate the medurally nuclei (10) and several cytokines in the liver are thought to play important roles in experimental liver injury (14, 23), it is of interest to study an effect of central neropeptides on the expression of these cytokines in the liver.

The liver is known to be richly innervated, and there have been abundant evidences which indicate important roles of the central and autonomic nervous system in hepatic function (7, 9, 16, 26). Although a little is revealed about central neuropeptides as a neurotransmitter inducing modulation of hepatic function (5, 31, 33-37), nothing is known about a role of endogenous neuropeptides in the hepatic physiological and pathophysiological regulations. In the present study, we have found that central administration of CRF receptor antagonist induces a partial hepatic cytoprotection against experimental liver injury through sympathetic-noradrenergic pathways and speculated that endogenous CRF acts in the brain as neurotransmitter to induce central modulation of experimental acute liver injury.

In summary, the present study indicate that CRF receptor antagonist injected intracisternally acts in
the brain to induce a partial hepatic cytoprotection at least partially through sympathetic-noradrenergic pathways. These findings provide the first evidence for a role of endogenous neuropeptides in the central nervous system in hepatic pathophysiological regulations.
References


Figure legends

Fig. 1. Effect of intracisternal α-helical CRF9-41 on CCl4-induced elevation of serum alanine aminotransferase (ALT) level (means ± SE). Saline or α-helical CRF9-41 (2 µg) was injected intracisternally just before and 6 h after CCl4 (2 ml/kg) administration. Control animals were intracisternally injected with saline just before and 6 h after CCl4 administration. Blood samples were collected before and 24 h after CCl4 administration. **P < 0.01 compared with respective control group.

Fig. 2. Dose response of intracisternal α-helical CRF9-41 effect on CCl4-induced elevation of serum ALT level (means ± SE). Saline or α-helical CRF9-41 (0.125, 0.25, 0.5, 1, 2, or 5 µg) was injected just before and 6 h after CCl4 (2 ml/kg) administration. *P < 0.05, ** < 0.01 compared with saline injection group.

Fig. 3. Dose response of intracisternal α-helical CRF9-41 effect on CCl4-induced elevation of serum aspartate aminotransferase (AST) level (means ± SE). Saline or α-helical CRF9-41 (0.125, 0.25, 0.5, 1, 2, or 5 µg) was injected just before and 6 h after CCl4 (2 ml/kg) administration. *P < 0.05, ** < 0.01 compared with respective saline injection group.

Fig. 4. Effect of intracisternal α-helical CRF9-41 on CCl4-induced histological changes. Saline or α-helical CRF9-41 (2 µg) was injected just before and 6 h after CCl4 (2 ml/kg) administration, and the liver tissues were obtained 24 h after CCl4 administration, and specimens were stained with hematoxylin and eosin. A: intracisternal saline injection (X 75), Necrotic area is indicated by arrows. B: intracisternal α-helical CRF9-41 (2 µg) injection (X 75).

Fig. 5. Effect of hepatic plexus denervation (A), 6-hydroxydopamine (6-OHDA; B), hepatic branch vagotomy (C) and atropine methyl nitrate (D) on intracisternal α-helical CRF9-41-induced inhibition of elevation of serum ALT levels (means ± SE) by CCl4. Hepatic plexus denervation by 85% phenol was performed 7 days before, 6-OHDA was intraperitoneally injected 7 days before (100 mg/kg) and 4 days before (80 mg/kg), hepatic branch vagotomy was performed 3 days before, and atropine methyl nitrate (0.15 mg/kg ip) was injected 30 min before CCl4. Saline or α-helical CRF9-41 (2 µg) was injected intracisternally just before and 6 h after CCl4 (2 ml/kg) administration. ** < 0.01 compared with respective control group.
Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>percentage of the degeneration and necrosis area (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>69 ± 1 **</td>
<td>5</td>
</tr>
<tr>
<td>α-helical CRF9-41</td>
<td>41 ± 3</td>
<td>5</td>
</tr>
</tbody>
</table>

Values are means ± SE of n fields. CRF antagonist, α-helical CRF9-41 (2 μg), or saline was injected intracisternally just before and 6 h after CCl4 administration. The liver tissue was obtained 24 h after CCl4 and stained with hematoxylin and eosin. Five fields (X75 magnification) per each slide were blindly evaluated under a light microscope, and the degeneration and necrosis areas surrounded by fatty degeneration were measured by a computerized image analyzer. ** P < 0.01
Table 2

Effect of intracisternal alpha-helical CRF injection on serum ALT levels with olive oil treatment instead of CCl4

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Before</th>
<th>24 h After</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
<td>5</td>
</tr>
<tr>
<td>α-helical CRF9-41</td>
<td>5 ± 1</td>
<td>7 ± 1</td>
<td>5</td>
</tr>
</tbody>
</table>

Values are means ± SE of n fields. ALT, alanine aminotransferase.
Instead of CCl4, olive oil (2 ml/kg) was injected subcutaneously. α-helical CRF9-41 (2 μg), or saline was injected intracisternally just before and 6 h after olive oil administration. Blood samples were collected just before and 24 h after olive oil administration.
Table 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Before</th>
<th>24 h After</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>$6 \pm 1$</td>
<td>$320 \pm 14$</td>
<td>5</td>
</tr>
<tr>
<td>$\alpha$-helical CRF9-41</td>
<td>$6 \pm 1$</td>
<td>$310 \pm 20$</td>
<td>6</td>
</tr>
</tbody>
</table>

Values are means ± SE of n fields. CRF antagonist, $\alpha$-helical CRF9-41 (2 μg), or saline was injected intravenously just before and 6 h after CCl4 administration. Blood samples were collected just before and 24 h after CCl4 administration.
Table 4

Effect of intracisternal α-helical CRF on intracisternal CRF-induced aggravation of liver injury by CCl4

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALT (IU/ml)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle sc + Saline ic + Saline ic</td>
<td>6 ± 2</td>
<td>4</td>
</tr>
<tr>
<td>CCl4 sc + Saline ic + Saline ic</td>
<td>48 ± 8</td>
<td>4</td>
</tr>
<tr>
<td>CCl4 sc + CRF ic + Saline ic</td>
<td>151 ± 30</td>
<td>4</td>
</tr>
<tr>
<td>CCl4 sc + CRF ic + α-helical CRF ic</td>
<td>52 ± 12</td>
<td>4</td>
</tr>
</tbody>
</table>

Values are means ± SE of n fields. CRF (10 μg) or saline was injected intracisternally just before and 6 h after after CCl4 administration. CRF antagonist, α-helical CRF (2 μg) or saline was injected intracisternally 5 min before the each CRF injection. Blood samples were collected just before and 24 h after CCl4 administration. ** P < 0.01
Fig. 1

![Graph showing the effect of CCl4 injection on ALT levels. The x-axis represents the injection time of α-helical CRF9-41 (h), and the y-axis represents ALT (IU/l). The graph includes bars for Saline, 0, 6, and 0 and 6 h after CCl4 injection, with error bars indicating standard error. The bars for 0 and 6 h have an asterisk, indicating a significant difference. The sample size (n) is 5-7.]
Fig. 2

![Graph showing ALT levels (IU/l) in response to different doses of α-helical CRF9-41 (µg). The x-axis represents the dose of α-helical CRF9-41 (µg) with values 0.125, 0.25, 0.5, 1, 2, and 5. The y-axis represents ALT levels ranging from 0 to 400 IU/l. The graph includes error bars for each dose level. The figure indicates a significant effect at doses of 0.25, 0.5, 1, 2, and 5 µg, denoted by asterisks (**). The sample size (n) is 5-7.](image-url)
Fig. 3

![Graph showing AST levels with different doses of α-helical CRF9-41 (μg).](image)

- **Saline**
- **0.125**
- **0.25**
- **0.5**
- **1**
- **2**
- **5**

**AST (IU/l)**

**n = 5-7**
Fig. 5B

![Graph showing ALT levels after different treatments.](image)

- **Saline**
- **α-helical CRF9-41 (2 µg)**

ALT (IU/L) vs. Vehicle treatment (Vehicle) vs. 6-OHDA

- **n = 5-6**

**Notes:**
- Significant difference indicated by ****.
Fig. 5C

![Bar graph showing ALT levels in Sham operation and Vagotomy groups with and without α-helical CRF9-41 (2 μg) administration.](image)

- **Saline**
- α-helical CRF9-41 (2 μg)

ALT (IU/l)  

Sham operation  |  Vagotomy

- **n = 5-7**
Fig. 5D

![Graph showing ALT levels with Vehicle treatment and Atropine with Saline and α-helical CRF9-41 (2 µg) comparison.](image)