RestRAINT stress delays solid gastric emptying via a central CRF and peripheral sympathetic neuron in rats

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Nakade, Yukiomi, Daisuke Tsuchida, Hiroyuki Fukuda, Masahiro Iwa, Theodore N. Pappas, and Toku Takahashi. Restraint stress delays solid gastric emptying via a central CRF and peripheral sympathetic neuron in rats. Am J Physiol Regul Integr Comp Physiol 288: R427–R432, 2005. First published September 30, 2004; doi: 10.1152/ajpregu.00499.2004.—Central corticotropin-releasing factor (CRF) delays gastric emptying through the autonomic nervous system. CRF plays an important role in mediating delayed gastric emptying induced by stress. However, it is not clear whether a sympathetic or parasympathetic pathway is involved in the mechanism of central CRF-induced inhibition of solid gastric emptying. The purpose of this study was to investigate whether 1) CRF inhibits solid gastric emptying via a peripheral sympathetic pathway and 2) stress-induced inhibition of solid gastric emptying is mediated via a central CRF and peripheral sympathetic pathways. Using male Sprague-Dawley rats, CRF was injected intracisternally with or without various adrenergic-blocking agents. To investigate whether central CRF-induced inhibition of solid gastric emptying is mediated via a peripheral sympathetic pathway, rats underwent celiac ganglionectomy 1 wk before the gastric emptying study. After solid meal ingestion (90 min), gastric emptying was calculated. To investigate the role of endogenous CRF in stress-induced delayed gastric emptying, a CRF type2 receptor antagonist, astressin2-B, was intracisternally administered. Rats were subjected to a restraint stress immediately after the feeding. Intracisternal injection of CRF (0.1–1.0 μg) dose-dependently inhibited solid gastric emptying. The inhibitory effect of CRF on solid gastric emptying was significantly blocked by guanethidine, propranolol, and celiac ganglionectomy but not by phentolamine. Restraint stress significantly delayed solid gastric emptying, which was improved by astressin2-B, guanethidine, and celiac ganglionectomy. Our research suggests that restraint stress inhibits solid gastric emptying via a central CRF type2 receptor and peripheral sympathetic neural pathway in rats.

blood-brain barrier; guanethidine; celiac ganglionectomy; corticotropin-releasing factor

CORTICOTROPIN-RELEASING FACTOR (CRF), one of the stress-related neuropeptides, is known to act in the brain to influence on the gastrointestinal tract. Exogenously applied CRF in the central nervous system (CNS) inhibits gastric emptying and acid secretion, whereas it stimulates colonic transit through the autonomic nervous system (5, 10, 24, 41). Restraint stress is known to increase CRF mRNA in the amygdala and paraventricular nucleus (PVN; see Refs. 18 and 21), resulting in alteration of gastrointestinal motor activities. Restraint stress-induced alterations of gastrointestinal motility are abolished by the central administration of CRF antagonist (26). These data suggest that endogenous CRF plays an important role in mediating stress-induced abnormalities of gastrointestinal motility.

Intracerebroventricular injection of CRF increases plasma insulin (37) and somatostatin levels (39) and accelerates colonic transit (24) in rats, which are abolished by vagotomy and atropine. It is suggested, therefore, that the stimulatory effects of central CRF on gastrointestinal function are mediated by a vagal pathway.

It remains controversial whether the inhibitory effect of central CRF on gastric emptying is mediated via a parasympathetic (4, 41) or sympathetic pathway (24). Tache et al. (41) reported that intracisternally applied CRF inhibits liquid gastric emptying and that truncal vagotomy prevents the inhibitory effect of CRF in rats. Other investigators have also shown a role of vagal pathways in the central inhibition of liquid gastric emptying induced by CRF in rats (4). On the other hand, Lenz et al. (24) reported that sympathetic blockade, but not vagotomy, prevented the inhibitory effect of CRF on liquid gastric emptying in rats.

In the hepatobiliary system, the autonomic nervous system influences hepatic metabolism and hemodynamics (12). Certain physiological stressors or continuous stimulation of sympathetic nerve aggravate hepatic injury (11, 16). For example, central administration of CRF aggravated carbon tetrachloride acute liver injury through a sympathetic pathway in rats (44). Acoustic stress inhibits gallbladder contraction via activation of central CRF and sympathetic efferent in dogs (25). These results suggest that release of norepinephrine, mediated by central CRF, is the common pathway producing hepatic injury and inhibition of gallbladder contraction during stress.

There are two distinct CRF receptors, which are coupled to G protein, subtype1 (CRF type1) and subtype2 (CRF type2). These have been cloned from distinct genes in rodents and humans (36). CRF type1 receptor activation is associated with the endocrine, behavioral, and autonomic responses to stress (15, 40). In contrast, the activation of the CRF type2 receptor by CRF agonist in the brain stem imitated stress-induced inhibition of gastric emptying (7, 30). Intracisternal administration of CRF or CRF-related peptide, sauvagine and urotensin I, inhibited solid gastric emptying (29). Vagal stimulation solid gastric emptying is delayed by intracisternal administration of CRF-related peptide, urocortin (7). The selective CRF type2 receptor antagonist, astressin2-B, dose dependently prevented urocortin action, whereas the CRF type1 receptor antagonist, NBI-27914, did not (7).

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It is generally accepted that gastric emptying of a liquid meal primarily reflects the activity of fundus (33). In contrast, gastric emptying of a solid meal is regulated by the coordination of the antrum, pylorus, and duodenum (14, 20, 33). It remains unknown whether a parasympathetic or sympathetic pathway is involved in mediating central CRF-induced inhibition of solid gastric emptying.

The present study examined whether a sympathetic nervous pathway is involved in central CRF-induced inhibition of solid gastric emptying in rats, using combined pharmacological and surgical approaches. We also examined whether endogenous CRF is involved in mediating stress-induced inhibition of solid gastric emptying, using CRF type2 receptor antagonist astressin2-B.

**MATERIALS AND METHODS**

**Animals**

Male Sprague-Dawley rats weighing 240–280 g were housed in group cages under conditions of controlled temperature (22–24°C) and illumination (12-h light cycle starting at 6:00 AM) for at least 7 days before experiments and maintained on laboratory chow and water. Before the experiment, rats were fasted for 24 h but given free access to water. Protocols describing the use of rats were approved by the Institutional Animal Care and Use Committee of Durham Veterans Affairs Medical Center and in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

**Substances and Treatments**

A rat/human CRF and astressin2-B (Sigma, St. Louis, MO) were kept in powder form at 70°C. Peptides were dissolved in 0.9% saline (CRF) or in double-distilled water (astressin2-B) immediately before use. Under a light anesthesia of isoflurane (3%), rats were placed in a stereotaxic apparatus (Kopf model 900; David Kopf Instruments, Tujunga, CA). Peptides or vehicles were injected intracisternally (10 μl/rat) by puncture of the occipital membrane with a 10-μl Hamilton microsyringe (Hamilton, Reno, NV). The presence of cerebrospinal fluid in the Hamilton syringe on aspiration before injection confirmed the accuracy of needle placement in the cisterna magna, as previously described (34).

**Experimental Design**

**Measurement of solid gastric emptying.** Rats were anesthetized with isoflurane (3%) and mounted on a stereotaxic apparatus. After an intracisternal injection of CRF (0.1–2.0 μg/rat) or saline (30 min), preweighed pellets (1.5 g) were given for 10 min, as previously reported (19, 20).

A previous report has shown that intracisternal injection of a high dose of CRF (5.0 μg/rat) reduced food intake from 7 g/2 h to 1 g/2 h (23). If rats did not eat all of the 1.5-g meal within 10 min, these rats were excluded from the experiment. There were no significant differences in the time of whole food intake between saline-injected rats (5.2 ± 1.3 min, n = 7) and CRF-injected rats (7.3 ± 2.1 min, n = 7). After the end of feeding (90 min), rats were killed by intraperitoneal injection of pentobarbital sodium (200 mg/kg). The stomach was surgically isolated and removed. The gastric content was recovered from the stomach, dried, and weighed. Solid gastric emptying was calculated according to the following formula, as previously described (19, 20):

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\text{Gastric emptying} = \frac{1 - (\text{dried weight of food recovered from stomach/weight of food intake})}{100}.
\]

**Pharmacological and surgical approaches.** To investigate whether the inhibitory effect of CRF on solid gastric emptying is mediated through a sympathetic pathway, guanethidine (5.0 mg/kg), phenolamine (1.0 mg/kg), and propranolol (1.0 mg/kg) were injected intraperitoneally 30 min before an intracisternal injection of CRF. We have previously proven that an intraperitoneal injection of these adrenergic blocking agents had no significant effects on gastric motility (42) and gastrointestinal transit (43) in normal conditions in rats.

To investigate whether the inhibitory effect of CRF on solid gastric emptying is mediated via a parasympathetic or sympathetic pathway, celiac ganglionectomy and truncal vagotomy were performed 1 wk before the peptide injection. Under pentobarbital sodium (45 mg/kg ip) anesthesia, truncal vagotomy was performed by cutting the vagal trunks around the abdominal esophagus, as previously described (35). A celiac ganglionectomy was carried out by deflecting the stomach and spleen to the right of the rat, which allowed identification of the nerves and celiac ganglion (35). Sham-operated rats served as controls.

**Restraint stress.** After 24 h of fasting, rats were anesthetized with isoflurane and mounted on a stereotaxic apparatus for an intracisternal injection of astressin2-B (10 μg) or saline (10 μl). We chose the dose of astressin2-B by the previous reports demonstrating that intracisternal injection of astressin2-B (10 μg) prevented restraint stress-induced inhibition of solid gastric emptying (7). After the astressin2-B injection (30 min), the rats were given a preweighed solid pellet (1.5 g) for 10 min. Immediately after the end of feeding, the rats were subjected to the restraint stress.

The rats were restrained in hemicylindrical, well-ventilated, Plexiglas tubes for 90 min. After the restraint stress loading, the rats were killed by pentobarbital sodium anesthesia (200 mg/kg ip). The gastric content was recovered, and the solid gastric emptying was calculated.

To investigate whether the sympathetic pathway is involved in restraint stress-induced inhibition of solid gastric emptying, we performed guanethidine pretreatment and celiac ganglionectomy. Guanethidine (5.0 mg/kg) was administered ip 30 min before an intracisternal injection of astressin2-B (10 μg) or saline (10 μl). Celiac ganglionectomy was performed 1 wk before the restraint stress loading.

To investigate whether peripheral administration of astressin2-B (10 μg) affects the stress-induced inhibition of solid gastric emptying, astressin2-B (10 μg) was subcutaneously injected 30 min before the feeding.

**Statistical Analysis**

All results were expressed as means ± SE. The comparison of the values before and after the restraint stress was calculated by paired Student’s t-test. A multiple-group comparison was performed by ANOVA followed by Scheffé’s test. A P value <0.05 was considered statistically significant.

**RESULTS**

**Effect of Sympathetic Nerve Blockade on the Inhibitory Effects of CRF on Solid Gastric Emptying**

In saline-treated rats, solid gastric emptying was 62 ± 4% (n = 6) 90 min after the feeding of 1.5 g rat chow. Intracisternal administration of CRF (0.1–1.0 μg) dose dependently inhibited solid gastric emptying (Fig. 1). Administration of 2.0 μg CRF did not further inhibit solid gastric emptying, indicating that the maximal effective dose of CRF on solid gastric emptying is 1 μg. Intracisternal administration of CRF (1 μg) significantly reduced solid gastric emptying to 25 ± 3% (n = 6).

Pretreatment with a noradrenergic blocking agent, guanethidine, partially attenuated CRF-induced inhibition of gastric emptying to 42 ± 4% (n = 6; Fig. 2A). Similarly, pretreatment with a β-adrenoceptor antagonist, propranolol, partially attenuated central CRF-induced inhibition of gastric emptying to...
injection of CRF (0.1–1.0 μg/rat) inhibited solid gastric emptying in a dose-dependent manner.

Our pharmacological approaches demonstrated that the central CRF-induced inhibition of solid gastric emptying was significantly restored by the treatment with guanethidine. This suggests that the adrenergic pathway is involved in mediating CRF-induced inhibition of solid gastric emptying. This is consistent with the previous study of Lenz et al. (24), who reported that delayed gastric emptying induced by central CRF was blocked by adrenergic blocking agents in rats. Adrenergic receptors are located in the brain and the myenteric plexus (9). It remains unclear whether CRF-induced inhibition of gastric emptying is via the peripheral or central adrenergic pathway. Although guanethidine does not cross the blood-brain barrier (BBB; see Ref. 6), it may act on the area postrema of the brain stem where there is a loose BBB structure.

The present study revealed that celiac ganglionectomy almost completely abolished the inhibitory effects of CRF on

Fig. 1. Dose-related inhibition of solid gastric emptying induced by intracisternal (ic) injection of corticotropin-releasing factor (CRF; 0.1–2.0 μg/rat) in 24-h-fasted rats. CRF or saline was given 10 min before a solid meal injection. Gastric emptying was measured 90 min after solid meal ingestion (n = 5–6 rats/group). *P < 0.05 and **P < 0.01 compared with vehicle-treated group.

50 ± 6% (n = 6; Fig. 2B). In contrast, pretreatment with an α-adrenoceptor antagonist, phentolamine, did not affect the central CRF-induced inhibition of gastric emptying (Fig. 2B). Celiac ganglionectomy itself had no significant effects on solid gastric emptying (67 ± 7%, n = 5) compared with rats undergoing sham ganglionectomy (65 ± 6%, n = 5). Celiac ganglionectomy completely eliminated the inhibitory effect of CRF on solid gastric emptying (Fig. 2C).

Truncal vagotomy itself almost completely abolished gastric emptying of solid food (3 ± 3%, n = 4) compared with rats that underwent sham vagotomy (62 ± 5%, n = 5). Therefore, we were unable to study whether central CRF has the inhibitory effect of solid gastric emptying in vagotomized rats.

**Effect of Intracisternal or Subcutaneous Injection of Astressin2-B on Restraint Stress-induced Inhibitory Effects of Solid Gastric Emptying**

Partial body restraint stress reduced solid gastric emptying to 30 ± 3% (n = 6). Intracisternal injection of astressin2-B (10 μg) by itself did not affect solid gastric emptying in the nonrestraint rats. In contrast, intracisternal injection of astressin2-B (10 μg) almost completely abolished restraint stress-induced inhibition of solid gastric emptying (Fig. 3A). Subcutaneous injection of astressin2-B (10 μg) did not affect restraint stress-induced inhibition of solid gastric emptying (32 ± 3%, n = 5).

**Effect of sympathetic nerve blockade on restraint stress-induced inhibitory effects of solid gastric emptying.** Guanethidine restored restraint stress-induced inhibition of solid gastric emptying to 44 ± 1% (n = 6; Fig. 3B). Intracisternal injection of astressin2-B did not show any further improvement of solid gastric emptying in guanethidine-treated groups (Fig. 3B). Restraint stress delayed gastric emptying to 35 ± 5% (n = 5) in the rats that underwent sham ganglionectomy. Celiac ganglionectomy almost completely abolished the restraint stress-induced inhibition of gastric emptying (Fig. 3C).

**DISCUSSION**

Martinez and colleagues (29) reported that intracisternal injection of CRF (0.1–1.0 μg) dose dependently inhibited solid gastric emptying. We also demonstrated that intracisternal
solid gastric emptying. We further demonstrated that central CRF-induced inhibition of solid gastric emptying was antagonized by propranolol, not phentolamine. The activation of β-adrenoceptors located in the smooth muscle cells induces a cholinergic formation, resulting in muscular relaxation of the stomach (3, 42). These results suggest that central CRF inhibits solid gastric emptying via a sympathetic pathway and β-adrenoceptors in conscious rats.

On the other hand, others suggested the involvement of vagal pathways in mediating central CRF-induced inhibition of gastric emptying. Intracisternal injection of CRF-induced inhibition of liquid gastric emptying is abolished by vagotomy (41). Intracisternal injection of CRF (210 pmol) to induce 31% inhibition of liquid gastric emptying, whereas Lenz et al. (24) used intracisternal injection of CRF (210 pmol) to induce 81% inhibition of liquid gastric emptying, respectively. Therefore, it seems that a lower dose of intracisternal injection of CRF (210 pmol) is more effective than a higher dose of intracerebroventricular injection of CRF (1 nmol) to inhibit liquid gastric emptying. Because the responsive site of central CRF seems to be a brain stem (1), it is conceivable that intracisternal injection of CRF is more potent to inhibit liquid gastric emptying than intracerebroventricular injection. The discrepancy mentioned above may be explained by the different procedure of CRF injection (iv vs. ic).

Two CRF receptor subtypes (type1 and type2 receptors) have recently been identified through molecular cloning from distinct genes in rat and human brains (27). CRF type1 receptor activation is believed to be associated with the endocrine, behavioral, and autonomic responses to stress (15, 40). The activation of CRF type2 receptor in the brain stem imitated stress-induced inhibition of gastric emptying (2, 32), which suggests the involvement of CRF type2 receptor in stress-induced inhibition of gastric emptying.

We showed that intracisternal injection of astressin2-B (10 μg) significantly improved restraint stress-induced inhibition of solid gastric emptying. Subcutaneous administration of a high dose of astressin2-B (200 μg/kg) prevented the restraint-induced delay in gastric emptying (32). It has been reported that peripheral CRF type2 ligand is expressed in the stomach (17). The possible leakage of astressin2-B from the brain to the periphery may interfere with stress-induced inhibition of solid gastric emptying. However, our current study showed that peripheral administration of astressin2-B (10 μg) did not affect restraint stress-induced inhibition of solid gastric emptying.
This indicates that intracisternal administration of astressin2-B (10 μg) does not affect stress-induced inhibition of solid gastric emptying via the peripheral circulation.

Our current study showed that guanethidine pretreatment antagonized stress-induced inhibition of solid gastric emptying. Similarly, celiac ganglionectomy antagonized stress-induced inhibition of solid gastric emptying. These results strongly suggest that the peripheral sympathetic system is involved in the mechanism of stress-induced inhibition of solid gastric emptying. Intracisternal injection of astressin2-B did not induce any further improvement in guanethidine-treated rats. This suggests that stress-induced inhibition of solid gastric emptying is mediated via central CRF type2 receptors.

The responsible neural pathway mediating activation of sympathetic neurons induced by central CRF remains to be investigated. CRF receptors and nerve fibers are widely distributed in CNS (8), concretely located in the nuclei in the medulla and PVN, which are the important sites of sympathetic nervous outflow (1). CRF type2 receptors are located in the nucleus tractus solitarius (NTS) (1).

Neurons within the NTS project to a number of brain stem areas thought to be involved in the regulation of sympathetic activity (13). NTS also projects efferent nerve to rostral ventrolateral medulla (RVLm; see Ref. 38). RVLm is involved in the regulation of the autonomic nervous system, sending projections to the sympathetic intermediolateral cell column of the thoracolumbar spinal cord (28). Noradrenergic nerve fibers originating from cell bodies found in the celiac ganglion innervate to the stomach (31). We injected CRF in the cisterna magna, which is close to the medulla. Therefore, it can be suggested that the site of action of CRF is the NTS. It is conceivable that central injection of CRF may act on the NTS, resulting in activation of RVLm to enhance sympathetic nerve activity.

In summary, the present study indicates that exogenous CRF injected intracisternally acts on the brain stem to inhibit solid gastric emptying. Restraint stress inhibits solid gastric emptying via a central CRF in conscious rats. CRF- and restraint stress-induced inhibition of solid gastric emptying is antagonized by guanethidine and celiac ganglionectomy. Our findings provide the first evidence for a role of the sympathetic nervous systems in the central CRF- and stress-mediated inhibition of solid gastric emptying.

GRANTS

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