Restraint stress augments postprandial gastric contractions but impairs antropyloric coordination in conscious rats

Yukiomi Nakade, Daisuke Tsuchida, Hiroyuki Fukuda, Masahiro Iwa, Theodore N. Pappas, and Toku Takahashi

Department of Surgery, Duke University Medical Center, and Durham Veterans Affairs Medical Center, Durham, North Carolina

Submitted 4 March 2005; accepted in final form 20 October 2005

A GROWING BODY OF EVIDENCE suggests a role for stress in gastrointestinal (GI) physiological and pathophysiological functions, such as gastric emptying, gastric ulcerations, gastric secretion, and intestinal transit (1, 18, 19, 23, 28, 34, 41).

Several brain neuropeptides are known to act as stress-related neurotransmitters that modify GI motor functions (8, 29, 33, 37, 39). Corticotropin-releasing factor (CRF), one of the stress-related neuropeptides, is known to act in the brain to influence GI functions. Exogenous administration of CRF into the central nervous system (CNS) delays gastric emptying and inhibits acid secretion, whereas it stimulates colonic transit through the autonomic nervous system (5, 22, 35). Restrained stress augments CRF mRNA in the amygdala and paraventricular nucleus (PVN) (13, 16), resulting in alteration of GI motor activities. Restrained stress-induced delayed gastric emptying is abolished by central administration of CRF antagonist (23), suggesting that endogenous CRF has an important role in mediating stress-induced delay of gastric emptying. We recently demonstrated that restraint stress delays solid gastric emptying via a central CRF and peripheral sympathetic neuron in rats (30).

Although CRF and restraint stress attenuate gastric emptying (22), little is known about the postprandial gastric motility mediated by restraint stress. It remains unclear how restraint stress modifies gastric motility to delay gastric emptying.

Liquid meal emptying primarily reflects the activity of the fundus (27), whereas gastric emptying of a solid meal is regulated by coordination of the antrum, pylorus, and duodenum (3, 11, 15, 27).

The postprandial gastric motor pattern in response to a solid food involves a grinding and an emptying period in humans (3), dogs (40), and rats (14, 15). Immediately after a feeding, a grinding period, i.e., significant contraction, is observed in the antrum and pylorus. In contrast, the contractile pattern of the antrum is significantly changed from 40 min after a feeding to the duodenum, is observed in this emptying period. Thus coordinated postprandial antropyloric contractions play an important role in the gastric emptying of solids in rats (14, 15).

We previously showed that hyperglycemia impairs antropyloric coordination and delays solid gastric emptying in conscious rats (15). We also demonstrated that injection of neuropeptide Y (NPY) into the CNS increased gastric motility but impaired antropyloric coordination, resulting in delayed solid gastric emptying in conscious rats (14). These results led us to speculate that restraint stress could impair antropyloric coordination and delay solid gastric emptying.

The present study was designed to examine the restraint stress-induced alteration of gastric motility from the viewpoint of postprandial antropyloric coordination. We assessed the mechanisms of restraint stress-mediated antropyloric coordination. In addition, we used a CRF type 2 receptor antagonist, astressin 2B, to examine the role of endogenous CRF in restraint stress-induced alteration of gastric motility.
STRESS IMPAIRS ANTROPYLORIC COORDINATION

R617

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (280–320 g body wt) were housed in group cages under conditions of controlled temperature (22–24°C), humidity, and illumination (12 h of light starting at 6 AM) for ≥7 days before the experiments and maintained on laboratory chow and water. After surgery, the rats were housed individually with access to a standard diet and tap water until the beginning of the experiments. All experiments were started at 9 AM. 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 National Institutes of Health guidelines.

Substances and Treatments

A rat/human CRF and astressin 2B (both from Sigma, St. Louis, MO) were kept in powder form, CRF at −70°C and astressin 2B at room temperature. Peptides were dissolved in 0.9% saline (CRF) or double-distilled water (astressin 2B) immediately before use. Under light (3%) isoflurane anesthesia, rats were placed in a stereotaxic apparatus (model 900; David Kopf Instruments, Tujunga, CA). Peptides or vehicles were injected intracisternally (5 μl/rat) by puncture of the occipital membrane with a needle of a 10-μl microsyringe (Hamilton, Reno, NV). The presence of cerebrospinal fluid in the syringe on aspiration before injection verified the accuracy of needle placement into the cisterna magna, as previously described (30, 31).

Experimental Design

Recording of antral and pyloric motility in restraint stress. After 12 h of fasting, the rats were anesthetized with pentobarbital sodium (45 mg/kg ip). An abdominal incision was performed, and two strain gauge force transducers were sutured onto the serosal surface of the antrum and pylorus for recording of circular muscle contractions, as previously described (14, 15). The wires to the transducers were run under the skin to an opening made in the back of the neck using a rat vagotomy. As shown in Fig. 8, CRF (0.5 μg ic) attenuated postprandial gastric motility in conscious rats. In our preliminary study, 60 μg of astressin 2B almost completely abolished CRF (0.5 μg ic)-induced inhibition of gastric motility.

Effect of Restraint Stress on Postprandial Antropyloric Coordination

At 90 min after feeding, the contraction maxima of the antrum occurred most often 3–6 s before the contraction maxima of the pylorus (Fig. 1), as previously reported (14, 15). The number of episodes of antropyloric coordination was also counted before and after CRF injection for 30 min. To investigate the involvement of sympathetic nerves on CRF-induced alteration of gastric motility, guanethidine (5.0 mg/kg ip) was injected 30 min before CRF injection.

Statistical Analysis

Values are means ± SE. Data from control animals and those subjected to restraint stress were compared by paired Student’s t-test. Data from sham-operated control rats and rats subjected to truncal vagotomy were compared by Student’s t-test. A multiple-group comparison was performed by ANOVA followed by Fisher’s protected least significant difference method. *P < 0.05 was considered statistically significant.

RESULTS

Effect of Restraint Stress on Postprandial Antropyloric Coordination

At 90 min after feeding, the contraction maxima of the antrum occurred most often 3–6 s before the contraction maxima of the pylorus (Fig. 1), as previously reported (14, 15). The number of episodes of antropyloric coordination was 2.4 ± 0.4/10 min before the restraint stress (Table 1). Restraint stress enhanced antral and pyloric motility compared with the prerestraint state. Restraint stress increased...
the peak amplitude of the antrum and pylorus to $137 \pm 11\%$ and $125 \pm 12\%$ of basal ($n = 6$), respectively. Restraint stress increased the frequency of antral and pyloric contractions from $2.2 \pm 0.5$ to $3.8 \pm 0.6$ and from $2.8 \pm 0.5$ to $4.1 \pm 0.7/min$, respectively. Thus the motility index of the antrum and pylorus was also increased from $23,724 \pm 2,467$ to $33,450 \pm 2,763$ and from $10,524 \pm 927$ to $14,102 \pm 1,242$ g/s for 30 min, respectively (Fig. 2). During the restraint stress loading, the time lag between the antral and pyloric contractions was not observed. The contraction maxima of the antrum and pylorus occurred simultaneously (Fig. 1). The number of episodes of antropyloric coordination was significantly reduced to $0.6 \pm 0.3/10$ min during the restraint stress (Table 1).

Restraint stress-induced augmentation of the antral and pyloric contractions returned to the basal levels within 3–5 min after termination of stress loading (Fig. 1). The time lag between the antral and pyloric contractions was also observed after termination of restraint stress loading (Fig. 1).

Table 1. Episodes on antropyloric coordination mediated by restraint stress or central CRF

<table>
<thead>
<tr>
<th>Condition</th>
<th>Episodes of Antropyloric Coordination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control + saline ic</td>
<td>$2.4 \pm 0.4$</td>
</tr>
<tr>
<td>Control + atressin 2B (60 µg ic)</td>
<td>$2.5 \pm 0.6$</td>
</tr>
<tr>
<td>Control + guanethidine (5 mg/kg ip)</td>
<td>$2.0 \pm 0.6$</td>
</tr>
<tr>
<td>Control + CRF (0.5 µg ic)</td>
<td>$0.9 \pm 0.3\dagger$</td>
</tr>
<tr>
<td>Restraint stress + saline (ic)</td>
<td>$0.6 \pm 0.3\dagger$</td>
</tr>
<tr>
<td>Restraint stress + atressin 2B (60 µg ic)</td>
<td>$1.6 \pm 0.3\ast$</td>
</tr>
<tr>
<td>Restraint stress + guanethidine (5 mg/kg ip)</td>
<td>$1.5 \pm 0.4\ast$</td>
</tr>
<tr>
<td>Restraint stress + guanethidine (5 mg/kg ip) + atressin 2B (60 µg ic)</td>
<td>$1.4 \pm 0.5\ast$</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as number per 10 min ($n = 6$). CRF, corticotropin-releasing factor. Number of episodes of antropyloric coordination was significantly reduced by restraint stress. Intracisternal (ic) atressin 2B attenuated impairment of antropyloric coordination. \* $P < 0.05$; \dagger $P < 0.01$ vs: control + saline.

Fig. 2. Effects of saline, guanethidine, atropine, and hexamethonium on restraint stress-induced augmentation of antral (A) and pyloric (B) motility in conscious rats. Motility indexes of antrum and pylorus were augmented by restraint stress. Motility index of antrum induced by restraint stress was further enhanced by guanethidine. Although motility index of pylorus induced by restraint stress was augmented, there was no significant difference compared with saline-treated group ($n = 4–6$). ** $P < 0.01$ compared with control. \# $P < 0.05$ compared with saline. Atropine and hexamethonium attenuated motility index. Restraint stress did not induce any change of motility mediated by atropine and hexamethonium. NS, not significant.
Effect of Guanethidine, Atropine, Hexamethonium, and Truncal Vagotomy on Restraint Stress-Induced Alteration of Antral and Pyloric Motility

Guanethidine enhanced restraint stress-induced augmentation of antral and pyloric contractions (Fig. 3). During the restraint stress, guanethidine increased the peak amplitude of the antrum and pylorus to 145±110% and 146±10% of basal (n=6), respectively. During the restraint stress, guanethidine increased the frequency of antral and pyloric contractions from 2.6±0.5 to 5.0±0.2 and from 3.3±0.8 to 5.1±0.1/min, respectively. The motility index of the antrum was also increased from 25,500±2,467 to 37,211±2,342 g/s for 30 min (Fig. 2A) during stress loading. The motility index of the pylorus tended to be enhanced by guanethidine, although this was not statistically significant (NS). Values are means ± SE (n=4–6). *P < 0.05; **P < 0.01 compared with saline. #P < 0.05 compared with saline/restraint stress.

Effect of Guanethidine, Atropine, Hexamethonium, and Truncal Vagotomy on Restraint Stress-Induced Alteration of Antral and Pyloric Motility

Guanethidine enhanced restraint stress-induced augmentation of antral and pyloric contractions (Fig. 3B). During the restraint stress, guanethidine increased the peak amplitude of the antrum and pylorus to 145±21% and 146±10% of basal (n=6), respectively. During the restraint stress, guanethidine increased the frequency of antral and pyloric contractions from 2.6±0.5 to 5.0±0.2 and from 3.3±0.8 to 5.1±0.1/min, respectively. The motility index of the antrum was also increased from 25,500±2,467 to 37,211±2,342 g/s for 30 min (Fig. 2A) during stress loading. The motility index of the pylorus tended to be enhanced by guanethidine, although the difference was not statistically significant (Fig. 2B). The number of episodes of antropyloric coordination was partially restored to 1.5±0.4/10 min by guanethidine during stress loading (Table 1).

Fig. 3. Effects of saline (A), guanethidine (Gua, B), atropine (Atr, C), and hexamethonium (Hex, D) on restraint stress-induced augmentation of antral and pyloric motility in conscious rats. Guanethidine enhanced restraint stress-induced augmentation of antral and pyloric motility. Atropine and hexamethonium attenuated antral and pyloric motility induced by restraint stress.

Fig. 4. A: effects of truncal vagotomy on restraint stress-induced augmentation of antral and pyloric motility in conscious rats. Truncal vagotomy reduced postprandial antral (B) and pyloric (C) motility indexes. Restraint stress did not augment antral and pyloric motility in vagotomized rats. Values are means ± SE (n=4–5). *P < 0.05 compared with control. **P < 0.01 compared with respective groups.

Fig. 5. Effect of intracisternal (ic) injection of astressin 2B on restraint stress-induced augmentation of antral (A) and pyloric (B) motility. Restraint stress-induced augmentation of antral and pyloric motility was further enhanced by astressin 2B (60 µg ic). Guanethidine also increased restraint stress-induced augmentation of antral motility. Intracisternal astressin 2B did not further increase antral and pyloric motility in guanethidine-treated rats. Guanethidine also tended to increase restraint stress-induced augmentation of pyloric motility, although this was not statistically significant (NS). Values are means ± SE (n=4–6). *P < 0.05 compared with control. **P < 0.01 compared with respective groups.
Pretreatment with atropine and hexamethonium reduced postprandial antral and pyloric contractions and abolished restraint stress-induced augmentation of the antral and pyloric contractions (Fig. 3, C and D). During the restraint stress loading, no significant changes were observed in the antrum and pylorus in atropine- and hexamethonium-treated rats (Fig. 2, A and B).

Postprandial antral and pyloric contractions were attenuated in vagotomized compared with sham-operated rats (Fig. 4, B and C). Restraint stress did not affect postprandial antral and pyloric contractions in vagotomized rats (Fig. 4).

**Effect of Intracisternal Injection of Astressin 2B on Restraint Stress-Induced Augmentation of Antral and Pyloric Motility**

Intracisternal astressin 2B (60 μg) did not affect postprandial antral and pyloric contractions (Fig. 5, A and B). Intracis-
ternal astressin 2B significantly enhanced restraint stress-in-
duced augmentation of antral and pyloric contractions (Fig. 6) and increased the motility index of antral and pyloric contrac-
tions stimulated by restraint stress (Fig. 5, A and B).

During restraint stress loading, there was an obvious time lag between the antral and pyloric contractions in astressin 2B-treated rats (Fig. 6). The number of episodes of antropyloric coordination was partially restored to 1.6 ± 0.3/10 min during the restraint stress loading (Table 1).

In guanethidine-treated rats, intracisternal astressin 2B did not further increase the motor activities stimulated by restraint stress (Fig. 5, A and B). The time lag between the antral and pyloric contractions was also observed in animals treated with astressin 2B + guanethidine (Fig. 7). The number of episodes of antropyloric coordination was also restored to 1.4 ± 0.5/10 min during the restraint stress loading (Table 1).
Intracisternal injection of saline or CRF (0.1 \( \mu \text{g} \)) did not affect postprandial antral contractions (Figs. 8, A and B, and 9). In contrast, intracisternal injection of CRF (0.25, 0.5, and 1.0 \( \mu \text{g} \)) inhibited postprandial antral contractions in a dose-dependent manner (Figs. 8, C–E, and 9). The inhibitory effects were observed for 30 min after CRF (1.0 \( \mu \text{g} \)) injection (Fig. 8E). The inhibitory effect of CRF on the pyloric contractions was also observed (data not shown).

Intracisternal injection of saline or CRF (0.1 \( \mu \text{g} \)) did not affect postprandial antral contractions (Figs. 8, A and B, and 9). In contrast, intracisternal injection of CRF (0.25, 0.5, and 1.0 \( \mu \text{g} \)) inhibited postprandial antral contractions in a dose-dependent manner (Figs. 8, C–E, and 9). The inhibitory effects were observed for 30 min after CRF (1.0 \( \mu \text{g} \)) injection (Fig. 8E). The inhibitory effect of CRF on the pyloric contractions was also observed (data not shown).

Intracisternal injection of CRF (0.5 \( \mu \text{g} \)) increased the number of simultaneous contractions of the antrum and pylorus (Fig. 10) and significantly attenuated the number of episodes of antropyloric coordination for up to 30 min after the peptide injection (Table 1).

Pretreatment with guanethidine almost completely abolished the inhibitory effects of CRF on postprandial antral and pyloric contractions (Fig. 11).

**Effect of Intracisternal Injection of CRF on Postprandial Antral and Pyloric Contractions**

**DISCUSSION**

A number of investigators reported that restraint stress delays gastric emptying of a liquid (4, 23, 26) and solid food (30). However, little is known about whether restraint stress modifies gastric motility. Although it has been reported that restraint stress does not affect gastric motility in a fasting state (42), it remains unclear whether restraint stress alters postprandial gastric motility.

Our present study demonstrated that restraint stress significantly augmented gastric contractions of the antrum and pylorus in response to solid food ingestion. We also showed that the augmented gastric motility was restored to basal levels immediately after termination of the restraint stress loading. Because atropine, hexamethonium, and vagotomy blocked restraint stress-induced augmentation of gastric motility, the vagal-cholinergic pathway might be involved in this event.

Previous reports indicate that the CNS produces several neuropeptides in response to restraint stress (16, 20). Cold-restraint stress increases thyrotropin-releasing hormone (TRH) in the brain stem, which stimulates gastric motility and liquid emptying (17, 36). It remains unknown whether endogenous TRH is involved in mediating restraint stress-induced augmentation of postprandial gastric contractions. Some other unknown factors might be involved in the mechanism of restraint stress-induced augmentation of gastric motility.

It is generally accepted that solid gastric emptying is regulated by coordination of the antrum, pylorus, and duodenum (11, 27). In particular, coordination between the antrum and pylorus is an important factor in the emptying of solid foods (3, 6, 14, 21, 27).

In pre- and postrestraint stress, a time lag was observed between the antral and pyloric contractions; most often, the contraction maxima of the antrum occurred 3–6 s before the contraction maxima of the pylorus.

During restraint stress loading, the time lag between the antral and pyloric contractions was not observed frequently and the contraction maxima of the antrum and pylorus occurred simultaneously. The number of episodes of antropyloric coordination was significantly reduced during the restraint stress. Simultaneous contractions of the antrum and pylorus are considered to impair the effective emptying process and delay gastric emptying of a solid food.

It remains unclear whether the augmented gastric contractions are well correlated with the acceleration of gastric empt-
tying. Pentagastrin significantly augments gastric antral contractions, but it does not accelerate gastric emptying (12, 25). Although restraint stress-induced augmentation of gastric contractions was further increased by guanethidine, gastric emptying was not fully restored (30). Thus augmented gastric motility does not necessarily improve delayed gastric emptying. The coordinated motor pattern between the antrum and pylorus seems to be more important than augmented gastric contractions in mediating gastric emptying of solid food.

CRF is known to act in the brain as a neurotransmitter to influence GI function through the autonomic nervous system (5, 7, 22, 30). The present study revealed that intracisternal injection of CRF dose dependently attenuated postprandial gastric motility of the antrum and pylorus for up to 30 min. Furthermore, we demonstrated that intracisternal injection of CRF (0.5 μg) produced simultaneous contractions, resulting in impairment of antropyloric coordination. These results indicated that administration of CRF into the CNS not only attenuates gastric motility but also impairs antropyloric coordination. It is conceivable that endogenous CRF may be involved in the impairment of antropyloric coordination in stress loading.

The pathway through which central CRF attenuates gastric motility remains to be elucidated. A previous report demonstrated that microinjection of CRF at the dorsal vagal complex attenuates bethanechol-stimulated gastric contractions in anesthetized rats (24). The inhibitory effect of CRF is mediated via activation of inhibitory nonadrenergic noncholinergic vagal input to the stomach (24). In contrast, our present study demonstrated that guanethidine abolished inhibition of postprandial gastric motility induced by intracisternal injection of CRF in conscious rats, suggesting involvement of the sympathetic pathway.

We also showed that intracisternal injection of the CRF type 2 receptor antagonist astressin 2B (60 μg) enhanced restraint stress-induced augmentation of gastric motility. The pathophysiological significance of the inhibitory effect of central CRF on postprandial gastric motility remains unclear. It has been reported that hypothalamic microinfusions of CRF increase bicarbonate content, decrease gastric acid content, and protect against cold restraint-induced gastric mucosal damage (10). It is conceivable that central CRF may act against stress-induced augmentation of gastric motility. This may provide new information about the role of endogenous CRF in stress-induced alteration of postprandial gastric motility.

Restraint stress-induced impairment of antropyloric coordination was partially restored by treatment with guanethidine. Intracisternal injection of astressin 2B did not produce any further changes in stress-induced impaired coordination in guanethidine-treated rats. These results suggest that the inhibitory effect of endogenous CRF on gastric motility is mediated via sympathetic pathways.
Although intracisternal injection of astressin 2B did not fully restore impaired antropyloric coordination induced by restraint stress (Table 1), our previous study showed that restraint stress-induced delay of gastric emptying was completely restored by astressin 2B (30). The reason for this difference remains to be elucidated. One possibility may be the difference in the stress models. In our previous study, partial body-tubing stress was applied (30); in the present study, partial body-wrapping stress was used. In the wrapping stress model, stress-induced delay of solid gastric emptying might not be completely inhibited by intracisternal injection of astressin 2B.

We cannot exclude the possibility that other stress-related neuronal factors, in addition to CRF, might be involved in the impaired antropyloric coordination induced by partial body-wrapping stress. Several central coding neuropeptides, such as NPY or TRH, seem to be involved in the mechanism of stress-induced alteration of gastric motor functions (14, 17, 20). We previously showed that centrally administered NPY impairs antropyloric coordination (14). It is of interest to examine whether stress-induced impairment of antropyloric coordination is mediated by these neuropeptides.

In summary, restraint stress augmented postprandial gastric motility but impaired antropyloric coordination in conscious rats. The augmented postprandial gastric motility is mediated via vagal cholinergic pathways. Central CRF in response to restraint stress may play an important role in impairment of postprandial antropyloric coordination. Delayed gastric emptying by restraint stress might be due to impairment of antropyloric coordination. These results provide new evidence that restraint stress modifies gastric motor function.

REFERENCES

This study was supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Grant RO1-DK-55808 (to T. Takahashi).

GRANTS

This study was supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Grant RO1-DK-55808 (to T. Takahashi).

REFERENCES


