Peripheral urocortin delays gastric emptying: role of CRF receptor 2

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CRF mediates its actions through activation of specific, seven transmembrane domain receptors, which are coupled to a guanine nucleotide stimulatory factor (Gs) signaling protein resulting in increased intracellular cAMP levels (6, 51). To date, two CRF receptor subtypes, designated CRF-R1 and CRF-R2, were identified through molecular cloning from distinct genes in the rat and human (6, 24). CRF-R2 exists in multiple forms (α and β) as splice variants differing in their amino acid NH2-terminal extracellular domains and distribution (6). In human brain, unlike in rat brain, another CRF-2y splice variant has been recently identified (18). CRF-R2α is located on brain neurons, whereas CRF-R2β is found in nonneuronal brain tissue and in the periphery (predominantly in the heart and the gastrointestinal tract in rats) (24, 36).

Urocortin, a new 40-amino acid mammalian member of the CRF family, was cloned recently from rat midbrain and detected at peripheral sites, including the rat gut and heart and human lymphocytes (1, 10, 54). Urocortin possesses the characteristics of an endogenous ligand for CRF-R2 (10, 54). In Chinese hamster ovary cells, which have a stable expression of CRF-R2β, rat urocortin, and nonmammalian members of the CRF family, sauvagine and urotensin I exhibit a much higher binding affinity and stimulation of cAMP than rat CRF, whereas urocortin, sauvagine, and urotensin I display almost similar binding affinity to CRF-R1 as to CRF (10, 37, 43, 54). Therefore, the distinct pharmacological profiles of existing ligands for the CRF-R1 and CRF-R2 have been used to discriminate their localization and involvement in biological actions of CRF (42, 43). In addition, recent advances have been made in the development of CRF receptor antagonists. Astressin, cyclo-(30–33)-[β-Phe12,Nle21,38,Glu30,Lys33] rat and human (r/h) CRF12–41, which has a low intrinsic activity and high affinity to both CRF-R1 and CRF-R2, is more potent than any other peptide CRF antagonists reported to date to antagonize intravenous CRF action at the pituitary (12, 37). Moreover, nonpeptide CRF antagonists such as NBI-27914, SC-241, CP-154,526, and the CP-154,526-related compound antalarmin display selectivity for CRF-R1 (7, 8, 43, 55), whereas the peptide antagonist α-helical CRF9–41 has a higher affinity at the CRF-R2 than CRF-R1 (17, 37). Convergent sets of evidence established that peripheral CRF-induced stimulation of ACTH secretion at the pituitary level involved CRF-R1 (46, 51), whereas the relaxing action of CRF on mesenteric artery or anti-inflammatory effect of systemically injected CRF and urocortin appear to be mediated by CRF-R2 (11, 42, 52).
To better define the inhibitory effects of peripheral CRF on gastric motor function, we studied the influence of the newly characterized mammalian member of the CRF family, rat urocortin injected intravenously, on gastric emptying of a nonnutrient liquid meal and compared its potency with that of rat CRF in conscious rats. Then we tested the blocking action of the CRF-R1/CRF-R2 antagonist astressin (37) or the selective CRF-R1 antagonists antalarmin (55) and NBI-27914 (7) administered peripherally against intravenous CRF-, intravenous urocortin-, and stress-related inhibition of gastric emptying caused by abdominal surgery.

**MATERIALS AND METHODS**

Animals. Male Sprague-Dawley rats (Harlan, San Diego, CA), weighing about 250 g (range 230–280 g), were housed under controlled conditions of 12:12-h light-dark cycle with room temperature maintained at 22 ± 1°C. Animals were allowed free access to food (Purina Rat Chow) and tap water. Before the experiment, rats were deprived of food for 18–20 h, whereas water was provided ad libitum up to the beginning of treatment. Experiments were performed under the Veterans Affairs Animal Component of the Research Protocol number 96-080-08.

Drugs. Rat/human CRF (r/hCRF), rat urocortin, and astressin, cyclo-[30-32]-(t-Phed2,Nle21-36,Glu38,Lys39)-r/hCRF23-41 (Salk Institute, Clayton Foundation Laboratories for Peptide Biology, La Jolla, CA), were synthesized and purified as previously described (12). Peptides were kept in powder form at −70°C and dissolved immediately before use. CRF and urocortin were dissolved in sterile saline and astressin in double-distilled water (adjusted to pH 7.0 and warmed to 37°C). NBI-27914 (Neurocrine Biosciences, San Diego, CA) was synthesized as a tosylate salt as previously described (7).

Before use NBI-27914 was dissolved in 100% DMSO at a concentration of 10 mM and further diluted with PBS, pH 7.4. Antalarmin was synthesized and dissolved as previously described (55) using 50% ethanol and 50% cremophor EL (PEG-35 castor oil) and then further diluted with distilled water.

Measurement of gastric emptying. Measurement of gastric emptying was performed as previously detailed (49). The liquid meal consisted of methyl cellulose (Sigma Chemical, St. Louis, MO) dispersed in hot water at a final concentration of 1.5% under continuous stirring in which phenol red (50 mg/100 ml, Sigma) was added as a nonabsorbable marker. The meal (1.5 ml/rat) was given to conscious rats by oral intubation using stainless steel cannulas, and 20 min later rats were euthanized by CO2 inhalation. The abdominal cavity was opened, the gastroesophageal junction and the pylorus were clamped, and then the stomach was extirpated and rinsed in 0.9% saline. After the clamps were removed the stomach was placed in 100 ml of 0.1 N NaOH and homogenized (Polytron, Brinkmann Instruments). The suspension was allowed to settle for 1 h at room temperature, and 5 ml of the supernatant were added to 0.5 ml of 20% TCA (wt/vol) and then centrifuged at 3,000 rpm at 4°C for 20 min. The supernatant was mixed with 4 ml of 0.5 N NaOH, and the absorbance of the sample read at 560 nm (Shimazu 260 Spectrophotometer). Phenol red recovered from stomachs immediately after the administration of the meal was used as standard (0% emptying). Percent emptying in the 20-min period was calculated according to the following equation: percent emptying = (1 − absorbance of sample/absorbance of standard) × 100.

Experimental protocols. The experimental design included vehicle and several doses of test substances evaluated on the same day.

Effects of intravenous CRF and urocortin on gastric emptying. Rat CRF, rat urocortin (0.4, 12, 2.4, or 4.0 µg/kg in 0.1 ml), or saline (0.1 ml) was injected intravenously through the jugular vein in rats under short enflurane anesthesia (5.5% vapor concentration in oxygen; Ethane-Anaquest, Madison, WI). The intravenous doses of CRF were based on previous dose-response studies (49). After intravenous injection animals were returned to their home cages, 10 min thereafter the methyl cellulose phenol red meal was administered per orogastric gavage in lightly restrained rats, and 20 min later animals were euthanized to measure gastric emptying.

Effects of peripheral CRF antagonists on intravenous CRF- and urocortin-induced inhibition of gastric emptying. In rats under short enflurane anesthesia, astressin (4, 12, 40, or 80 µg/kg in 0.1 ml) or its vehicle (1 ml distilled water) or NBI-27914 (400 µg/kg in 0.1 ml) or its vehicle (16% DMSO and 84% PBS in 0.1 ml) was injected intravenously immediately before that of CRF (2.4 µg/kg in 0.1 ml), urocortin (1.2 or 2.4 µg/kg, or 0.1 ml), or saline. In other groups, antalarmin (20 mg/kg in 0.3 ml) or its vehicle (distilled water containing 8% cremophor EL and 8% ethanol in 0.3 ml) was injected intraperitoneally in conscious rats 1 h before intravenous injection (0.1 ml) of CRF (2.4 µg/kg), urocortin (1.2 µg/kg), or saline under enflurane anesthesia. The intravenous doses of astressin were based on the previous dose-related antagonism of CRF action on gastric emptying on intracisternal injection of both peptides (25). Antalarmin was administered under similar conditions previously reported to block ACTH release induced by systemic CRF in rats (55) and that of NBI-27914 on in vitro inhibition of CRF binding in cells stably transfected with human CRF-R1 receptor (7). After the administration of CRF, urocortin, or saline, rats were returned to their home cages, 10 min later the liquid meal was administered, and 20 min later animals were euthanized to measure gastric emptying.

Effects of CRF antagonists on abdominal surgery-induced inhibition of gastric emptying. Under a 10-min exposure to enflurane anesthesia (5.5% vapor concentration in oxygen), groups of rats were injected intravenously either with astressin (4, 12, or 40 µg/kg, 0.1 ml) or distilled water (0.1 ml) or NBI-27914 (400 µg/kg in 0.1 ml) or its vehicle (16% DMSO and 84% PBS in 0.1 ml) immediately before abdominal surgery with cecal manipulation performed as previously described (3). In other groups antalarmin (20 mg/kg in 0.3 ml) or its vehicle (distilled water containing 8% cremophor EL and 8% ethanol in 0.3 ml) was injected intraperitoneally in conscious rats 1 h before the abdominal surgery with manipulation of the cecum. Briefly, abdominal surgery consisted of a medial celiotomy (3–4 cm) and cecal exteriorization and handling in gauze soaked with saline for a 1-min period, and then the cecum was returned to the abdominal cavity. The linea alba and the skin were closed separately with 3-0 silk sutures. Sham-operated control groups were exposed to similar duration of enflurane anesthesia (10 min) and had only a skin incision with no laparotomy and manipulation of the cecum. The phenol red methyl cellulose meal was administered at 160 min after the end of the surgery and gastric emptying was determined 20 min later.

Statistical analysis. All results represent means ± SE. For two-group comparisons, data were analyzed by Student’s t-test. Comparisons between multiple groups were performed using one-way ANOVA followed by a Student-Newman-Keuls...
Effects of intravenous CRF and urocortin on gastric emptying. In rats injected intravenously with saline, 52.9 ± 1.8% (n = 20) of the noncaloric methyl cellulose liquid meal was emptied after 20 min. Rat CRF injected intravenously (1.2–2.4 µg/kg) inhibited gastric emptying in a dose-dependent manner [ANOVA, F(4,37) = 10.393, P < 0.001; Fig. 1]; a plateau inhibitory response was observed at 2.4 and 4.0 µg/kg (24.2 ± 7.9%, n = 5, and 25.5 ± 4.5%, n = 6, respectively), whereas at 0.4 µg/kg intravenous CRF did not significantly reduce gastric emptying (42.8 ± 5.4%, n = 7; Fig. 1).

Rat urocortin (1.2–4.0 µg/kg) inhibited gastric emptying in a dose-dependent manner [ANOVA, F(4,40) = 34.435, P < 0.001]. The intravenous urocortin doses of 1.2, 2.4, and 4.0 µg/kg significantly inhibited gastric transit to 23.0 ± 4.0% (n = 9), 12.4 ± 7.7% (n = 5), and 7.1 ± 3.1% (n = 5), respectively, whereas 0.4 µg/kg had no significant effect (Fig. 1). The 50% dose for CRF (2.5 µg/kg, 529 pmol/kg), 95% confidence intervals (1.3–4.7 µg/kg, r² = 0.963) was 2.3-fold higher than that of urocortin (1.1 µg/kg, 227 pmol/kg), 95% confidence intervals (0.96–1.2 µg/kg, r² = 0.998).

Effects of intravenous astressin on intravenous CRF- and urocortin-induced inhibition of gastric emptying. In rats injected intravenously with water followed by saline (0.1 ml each), 54.4 ± 2.7% (n = 12) of the nonnutrient meal was emptied from the stomach 20 min after its administration. CRF (2.4 µg/kg) or urocortin (2.4 µg/kg) significantly reduced gastric emptying to 23.9 ± 6.1% [ANOVA, F(6,40) = 7.22, P < 0.001, n = 6] and 12.7 ± 2.0% [ANOVA, F(6,47) = 25.5, P < 0.0001, n = 13], respectively, in intravenous water-pretreated rats (Fig. 2). Astressin (40 or 80 µg/kg iv) had no significant effect on basal gastric emptying, although at 80 µg/kg there was a tendency to reduce gastric emptying (Table 1).

A stressin (4–40 µg/kg iv) dose dependently antagonized CRF (2.4 µg/kg iv)-induced inhibition of gastric transit with a partial prevention at 4 µg/kg (40.5 ± 1.3%, n = 5, P < 0.05 vs. intravenous water and CRF) and complete normalization at 12 or 40 µg/kg (56.0 ± 7.9% vs. respective CRF group (ANOVA followed by Student-Newman-Keuls multiple-comparison test).
Effects of peripheral antalarmin or NBI-27914 on intravenous CRF- or urocortin-induced inhibition of gastric emptying. CRF (2.4 µg/kg) or urocortin (1.2 µg/kg) injected intravenously significantly inhibited gastric emptying in vehicle-pretreated rats (Table 2). Antalarmin (20 mg/kg ip) modified neither basal nor intravenous CRF- or urocortin-induced inhibition of gastric emptying (Table 2). No antagonist-to-agonist ratio could be deduced due to the different routes of administration. NBI-27914 (400 µg/kg iv) had no significant effect on the basal gastric emptying or intravenous CRF- and urocortin-induced gastric stasis (Table 2). The dose of NBI-27914 tested corresponds to an antagonist-to-agonist ratio (µg/kg) of 167:1 for CRF and 333:1 for urocortin.

Effects of CRF antagonists on abdominal surgery-induced inhibition of gastric emptying. For control rats exposed to 10 min of anesthesia in which an intravenous injection of water followed by abdominal skin incision without laparotomy were performed, gastric emptying was 59.5 ± 2.8% (n = 10) as measured during the 160- to 180-min period after the end of anesthesia. Abdominal surgery and cecal manipulation in rats injected intravenously with water inhibited gastric emptying to 27.5 ± 3.2% at the 160- to 180-min period after surgery [P < 0.05 vs. vehicle plus sham surgery, n = 11, ANOVA, F(6, 35) = 33.86, P < 0.0001]. Astressin at intravenous doses of 12 or 40 µg/kg completely antagonized postoperative gastric ileus (58.0 ± 1.2 and 54.1 ± 4.6%, respectively, P < 0.05 vs. vehicle plus surgery, n = 4–5), whereas it had no influence on basal gastric emptying in sham control (58.1 ± 3.7 and 53.2 ± 8.0%, respectively, n = 3–4 for each dose, P > 0.05 vs. water plus sham operation; Fig. 3). The CRF-R1 antagonists antalarmin (20 mg/kg ip) or NBI-27914 (400 µg/kg iv) did not alter abdominal surgery-induced inhibition of gastric emptying (Table 3).

DISCUSSION

Present and previous studies established that CRF decreases gastric emptying of a nonnutrient liquid meal in rats, mice, and dogs after intravenous (4, 34, 38, 45, 49, 56) as well as intraperitoneal or subcutaneous administration in rats or mice (5, 20, 45). We showed that rat urocortin, a novel mammalian member of the CRF family, injected intravenously also inhibits gastric emptying of a noncaloric liquid meal in rats.
and antalarmin on abdominal surgery-induced gastric emptying in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Gastric Emptying, %</th>
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<tbody>
<tr>
<td>Vehicle + sham surgery</td>
<td>53.3 ± 2.1</td>
</tr>
<tr>
<td>Antalarmin + sham surgery</td>
<td>53.3 ± 7.4</td>
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<tr>
<td>Vehicle + abdominal surgery</td>
<td>27.6 ± 0.9*</td>
</tr>
<tr>
<td>Antalarmin + abdominal surgery</td>
<td>19.1 ± 4.3*</td>
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<tr>
<td>Vehicle + sham surgery</td>
<td>63.5 ± 5.1</td>
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<tr>
<td>NBI-27914 + sham surgery</td>
<td>54.6 ± 1.5</td>
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<tr>
<td>Vehicle + abdominal surgery</td>
<td>21.4 ± 2.6*</td>
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<tr>
<td>NBI-27914 + abdominal surgery</td>
<td>23.1 ± 6.4*</td>
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Values are means ± SE; n = no. of rats. Vehicle (distilled water containing ethanol and cremophor EL) or antalarmin (20 mg/kg) was injected intraperitoneally 1 h before either sham or abdominal surgery performed under 10-min enflurane anesthesia. In other groups of anesthetized rats exposed for 10 min to enflurane, either vehicle (16% DMSO and 84% PBS, 0.1 ml) or NBI-27914 (400 µg/kg) was injected intravenously and sham surgery or laparotomy with colic manipulation was performed. The 20-min gastric emptying was determined during the 160- to 180-min period after surgery in conscious rats. *P < 0.05 compared with vehicle + sham surgery. ANOVA was followed by Student-Newman-Keuls multiple-comparison test.

Urocortin is structurally related to sucker fish urotensin I (63% sequence identity) (19) and shares 45% sequence identity with r/hCRF and 35% with sauvgaine (10, 54). Likewise, urotensin I and sauvgaine injected subcutaneously suppress gastric emptying in rats (5, 15). Taken together, these findings indicate that mammalian and nonmammalian homologs of CRF display a similar pattern of action on gastric motor function when injected peripherally. Urocortin inhibits gastric emptying at a similar or lower intravenous dose range (100–500 pmol/kg) than cholecystokinin, peptide YY, and glucagon-like peptide 1 (14, 35, 39). Only a few studies have reported biological actions of peripherally administered urocortin at such a dose range, and they relate to the modulation of pituitary secretion, blood pressure, and inflammation in rats (52, 54). The present data provide the first evidence that peripherally injected urocortin influences gut function.

Defined differences in CRF-R1 and CRF-R2 pharmacology provide a tool with which to distinguish the involvement of the two receptor subtypes through which CRF actions are mediated (6). In particular, urocortin, sauvgaine, and urotensin I exhibit much greater affinity than CRF for the CRF-R2 isoforms (10, 37, 54). In the present study, urocortin was found to be 2.3-fold more potent than CRF to inhibit gastric emptying. Because both peptides used were derived from the rat sequence, possible differences related to nonhomologous species comparison can be ruled out. A rank order of potency of nonmammalian CRF-related peptides (sauvgaine > urotensin I > CRF) to inhibit gastric emptying of a nonnutrient liquid meal after subcutaneous injection in rats was also reported (15, 25). Compared with CRF, the greater potency of urocortin, urotensin I, and sauvgaine injected peripherally to delay gastric emptying is in keeping with their relatively higher affinities at the CRF-R2 (10, 43, 54).

In addition, the use of CRF receptor antagonists further supports the speculation that the inhibition of gastric emptying induced by members of the CRF family may be mediated through CRF-R2. Both antalarmin and NBI-27914 are selective CRF-R1 antagonists (7, 22, 55) and did not modify intravenous CRF- or urocortin-induced gastric stasis. Antalarmin is a derivative of the well-established CRF-R1 antagonist CP-154,526 (8, 55) and was administered under identical conditions to those inhibiting the CRF-R1-mediated effect of intravenous CRF (4.7 µg/rat) on pituitary ACTH release (55). NBI-27914 (606 mol wt) was administered at a 193-fold higher molar dose than antalarmin. Because both astressin and NBI-27914 have a similar affinity at the CRF-R1 (K, in the 2 nM range) (7, 12, 37), we can assume that the NBI-27914 at the intravenous dose used should be efficient to block peripheral CRF-R1. By contrast, astressin, which exhibits a mixed CRF-R1/CRF-R2 antagonist profile (12, 37), completely abolished intravenous CRF-induced 50% inhibition of gastric emptying when injected intravenously at a low dose (12 µg/kg). This shows the high potency of the new CRF antagonist as previously established in vitro and in vivo mainly against intravenous CRF-induced ACTH release (40). The higher ratio of astressin to urocortin (67:1) than astressin to CRF (5:1) required to antagonize intravenous urocortin action is consistent with the greater affinity of urocortin for CRF-R2 (α and β) compared with CRF (10, 37, 43, 54). Recent studies indicate that α-helical CRF9–41 is more selective to antagonize CRF-R2 based on its much greater affinity at CRF-R2 than CRF-R1 (37) and its differential antagonistic activity for intravenous CRF actions on the pituitary (antagonist-to-agonist ratio of 3,000:1) vs. cardiovascular system (6:1) (11). We previously showed that α-helical CRF9–41 injected intravenously prevented intravenous CRF at a much lower ratio (100:1) than required to block intravenous CRF-induced ACTH release (4, 11). With the use of rank order of potency of CRF-related peptides and α-helical CRF9–41-to-CRF ratio as a strategy to discriminate CRF receptor subtypes, as well as the presence of CRF-R2 in the heart and vessels, the peripheral cardiovascular effects of CRF were suggested to involve CRF-R2 (11, 17, 42), whereas the effect of intravenous CRF to stimulate pituitary ACTH release was mediated by CRF-R1 (46, 51). Taken together, the present data provide convergent evidence that CRF-R2 may be preferentially involved in intravenous CRF-induced delay of gastric emptying and that such a response is not secondary to pituitary activation because nonpeptide CRF-R1 antagonists have no effect. However, the role of CRF-R2 will ultimately be assessed when CRF-R2 antagonists/agonists devoid of affinity for CRF-R1 are developed.

CRF injected into the cerebrospinal fluid at a similar dose range inhibits gastric motor function, and CRF-R2 in the brain was suggested to mediate CRF action (25). However, it is unlikely that the inhibition of gastric emptying induced by intravenous CRF reflects a central action due to the entry of CRF across the blood-brain barrier (2). First, the characteristics of pharmaco-
kinetic studies in mice indicate that peripheral CRF is not transported from the blood to the brain (28). Second, astressin (40 µg/kg) injected intravenously did not alter the 50% inhibition of gastric emptying induced by intracisternal injection of CRF (26), whereas it prevented the effect of intravenous CRF (present study). The localization of peripheral CRF-R2 receptors on which CRF and urocortin act needs to be further assessed. CRF-R2β are expressed in the gastrointestinal tract (36), although the exact cellular distribution has not yet been established. In addition, CRF binding sites have been found on the subdiaphragmatic vagus (31). These pathways may have relevance as vagotomy attenuated peripheral CRF-induced delay of gastric emptying in rats (5), whereas blockade of sympathetic nervous system by adrenalectomy, celiac ganglionectomy, or bretylium had no effect (20, 38). The extrinsic and/or intrinsic neural pathways vs. direct action of intravenous urocortin on gastric smooth muscles needs to be further investigated.

Astressin injected intravenously did not influence gastric emptying, suggesting that peripheral CRF receptors are not involved in the basal regulation of gastric emptying of a nonnutrient liquid meal. By contrast, under stress-related conditions, such as abdominal surgery, known to activate CRF pathways and to increase circulating CRF levels (32), the activation of peripheral CRF receptors plays a role in the alterations of gastric motor function. In the present study, laparotomy followed by 1-min manipulation of the cecum results in 47% reduction of gastric transit monitored 3 h later in agreement with our previous studies (3, 4). Intravenous injection of astressin at 12 µg/kg abolished the abdominal surgery-induced inhibition of gastric emptying monitored 3 h later, whereas nonpeptide CRF-R1 antagonists have no effect. In a previous study, α-helical CRF9–41 injected intravenously at 200 µg/kg normalized only by 70% gastric transit 3 h after abdominal surgery while completely reversing intravenous CRF-induced similar inhibition of gastric emptying (4). The enhanced potency of astressin compared with α-helical CRF9–41 may be related to the unique properties of astressin which has low intrinsic activity and binding affinity to the CRF binding protein as well as a greater affinity at CRF receptors than α-helical CRF9–41 (12, 37). These results indicate that peripheral CRF receptors, most likely the CRF-R2 located in the periphery (23), may play a key role in mediating acute postoperative gastric ileus.

We previously reported that intracisternal injection of astressin at a similar dose range also prevented abdominal surgery- and cecal manipulation-induced gastric ileus assessed 3 h after surgery (27). These findings would suggest that both central and peripheral CRF receptors are involved in postoperative gastric ileus (Ref. 3 and present study). As recent reports demonstrated an active transport of CRF from the brain to the periphery, where the peptide could directly affect a peripheral organ (28, 29), a possible dual action of peptide receptor antagonists needs to be further investigated using intracisternal injection of astressin and peripheral administration of CRF. This will allow us to further ascertain central and/or peripheral sites of astressin action to reverse postoperative gastric ileus particularly since experiments were performed during a 3-h period, which allows maximal peptide transport from the brain to the periphery as shown by kinetic studies (28, 29). By contrast, other acute stressors (restraint, forced swimming, ether, intravenous interleukin-1) that induce inhibition of gastric emptying have been reported to be selectively blocked by central, but not by peripheral, injection of α-helical CRF9–41 or D-Phe12 CRF12–41 antagonists (9, 21, 41, 47, 48). These findings would suggest that these stressors, unlike abdominal surgery, selectively activate brain CRF pathways.

In summary, urocortin, the new mammalian member of the CRF family characterized as the endogenous ligand for CRF-R2, injected intravenous, dose dependently delays gastric emptying of a nonnutrient meal in conscious rats with a rank order of potency showing rat urocortin > rat CRF. The mixed CRF-R1/CRF-R2 antagonist astressin prevented CRF and urocortin inhibitory effects, whereas relatively large doses of the selective nonpeptide CRF-R1 antagonist antalarmin and NB1-27914 have no effect. In addition, we showed that peripheral injection of a low dose of astressin (12 µg/kg), which by itself did not influence basal gastric emptying, completely abolished gastric stasis observed 3 h after abdominal surgery while the CRF-R1 antagonists were inactive. These results suggest an important role of peripheral CRF and urocortin acting through CRF-R2 as part of the mechanisms involved in acute postoperative gastric ileus.

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