0022-3565/99/2902-0629$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 290, No. 2
Printed in U.S.A.

JPET 290:629–634, 1999

Peripheral Injection of a New Corticotropin-Releasing Factor (CRF) Antagonist, Astressin, Blocks Peripheral CRF- and Abdominal Surgery-Induced Delayed Gastric Emptying in Rats[1]

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Accepted for publication March 9, 1999 This paper is available online at http://www.jpet.org

ABSTRACT

The effect of the corticotropin-releasing factor (CRF) receptor antagonists astressin and D-Phe CRF_{12-41} injected i.v. on CRF-induced delayed gastric emptying (GE) was investigated in conscious rats. Gastric transit was assessed by the recovery of methyl cellulose/phenol red solution 20 min after its intragastric administration. The 55% inhibition of GE induced by CRF (0.6 μg i.v.) was antagonized by 87 and 100% by i.v. astressin at 3 and 10 μg, respectively, and by 68 and 64% by i.v. D-Phe CRF_{12-41} at 10 and 20 μg, respectively. CRF (0.6 μg)-injected intracisternally (i.c.) induced 68% reduction of GE was not modified by i.v. astressin (10 μg) whereas i.c. astressin (3 or 10 μg) blocked by 58 and 100%, respectively, i.v. CRF inhibitory action. Abdominal surgery with cecal manipulation reduced GE to 7.1 $\pm$ 3.1 and 27.5 $\pm$ 3.3% at 30 and 180 min postsurgery, respectively, compared with 40.3 $\pm$ 4.3 and 59.5 $\pm$ 2.9% at similar times after anesthesia alone. Astressin (3 μg i.v.) completely and D-Phe CRF_{12-41} (20 μg i.v.) partially (60%) blocked surgery-induced gastric stasis observed at 30 or 180 min. The CRF antagonists alone (i.v. or i.c.) had no effect on basal GE. These data indicate that CRF acts in the brain and periphery to inhibit GE through receptor-mediated interaction and that peripheral CRF is involved in acute postoperative gastric ileus; astressin is a potent peripheral antagonist of CRF when injected i.v. whereas i.c. doses $\geq$3 μg exert dual central and peripheral blockade of CRF action on gastric transit.

Corticotropin-releasing factor (CRF) in the brain plays an important role in the behavioral, neuroendocrine, autonomic, immunologic, and visceral responses to stress (Irwin et al., 1990; Heinrichs et al., 1995). In particular, central CRF is involved in stress-related alterations of gastrointestinal (GI) motor function (Taché et al., 1993; Martinez et al., 1997). It is also becoming increasing apparent that CRF administered peripherally can induce a similar pattern of GI responses in rats, mice, dogs, and humans as when injected centrally in rats or dogs (Pappas et al., 1985; Sheldon et al., 1990; Taché et al., 1993; Fukudo et al., 1998). Namely, CRF injected peripherally at similar dose ranges effective into the cerebrospinal fluid (CSF) inhibits gastric contractility and emptying and slows small intestine transit while stimulating colonic motility, transit, and fecal pellet output in rats and mice (Williams et al., 1987; Lenz et al., 1988a,b; Sheldon et al., 1990; Gué et al., 1991; Martinez et al., 1997). However, less is known about the mechanism of action and role of peripheral, compared with central, CRF in visceral function (Taché et al., 1993).

CRF exerts its biological effects by binding to specific cell surface receptors on target tissues. CRF receptors are part of a subfamily of seven transmembrane domain receptors that are coupled to adenylate cyclase via a guanine nucleotide stimulatory factor-signaling protein (Turnbull and Rivier, 1997). The CRF receptor subtypes 1 (CRF-R1) and 2 (CRF-R2) have been cloned and shown to be encoded by two distinct genes (Dieterich et al., 1997). CRF-R2 exists in multiple forms as splice variants differing in their amino terminus domains and distributions (Dieterich et al., 1997). CRF-R2α is found in the brain whereas CRF-R2β is located in non-neuronal brain cells and the periphery, including the GI tract in rats and humans (Dieterich et al., 1997). The predominant receptor subtype in the pituitary gland is CRF-R1 (Chalmers et al., 1995), consistent with pharmacologic and binding studies showing that the peripheral action of CRF to stimulate

Received for publication November 20, 1998.
[1] This work was supported by the National Institute of Diabetes and Digestive and Kidney Diseases, Grants DK-33061 (Y.T.), DK-26741 (J.R.), and DK-41301 (Animal Core, Y.T.).
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ABBREVIATIONS: CRF, corticotropin-releasing factor; CRF-R1 and CRF-R2, corticotrophin-releasing factor receptor subtypes 1 and 2, respectively; CSF, cerebrospinal fluid; D-Phe CRF_{12-41}, [D-Phe^{12},$Nle^{21,38}$,$C^{\alpha}MeLeu^{37}$]r/h CRF_{12-41}; i.c., intracisternal; GE, gastric emptying; GI, gastrointestinal; ACTH, adrenocorticotropin; r/hCRF, rat/human CRF.

pituitary adrenocorticotropin (ACTH) release is mediated primarily by CRF-R1 and the recent use of CRF-R1 knockout mice (Turnbull and Rivier, 1997; Smith et al., 1998; Timpl et al., 1998). Indirect pharmacologic evidence suggests that CRF-R2 mediates peripheral CRF-induced relaxation of mesenteric small arteries (Rohde et al., 1996) and delayed gastric emptying (GE; Nozu et al., 1999), although this needs to be ascertained further using selective CRF receptor subtype antagonists.

Three generations of CRF analogs with specific competitive antagonist activity to the CRF receptors have been developed including α-helical CRF_{9-41}, the first reported CRF antagonist (Rivier et al., 1984), followed by D-Phe CRF_{12-41} and, more recently, the constrained astressin and its analogs (Hernandez et al., 1993; Gulyas et al., 1995; Miranda et al., 1997; Rivier et al., 1998). α-Helical CRF_{9-41} blocked various central and peripheral biological actions of CRF while lacking potency to antagonize CRF-induced pituitary ACTH release, suggesting that this CRF analog preferentially is a CRF-R2 antagonism (Fisher et al., 1991; Kishimoto et al., 1995; Rivier et al., 1996; Turnbull et al., 1996). By contrast, D-Phe CRF_{12-41} and astressin display high affinity to both CRF-R1 and CRF-R2α/β in vitro and prevent ACTH release induced by i.v. CRF in rats (Gulyas et al., 1995; Perrin et al., 1995; Rivier et al., 1996).

To date, the influence of peripheral administration of CRF antagonists on systemic injection of CRF- or stress-induced alterations of GI motor function has received little attention, and most of the information is derived from the influence of α-helical CRF_{9-41} tested at one dose (Williams et al., 1987; Barquist et al., 1992; Hernandez et al., 1993). Abdominal surgery-induced acute postoperative gastric ileus was prevented partly by i.v. injection of α-helical CRF_{9-41} at a similar dose, which fully reverses i.v. CRF in rats (Barquist et al., 1992; Hernandez et al., 1993). Williams et al. (1987) also showed that α-helical CRF_{9-41} injected at a similar dose either i.c.v. or i.v. prevented restraint-induced stimulation of colonic transit. These findings indicate that peripheral administration of CRF antagonist also counteracts stress-related alterations of GI motor function.

To assess further the role of peripheral CRF in the delayed GE induced by surgical stress in rats, we first investigated the antagonist activity of i.v. injection of the constrained CRF analog, astressin, compared with D-Phe CRF_{12-41} (Gulyas et al., 1995) on i.v. CRF-induced delayed gastric transit. Second, in view of growing evidence of exchanges of peptides across the blood-brain barrier, including transport of CRF from the brain into the circulation (Banks and Kastin, 1996; Martins et al., 1997) and the aforementioned similar potency of α-helical CRF_{9-41} given centrally or peripherally, we tested whether astressin injected i.v. influences i.c. CRF-induced delay in GE (Taché et al., 1993) and, conversely, whether astressin injected into the cisterna magna antagonizes i.v. CRF action. Last, we assessed the influence of peripheral injection of astressin and D-Phe CRF_{12-41} on abdominal surgery-induced acute postoperative gastric ileus.

Materials and Methods

Animals

Adult male Sprague-Dawley rats (Harlan, San Diego, CA) weighing 250 to 280 g were housed in group cages with free access to food (Purina Rat Chow) and tap water. Animals were maintained at a 12-h light/dark cycle and under controlled temperatures (21–23°C). Rats were fasted, but had free access to water for 18 to 20 h before experiments, which were conducted under the Veteran Administration Animal Component of Research Protocol number 96-080-08.

Peptides and Injections

Rat/human CRF (r/hCRF), astressin [cyclo(30–33) [D-Phe^{12},$Nle^{21,38}$,Glu^{30},Lys^{33}]r/hCRF_{12-41}], and D-Phe CRF_{12-41}, [D-Phe^{12},$Nle^{21,38}$,$C^{\alpha}MeLeu^{37}$]r/hCRF_{12-4} were synthesized by using the solid-phase approach and the Boc strategy and purified as described previously (Gulyas et al., 1995; Rivier et al., 1998). Peptides were stored in powder form at −70°C, and immediately before the experiments CRF was dissolved in sterile saline, and astressin and D-Phe CRF_{12-41} were dissolved in double-distilled water (pH 7.0, warmed to 37°C).

Intravenous injections were performed under short enflurane anesthesia (3–5 min, 5.5% vapor concentration in O_2; Ethrane-Anaquest, Madison, WI) by delivering vehicle and peptides in 0.1 ml through the right jugular vein. Intracisternal (i.c.) injections were performed acutely under short enflurane anesthesia (2–3 min) by puncture of the occipital membrane with a 50-μl Hamilton syringe in rats placed in a stereotaxic equipment. Presence of CSF into the Hamilton syringe upon aspiration before injection ensured correctness of needle placement in the cisterna magna.

Measurement of GE

GE of a non-nutrient viscous meal was determined by the phenol red method as described previously (Maeda-Hagiwara and Taché, 1987). The noncaloric liquid meal consisted of a viscous suspension of continuously stirred 1.5% methyl cellulose (w/v; Sigma Chemical Co., St. Louis, MO) containing phenol red (50 mg/100 ml; Sigma) given intragastrically (1.5 ml) to conscious rats. Twenty minutes after administration of the meal, rats were euthanized by CO_2 inhalation. The abdominal cavity was opened, the gastroesophageal junction and the pylorus were clamped, and the stomach was isolated and rinsed in 0.9% saline. After removing the clamps, the stomach was placed in 100 ml of 0.1 N NaOH and homogenized (Polytron; Brinkman Instruments, Inc., Westbury, NY). The suspension was allowed to settle for 1 h at room temperature, and then 5 ml of the supernatant was added to 0.5 ml of 20% trichloroacetic acid (w/v; Sigma) and then centrifuged at 3000 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 was read at 560 nm (Shimazu 260 Spectrophotometer). Phenol red recovered from animals euthanized immediately after the administration of the liquid meal was used as a standard (0% emptying). Percent emptying in the 20-min period was calculated according to the following equation: % emptying = (1 − absorbance of test sample/absorbance of standard) × 100.

Experimental Procedures

In each daily experiment, a vehicle and 2 to 3 doses of each test substance were included and repeated on multiple days on different animals. The i.c. and i.v. doses of CRF were selected based on previous dose-response studies showing a 50 to 70% inhibition of GE in doses ranging from 0.3 to 0.6 μg/rat (Taché et al., 1987; Martinez et al., 1997). Doses of astressin were selected based on previous i.c. dose-related antagonism of i.c. CRF (Martinez et al., 1997). After the

i.v. injections, animals were returned to their home cages and, 10 min later, except otherwise mentioned, the noncaloric viscous meal was administered per oral intubation to awake rats, and the content of the stomach was assessed 20 min later to calculate GE.

Influence of i.v. CRF Antagonists on i.v. CRF-Induced Inhibition of GE. Under short enflurane anesthesia, either astressin (1, 3, or 10 μg), D-Phe CRF_{12-41} (1, 3, 10, or 20 μg), or water (0.1 ml) was injected i.v. immediately before the i.v. injection of either CRF (0.6 μg) or saline (0.1 ml) in fasted rats.

Influence of i.v. CRF Antagonists on i.c. CRF-Induced Inhibition of GE. Under enflurane anesthesia, astressin (10 μg), D-Phe CRF_{12-41} (20 μg), or water (0.1 ml) was injected i.v. immediately before the i.c. injection of CRF (0.6 μg) or saline (10 μl).

Influence of i.c. Astressin on i.v. CRF-Induced Inhibition of GE. Under short enflurane anesthesia, astressin (1, 3, or 10 μg) or water (10 μl) was administered i.c. immediately before the i.v. injection of either CRF (0.6 μg) or saline (0.1 ml).

Influence of i.v. CRF Antagonists on Abdominal Surgery-Induced Inhibition of GE. In rats exposed to enflurane anesthesia for 10 min, either water (0.1 ml), astressin (1, 3, or 10 μg), or D-Phe CRF_{12-41} (1, 3, 10, or 20 μg) was injected i.v. and abdominal surgery with cecal manipulation was performed as described previously (Martinez et al., 1997). Abdominal surgery consisted of a medial celiotomy (3–4 cm) and exteriorization of the cecum, which was handled in gauze soaked with saline for a 1-min period. The cecum then was returned to the abdominal cavity. The linea alba and the skin were sutured separately with 3-0 silk suture. The noncaloric meal was administered intragastrically at 10 or 160 min after removal of the anesthetic, and GE was monitored 20 min later.

Statistical Analysis

Results are expressed as mean ± S.E. Comparisons within groups were performed using ANOVA followed by a Student-Newman-Keuls multiple-comparison test. P values $< .05$ were considered statistically significant.

Results

Influence of i.v. Astressin and D-Phe CRF_{12-41} on i.v. CRF-Induced Inhibition of GE. In the control group (n = 11), rats injected i.v. with water plus saline (0.1 ml each), 55.7 ± 3.3% of the noncaloric viscous meal was emptied in 20 min. The i.v. injections of water followed by CRF (0.6 μg) reduced GE to 24.9 ± 2.9% (n = 11) ($P < .05$ versus control; ANOVA: $F_{6,43} = 8.211$) (Fig. 1). Astressin (1, 3, and 10 μg) injected i.v. immediately before that of CRF (0.6 μg) dose-dependently prevented CRF inhibitory action (Fig. 1). A partial blockade of i.v. CRF was induced by astressin at 1 μg, as shown by the increase in GE to 41.2 ± 8.8% (n = 6, $P < .05$ versus i.v. water + CRF), whereas at higher doses (3 or 10 μg) astressin completely antagonized the CRF inhibitory effect (GE: 51.6 ± 3.2%, n = 6, and 58.4 ± 5.6%, n = 8, respectively, $P < .01$ versus water + CRF; $P > .05$ versus water + saline). Astressin alone (3 or 10 μg i.v.) did not modify basal GE (51.0 ± 3.8 and 54.7 ± 7.8%, respectively, n = 4 for each dose). D-Phe CRF_{12-41} injected i.v. at 1 or 3 μg did not modify i.v. CRF-induced inhibition of GE (23.1 ± 2.1%, n = 3, and 24.6 ± 1.3%, n = 5, respectively; Fig. 1) whereas at 10 and 20 μg, GE was increased similarly to 45.7 ± 3.3 and 44.5 ± 1.1% (n = 5; for each group, $P < .01$ versus vehicle + CRF; $P > .05$ versus water + saline; $F_{5,26} = 12.859$; Fig. 1). D-Phe CRF_{12-41} (20 μg i.v.) alone did not influence the basal GE (53.0 ± 7.8%, n = 4; $P > .05$ versus water + saline).

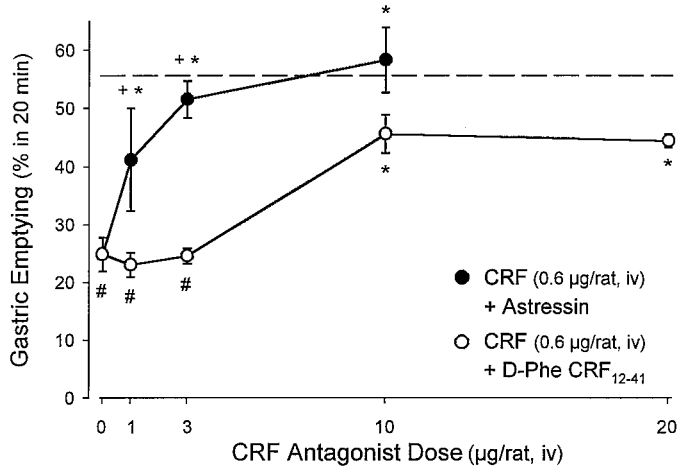

Fig. 1. Intravenous injection of the CRF antagonists D-Phe CRF_{12-41} and astressin dose-dependently reversed the inhibition of GE induced by i.v. CRF in conscious rats. Rats under short enflurane anesthesia were injected i.v. either with water, D-Phe CRF_{12-41}, or astressin and, immediately after, with i.v. saline or CRF. A noncaloric viscous meal was administered 10 min after the last i.v. injection, and GE was monitored 20 min after. Data represent the mean ± S.E. of 3 to 11 animals per dose. $^{*}P < .05$ versus the water + CRF (CRF antagonist dose of 0); $^{+}P < .05$ versus i.v. D-Phe CRF_{12-41} + i.v. CRF, at the same dose; $^{\#}P < .05$ versus i.v. water + i.v. saline; broken line: 55.7 ± 3.3%.

Influence of i.v. CRF Antagonists on i.c. CRF-Induced Inhibition of GE. In control rats injected i.v. with water followed by i.c. injection of saline (n = 4), 53.0 ± 4.7% of the non-nutrient meal was emptied 20 min after its administration. The i.c. injection of CRF (0.6 μg) immediately after the i.v. injection of water decreased GE to 17.1 ± 4.1% (n = 4) ($P < .001$ versus control; $F_{3,13} = 24.612$) (Fig. 2). Astressin injected i.v. (10 μg) did not modify i.c. CRF-induced inhibition of emptying (20.7 ± 4.5%, n = 5; $P > .05$ versus i.v. water + i.c. CRF; Fig. 2). D-Phe CRF_{12-41} (20 μg i.v.) also did not affect i.c. CRF (0.6 μg)-induced inhibition of GE [i.v. D-Phe CRF_{12-41} + i.c. vehicle: 52.3 ± 6.2%, n = 3; i.v. vehicle

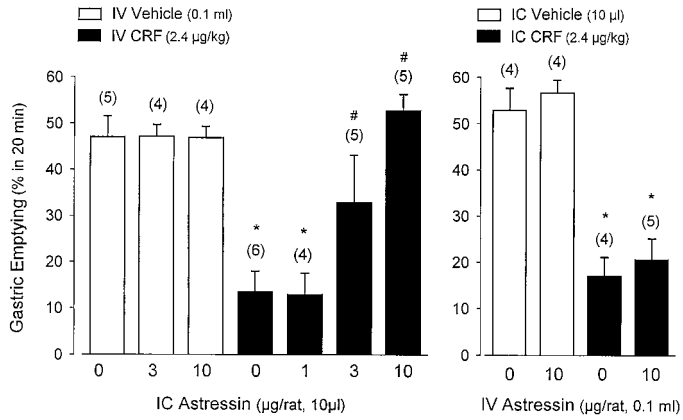

Fig. 2. Effect of i.c. and i.v. injection of astressin on CRF-induced inhibition of GE in conscious rats. Rats, under enflurane anesthesia, were injected i.c. with water or astressin followed by the i.v. injection of saline or CRF. In a separate experiment, water or the highest effective dose of astressin blocking i.v. CRF inhibitory effect was injected i.v. immediately before the i.c. injection of either saline or CRF. The GE of a noncaloric viscous meal was determined during the 10- to 30-min period after peptide administration. Each column represents the mean ± S.E. of the number of animals shown at the top. $^{*}P < .05$ versus vehicle controls; $^{\#}P < .05$ versus i.c. saline + i.v. CRF.

+ i.c. CRF: 22.4 ± 5.3%, $n = 2$; i.v. D-Phe CRF_{12-41} + i.c. CRF: 30.0 ± 1.0%, $n = 4$; $F_{2,6} = 13.351$, $P = .006$].

Influence of i.c. Astressin on i.v. CRF-Induced Inhibition of GE. The i.c. injection of water (10 μl) immediately followed by the i.v. injection of CRF (0.6 μg) reduced gastric transit of the non-nutrient meal to 13.6 ± 4.4% ($n = 6$) compared with 47.1 ± 4.4% ($n = 5$) in i.c. water plus i.v. saline-treated animals ($P < .01$; $F_{6,26} = 9.552$; Fig. 2). Astressin (1, 3, and 10 μg) injected i.c. dose-dependently inhibited i.v. CRF-induced delayed GE. The lower i.c. dose of astressin did not modify i.v. CRF inhibitory action on GE (13.0 ± 4.6%, $n = 4$; $P > .05$ versus i.c. water + i.v. CRF) whereas i.c. astressin at 3 and 10 μg resulted in a partial (32.9 ± 10.0%, $n = 5$) and complete (52.8 ± 3.5%, $n = 5$) normalization of GE values in i.v. CRF-injected rats (Fig. 2).

Influence of i.v. Astressin and D-Phe CRF_{12-41} on Abdominal Surgery-Induced Inhibition of GE. In animals maintained for 10 min under enflurane anesthesia and injected i.v. with water, the GE of the non-nutrient meal reached 40.3 ± 4.3% ($n = 5$) during the 10- to 30-min period postanesthesia (Fig. 3). Abdominal surgery (laparotomy and 1-min cecal manipulation) performed under 10 min of enflurane anesthesia reduced gastric transit to 7.1 ± 3.1% ($n = 5$) as determined during the 10- to 30-min period after anesthesia plus surgery (Fig. 3). Astressin (1, 3, and 10 μg i.v.) injected before the surgery dose-dependently reversed the inhibitory effect of abdominal surgery (GE values were 21.7 ± 1.9, 34.8 ± 4.2, and 30.2 ± 2.9%, respectively; $n = 4$ to 5 per group, $P < .05$ versus water + surgery; Fig. 3).

In rats maintained under enflurane anesthesia during 10 min and injected i.v. with water, the GE assessed during the 160- to 180-min period postanesthesia was 59.5 ± 2.9% ($n = 10$) in rats. Abdominal surgery (laparotomy and 1-min cecal manipulation) performed under similar duration of anesthesia reduced gastric transit to 27.5 ± 3.3% as assessed during the 160- to 180-min period anesthesia plus postsurgery [$n = 11$, $P < .01$ versus enflurane alone or astressin alone; ANOVA: $F_{6,35} = 13.019$, $P < .001$; Fig. 4]. Astressin injected i.v. at 1 μg partially prevented and, at 3 and 10 μg, completely normalized abdominal surgery-induced delayed gastric transit (GE values were 44.1 ± 5.4%, $n = 5$; 58.0 ± 1.2%, $n = 5$; and 54.1 ± 4.6%, $n = 4$, respectively, $P < .001$ versus water + surgery; Fig. 4). D-Phe CRF_{12-41} (1 or 3 μg i.v.) did not modify abdominal surgery-induced inhibition of GE (19.8 ± 4.3%, $n = 3$, and 24.3 ± 1.1%, $n = 5$, respectively). Whereas at 10 and 20 μg, the CRF antagonist partially suppressed the inhibitory effect of surgery, GE values were increased to 47.6 ± 2.0 and 46.7 ± 1.7%, respectively ($n = 4$ for each dose; $P < .01$ versus water + surgery; $P < .05$ versus i.v. water + anesthesia; Fig. 4). Astressin (12 or 40 μg i.v.) or D-Phe CRF_{12-41} (20 μg i.v.) alone did not influence significantly basal GE monitored during the 160- to 180-min period after i.v. injection (58.1 ± 3.7%, $n = 4$, and 53.2 ± 8.0% and 47.1 ± 2.4%, $n = 3$, respectively; $P > .05$ versus i.v. water).

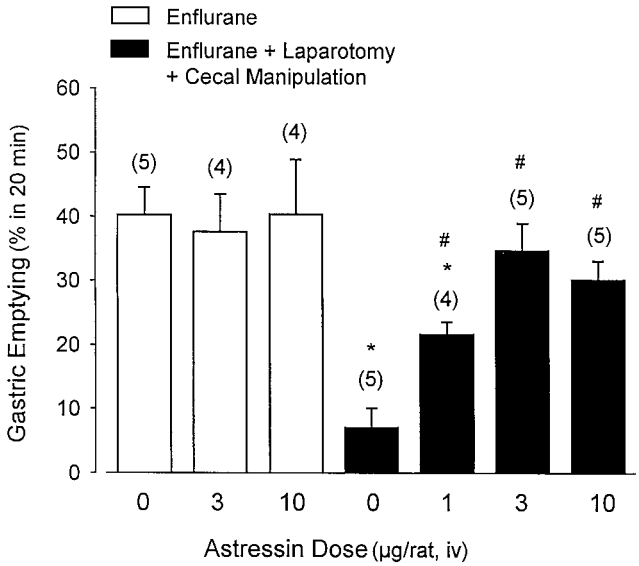

Fig. 3. Intravenous injection of the CRF antagonist astressin dose-dependently blocked the inhibition of GE observed at 30 min after laparotomy and 1-min cecal manipulation in conscious rats. Rats, under enflurane anesthesia for 10 min, were injected i.v. either with water or astressin immediately before the surgical procedure. The GE was determined during the 10- to 30-min period after surgery. Each column represents the mean ± S.E. of the number of rats shown at the top. *$P < .05$ versus all other experimental groups (ANOVA: $F_{6,25} = 7.24$, $P < .0001$). #$P < .05$ versus water + abdominal surgery.

Discussion

CRF injected i.v. inhibited GE of a noncaloric meal as reported previously using peripheral (i.v., i.p., or s.c.) administration in rats, mice, or dogs (Pappas et al., 1985; Williams et al., 1987; Lenz et al., 1988a,b; Broccardo and Improta, 1990; Raybould et al., 1990; Barquist et al., 1992). Astressin injected i.v. at 1, 3, and 10 μg antagonized CRF action by 53, 87, and 100%, respectively, whereas D-Phe CRF_{12-41} at 10 or 20 μg partially reversed CRF, and lower doses had no effect. Previously, i.v. α-helical CRF_{9-41} at 50 μg completely blocked i.v. CRF-induced delay GE under the same conditions (Barquist et al., 1992). These findings show a greater efficacy of astressin to block the biological action of systemic injection of CRF on visceral function. In vivo, the antagonist action of astressin and D-Phe CRF_{12-41} injected peripherally has been explored only in regard to the blockade of CRF-dependent increase in pituitary ACTH release in which astressin was found to be >10-fold more potent than any other CRF receptor antagonists (Hernandez et al., 1993; Gulyas et al., 1995; Rivier et al., 1996; Turnbull and Rivier, 1997; Perrin et al., 1999).

CRF injected i.c. or i.v. in vehicle-treated rats resulted in a similar 68 to 71% inhibition of GE. These results corroborate previous reports showing that CRF inhibits gastric transit with a similar potency when delivered into the circulation or the CSF in rats (Taché et al., 1987; Williams et al., 1987). Although an exchange of peptides across the blood-brain barrier exists (Banks and Kastin, 1996), the absence of transport of CRF from the peripheral circulation into the brain has been established (Martins et al., 1996, 1997). In addition, astressin injected i.v. at a dose that completely blocked the action of CRF injected i.v. did not influence i.c. CRF-induced gastric stasis. Therefore, circulating astressin does not seem to enter the brain after i.v. administration and would not be able to antagonize a direct central action of CRF at the doses tested. Taken together, these results indicate that the inhibition of GE induced by i.c. injection of CRF at 0.6 μg is centrally mediated and not related to a peripheral action after leakage into the circulation. Likewise, CRF antibody or α-helical CRF_{9-41} injected peripherally, at doses blocking

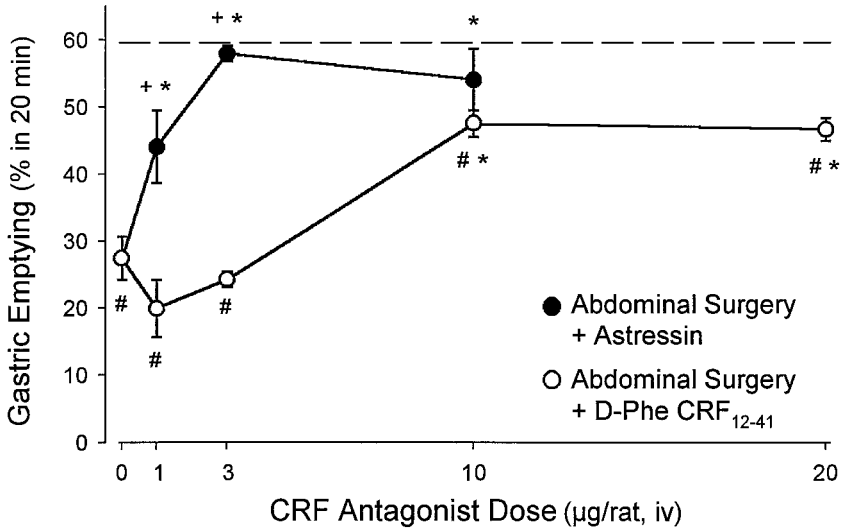

Fig. 4. Intravenous injection of the CRF antagonists D-Phe CRF_{12-41} and astressin dose-dependently reversed the inhibition of GE observed 180 min after laparotomy and 1-min cecal manipulation in conscious rats. Rats, under enflurane anesthesia for 10 min, were injected i.v. either with water, astressin, or D-Phe CRF_{12-41} immediately before the surgical procedure. The GE was determined during the 160- to 180-min period after surgery. Data represent mean ± S.E. of 3 to 11 animals per dose. $^{*}P < .05$ versus i.v. water + surgery group (CRF antagonist dose of 0); $^{+}P < .05$ versus i.v. D-Phe CRF_{12-41}; $^{\#}P < .05$ versus the i.v. water + anesthesia alone (broken line: 59.5 ± 2.9%).

peripherally administered CRF-induced delayed gastric transit, did not influence the inhibitory action of CRF injected into the CSF in rats or mice (Taché et al., 1987; Lenz et al., 1988b; Sheldon et al., 1990; Riviere et al., 1994).

By contrast, astressin injected i.c. at 3 and 10 μg was able to block i.v. CRF-induced inhibition of GE by 58 and 100%, respectively, whereas at 1 μg, it had no effect. We reported previously that astressin injected i.c. at 1, 3, and 10 μg antagonized by 33, 100, and 100% i.c. CRF-induced inhibition of GE tested under the same conditions (Martinez et al., 1997). The mechanisms through which i.c. injected astressin antagonizes peripheral CRF remain to be elucidated in relation to active transport and/or leakage from the brain to the periphery as established for CRF or other peptides (Banks and Kastin, 1996; Martins et al., 1997). Irrespective of the mechanism, these data indicate that astressin injected i.c. can antagonize CRF action on gastric motor function at both central and peripheral sites, particularly when i.c. doses ≥3 μg of peptide are used.

In experimental animals and humans, surgical stress is known to induce gastric stasis (Taché et al., 1991; Resnick et al., 1997) and to increase CRF release in the brain and the circulation (Giuffre et al., 1988; Naito et al., 1991; Bonaz and Taché, 1994). Abdominal surgery and cecal manipulation inhibited GE by 82 and 53% compared with anesthesia alone at 30 and 180 min, respectively, after the end of surgery, in agreement with our previous reports in rats (Taché et al., 1991; Barquist et al., 1992, 1996; Martinez et al., 1997). Astressin injected i.v. at a low dose (3 μg) completely blocked abdominal surgery-induced delayed GE either at 30 or 180 min. The use of i.v. astressin shows that peripheral CRF receptors play a primary role in the postoperative gastric ileus. By contrast, D-Phe CRF_{12-41} injected i.v. at 3.3- or 6.6-fold-higher doses than astressin resulted in partial (63–60%) blockade. In a previous study, we showed that α-helical CRF_{9-41} injected i.v. at 50 μg also result in a 60% inhibition of postoperative ileus under the same conditions (Barquist et al., 1992). These data indicate that astressin is a more potent antagonist of both endogenous and exogenous CRF action on gastric motor function than previously developed CRF receptor antagonists. The high potency of astressin, compared with that of the previous generation of CRF antagonists, may be related to its very low intrinsic activity and/or binding affinity to the CRF-binding protein; metabolic stability leads to an increased duration of action (Gulyas et al., 1995; Miranda et al., 1997; Perrin et al., 1999). The antagonistic effect of astressin injected i.v. was assessed previously mainly in relation with endogenous CRF-dependent stimulation of ACTH release induced by adrenalectomy, electroshock, ethanol, or lipopolysaccharide, although higher i.v. doses were required to exert a antagonist action over a 90-min period (Gulyas et al., 1995; Rivier et al., 1996; Aubry et al., 1997).

Astressin injected into the cisterna magna at a dose range similar to that injected i.v. in the present study also reverted abdominal surgery-induced gastric ileus assessed after 180 min (Martinez et al., 1997). These results indicate that CRF receptor activation is involved at both peripheral and central sites to induce postoperative gastric ileus. However, whether the complete prevention of postoperative gastric ileus induced by astressin injected i.c. at 3 and 10 μg (Martinez et al., 1997) results solely from a central action is difficult to ascertain based on the present demonstration that at such i.c. doses, astressin antagonized peripheral CRF as well. The delayed GE induced by stressors of immunological (i.v. interleukin 1β; Sütö et al., 1996), psychological (partial restraint; Williams et al., 1987; Lenz et al., 1988b), physical (swim; Coşkun et al., 1997), chemical (ether; Taché et al., 1991), or nociceptive (i.p. injection of acetic acid; Riviere et al., 1994) nature was prevented by CSF, unlike peripheral injection of α-helical CRF_{9-41} in rats (Williams et al., 1987; Lenz et al., 1988b; Riviere et al., 1994; Sütö et al., 1996). Whether peripheral CRF receptors are selectively recruited by abdominal surgery/visceral manipulation compared with other stressors needs to be further assessed.

In summary, the newly developed CRF antagonist, astressin, injected i.v. blocked i.v. CRF-induced inhibition of GE at a low (3:0.6 μg/rat) antagonist/agonist dose ratio and displayed higher potency than D-Phe CRF_{12-41} in conscious rats. The lack of reversal of i.c. CRF-induced delay GE by i.v. astressin indicates that i.c. CRF inhibitory action is centrally mediated and that i.v. astressin antagonist action is exerted at the periphery. By contrast, astressin injected i.c. exerts a dual central and peripheral CRF antagonist action that

needs to be taken into consideration when i.c. doses ≥3 μg of astressin are used. In addition, the blockade of abdominal surgery-induced delayed GE by i.v. astressin indicates that peripheral CRF receptors play an important role in acute postoperative gastric ileus. Taken together, these data show that astressin is a valuable new tool to assess the role of peripheral CRF in the GI motor response to somatovisceral stress.

Acknowledgments

We thank Paul Kirsch for his help in the preparation of the manuscript.

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