Central Injection of a New Corticotropin-Releasing Factor (CRF) Antagonist, Astressin, Blocks CRF- and Stress-Related Alterations of Gastric and Colonic Motor Function

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ABSTRACT

The influence of central injection of a new corticotropin releasing factor (CRF) antagonist, astressin, (cyclo(30–33)[D-Phe18,21,28, Glu30, Lys33,36,38]r/hCRF12–41), on exogenous and endogenous CRF-induced gastric ileus and stimulation of bowel discharges was investigated in conscious rats. Intracisternal (ic) CRF (0.6 μg) reduced gastric emptying of a noncalcitic solution to 17.1 ± 4.9% compared with 50.1 ± 4.6% in control group injected ic with vehicle. Astressin (1, 3 and 10 μg, ic) dose dependently prevented ic CRF-induced delayed gastric emptying by 33, 100 and 100%, respectively, and had no effect on basal gastric emptying. Abdominal surgery with cecal manipulation (1 min) reduced gastric emptying to 19.8 ± 5.5% 3 hr postsurgery compared with 59.9 ± 5.2% after anesthesia alone plus ic vehicle. Astressin (1, 3 and 10 μg, ic) prevented postoperative gastric ileus by 56, 93 and 92%, respectively. Intracerebroventricular CRF (0.6 μg) and water-avoidance stress stimulated pellet output (number/60 min) to 5 ± 1 and 11 ± 2, respectively, compared with no fecal pellet output after icv vehicle and no exposure to stress. Astressin (3 and 10 μg, icv) blocked exogenous CRF action by 47 and 63%, respectively, and colonic response to stress by 0 and 54%, respectively. These data indicate that astressin injected into the CSF at low doses (1–10 μg) has an antagonistic action against CRF and stress-related alterations of gastrointestinal motor function, without an intrinsic effect in these in vivo systems. Astressin may be a useful tool to explore functional CRF-dependent physiological pathways in specific brain nuclei.

CRF in the brain is recognized as a key mediator in a wide range of behavioral, neuroendocrine, autonomic, immunologic and visceral responses to stress (Dunn and Berridge, 1990; Morimoto et al., 1993; Owens and Nemeroif, 1991; Swanson et al., 1986). In particular, convergent reports in rodents indicate that CRF acts within the central nervous system to elicit similar changes in GI motor functions as those induced by stress (Taché et al., 1993). Namely, central administration of CRF inhibits gastric motility and emptying, and accelerates colonic motility and transit and fecal pellet output in rodents (Heymann-Monnikes et al., 1991; Lenz et al., 1988a; Martinez and Bueno, 1991; Monnikes et al., 1992, 1993, a and b, 1994; Sheldon et al., 1990; Taché et al., 1987). These effects are mediated through the interaction with CRF receptors, because central administration of specific CRF antagonists blocked CRF action (Gué et al., 1991; Lenz et al., 1988b; Sutó et al., 1994; Taché et al., 1991; Williams et al., 1987). Similarly, a variety of psychological (aversive stimuli), physical (surgery, restraint), immunological (interleukin-1β) or chemical (anesthesia) stressors inhibit gastric motor function and/or accelerate colonic transit and these effects can be blunted or blocked by the central administration of CRF antagonists (Bonaz and Taché, 1994; Gué et al., 1991; Fargeas et al., 1993; Hernandez et al., 1993; Lenz et al., 1988b; Monnikes et al., 1992, 1993, a and b; Sutó et al., 1994; Taché et al., 1991; Williams et al., 1987). Therefore the availability of specific and potent CRF antagonists provide valuable tools to elucidate the role of endogenous brain CRF in stress-related alterations of GI motor functions.

To date, two generations of CRF analogs with antagonist activity have been developed, α-helical CRF9–41.

ABBREVIATIONS: ACTH, adrenocorticotropic hormone; ANOVA, analysis of variance; CSF, cerebrospinal fluid; CRF, corticotropin-releasing factor; r/hCRF, rat/human corticotropin-releasing factor; ic, intracisternal; icv, intracerebroventricular; α-helical CRF9–41, [Met18,Lys36,Glu27,29,40, Ala32,41, Leu33,36,38]r/hCRF9–41; D-Phe18,21,28, Glu30, Lys33,36,38]r/hCRF9–41; D-Phe18,21,28, Glu30, Lys33,36,38]r/hCRF9–41; D-Phe18,21,28, Glu30, Lys33,36,38]r/hCRF12–41; [D-Phe12, Nle21,38, C “MeLeu25]CRF12–41; GI, gastrointestinal; PVN, paraventricular nucleus of the hypothalamus.
[Met^{18}, Lys^{23}, Glu^{27,29,40}, Ala^{32,41}], r/hCRF_{12-41}; and more recently, the constrained D-Phe CRF_{12-41} analog, [D-Phe^{12}, Nle^{21,38}, C"MeLeu^{37}]r/hCRF_{12-41} (Fisher et al., 1991; Gulyas et al., 1995; Hernandez et al., 1993; Menzaghi et al., 1994). These peptides, particularly \( \alpha \)-helical CRF_{12-41}, have been used extensively \textit{in vivo} to assess the physiological role of CRF in endocrine, autonomic and behavioral responses to various stressors as well as stress-related changes in GI functions (Brown et al., 1986; Fisher et al., 1991; Hernandez et al., 1993; Menzaghi et al., 1994; Rivier et al., 1984; Taché et al., 1993; Tazi et al., 1987). However, they display some limitations due to their poor solubility, persistence of intrinsic activity, and weak potency at the hypophysyal site of action (Fisher et al., 1991; Gulyas et al., 1995; Menzaghi et al., 1994). Recently, further search in achieving conformational stability of CRF antagonists resulted in the development of astressin, cycl[30–33][D-Met^{18}, Lys^{33}, 36, 38]r/hCRF_{12-41} (Fisher et al., 1991). Astressin’s main characteristics are its low intrinsic activity, high solubility in aqueous solutions, increased metabolic stability and high affinity to both CRF\(_1\) and CRF\(_2\) receptor subtypes (Gulyas et al., 1995; Perrin et al., 1995). A recent report indicates that astressin displays about 32% and 100% higher potency compared with D-Phe CRF_{12-41} and \( \alpha \)-helical CRF_{12-41}, respectively, to inhibit ACTH secretion from pituitary cell culture \textit{in vitro} (Gulyas et al., 1995). Moreover, the same study shows that after peripheral administration in rats, astressin is >10 times more potent than any other CRF antagonists reported to date to inhibit stress-induced increase in ACTH plasma levels (Gulyas et al., 1995).

The antagonistic activity of astressin at a central level, on stress-related alterations of the GI function has not been determined. Therefore, our study was designed to characterize the ability of the new CRF antagonist, astressin, injected into the CSF to block established changes in gastric emptying and bowel discharges induced by CSF injection of CRF and physical (surgery) or psychological (water avoidance) stress.

In addition, possible intrinsic activity of CSF injection of astressin on these parameters was also tested.

**Materials and Methods**

**Animals**

Adult male Sprague-Dawley rats (Harlan, San Diego, CA) weighing 250 to 280 g were maintained at a 12:12-hr light-dark-cycle, under conditions of controlled temperature (21–23°C). Animals were housed in group cages with food (Purina Rat Chow, St. Louis, MO) and tap water \textit{ad libitum}. Experiments on gastric emptying were performed in 18 to 20 hr fasted rats with free access to water up to the beginning of treatments. In studies on colonic discharges, rats were not deprived of food and water before the treatments.

**Peptides**

Rat/human CRF and astressin were synthesized using the solid-phase approach and the tert-butoxycarbonyl strategy and were purified as previously described (Gulyas et al., 1995). Peptides were kept in powder form at -70°C and dissolved immediately before the experiments. CRF was dissolved in sterile saline and astressin in double-distilled water (adjusted to pH 7.0, warmed to 37°C).

**Measurement of Gastric Emptying**

Gastric emptying was determined by the phenol red method as previously described (Barquist et al., 1996; Taché et al., 1987). A suspension of continuously stirred 1.5% methyl cellulose (Sigma Chemical Co., St Louis, MO) and phenol red (0.5%, Sigma) was given intragastrically (1.5 ml) to conscious rats. After a 20-min period, rats were euthanized by CO\(_2\) inhalation. The abdominal cavity was opened, and the gastroesophageal junction and the pylorus clamped, then the stomach was removed, rinsed in 0.9% saline and placed in 100 ml of 0.1 N NaOH and homogenized (Polytron, Brinkmann Instruments, Westbury, NY). The suspension was allowed to settle for 1 hr at room temperature and 5 ml of the supernatant were added to 0.5 ml of 20% trichloroacetic acid (w/v) 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 read at 560 nm (Shimazu 260 Spectrophotometer, Cole Scientific Inc., Moopark, CA). Phenol red recovered from animals euthanized immediately after the administration of the methyl-cellulose solution was used as standards (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.

**CSF Injections**

**Intracerebroventricular.** Animals were anesthetized with a mixture of ketamine hydrochloride (75 mg/kg, i.p.; Ketaset, Fort Dodge Laboratories Inc., Fort Dodge, IA) and xylazine (5 mg/kg, i.p.; Rompun, Mobay Corporation, Shawnee, KS). A chronic guide cannula (22 ga, Plastic One Products, Roanoke, VA) was implanted into the right lateral brain ventricle according to coordinates obtained from Paxinos and Watson (Paxinos and Watson, 1986) (mm from bregma: antero-posterior, -0.8; lateral, -1.5; dorsoventral, -3.5). The guide cannula was maintained in place by dental cement anchored by four stainless steel jewelry screws fixed to the skull. After the surgery, animals were housed individually with direct bedding. Experiments were performed in conscious, nonfasted animals, starting 7 days after the icv cannulation. During this time, rats were habituated to the manipulation of the cannula and were handled daily for a period of about 5 min. Intracerebroventricular injections were performed in conscious lightly restrained animals. A 28 ga cannula, 1 mm longer than the guide cannula, was connected to a 50 \( \mu \)l Hamilton syringe by a PE-50 catheter (Intramedic Polyethylene Tubing, Clay Adams, Sparks, MD) filled with distilled water. A small air bubble (1 \( \mu \)l) was drawn at the distal end of the PE-50 catheter to separate the injected solution from the water and for visual inspection of the icv injection, performed slowly over a 30- to 45-sec period. At the end of the experiments, animals were euthanized by CO\(_2\) inhalation, followed by decapitation and the correct location of the cannula into the lateral ventricle was verified by injecting 10 \( \mu \)l of dye (0.1% toluidine blue). Visualization of dye on the wall of the lateral ventricle indicated correct icv injections.

**Intracisternal.** Intracisternal injections were performed acutely under short enflurane anesthesia (2–3 min) by puncture of the occipital membrane with the needle of a 50-\( \mu \)l Hamilton syringe, in rats placed in ear bars of stereotaxic equipment. The presence of CSF in the Hamilton syringe upon aspiration before injection insured correctness of needle placement into the cisterna magna.

**Experimental Procedures**

**Intracisternal CRF-induced inhibition of gastric emptying.** Under short enflurane anesthesia either astressin (1, 3 or 10 \( \mu \)g/rat in 5 \( \mu \)l) or vehicle (5 \( \mu \)l) was injected ic immediately before the ic injection of either CRF (0.6 \( \mu \)g/rat in 5 \( \mu \)l) or vehicle (5 \( \mu \)l) in fasted rats. The ic dose of CRF was selected to produce a 60 to 70% inhibition of gastric emptying when dose response studies were performed under the same experimental conditions (Taché et al., 1987). After ic injections, animals were returned to their home cages and 10 min later, the marker used for the measurement of gastric emptying of noncaloric liquid meal was administered per orogastric gavage in awake rats, lightly restrained. The rate of gastric emptying was determined 20 min after the administration of the marker.
Abdominal surgery-induced inhibition of gastric emptying. Under 10-min exposure of enflurane anesthesia (5.5% vapor concentration in O2; Ethrane-Anaquest, Madison, WI) either vehicle (10 µl) or astressin (1, 3 or 10 µg/rat in 10 µl) was injected ic and abdominal surgery with cecal manipulation was performed as previously described (Barquist et al., 1996; Hernandez et al., 1993). Abdominal surgery consisted of a medial celiotomy (3–4 cm) and cecal exteriorization with cecal handling for a period of 1 min in gauze soaked with saline. The cecum was then returned to the abdominal cavity. The linea alba and the skin were closed separately with 3–0 silk sutures. The 20-min rate of gastric emptying was measured at 160 min after the completion of abdominal surgery.

Intracerebroventricular CRF-induced fecal pellet output. Either vehicle (5 µl) or astressin (3, 10 µg/rat in 5 µl) was injected ic in conscious, fed rats with chronic icv cannula. The animals were returned to their home cages and 10 min later a second ic injection of either vehicle (5 µl) or CRF (0.6 µg/rat in 5 µl) was performed. Animals were returned to their home cages and the number of pellets excreted during the next 60 min was counted at 15 min intervals. The animals were used four to five times, allowing a period of at least 4 days between consecutive experiments. None of the animals received the same treatment more than once.

Psychological stress-induced fecal pellet output. Rats implanted with chronic icv cannulae were injected with either vehicle (10 µl) or astressin (3, 10 µg/rat in 10 µl) and returned to their home cages. Ten min later, animals were subjected to a session of water-avoidance stress for a period of 60 min, as previously described (Bonaz and Taché, 1994; Mönikes et al., 1993b). Rats were put individually on a plastic platform (8 × 6 × 6 cm) placed in the middle of a home cage filled with water up to 7 cm of the height of the platform. To avoid direct contact with the water, rats stand on the platform for the 1-hr experimental period. Control animals received the same icv injection but were maintained in a similar cage without water for 1 hr. Fecal pellet output was counted every 15 min for 1 hr. Due to the habituation of animals to the stress stimuli, they were exposed only twice to the water avoidance stress at a 4- to 5-day interval between the two sessions of stress.

Statistical Analysis
Results are expressed as mean ± S.E. Comparisons between groups were performed using one-way ANOVA followed by a Student-Newman-Keuls multiple-comparison test. P < .05 were considered statistically significant.

Results
Intracisternal CRF-induced inhibition of gastric emptying. In rats receiving ic injections twice with vehicles, the 20-min rate of gastric emptying of a noncaloric solution was 50.1 ± 4.6% (n = 11). The ic injection of vehicle followed by CRF (0.6 µg/rat) inhibited the rate of gastric emptying by 66% compared with the control group [17.1 ± 4.9%, n = 7, P < .01 vs. vehicle + vehicle group; ANOVA: F(6,36) = 4.113, P = .003] (fig. 1). The ic injection of the CRF antagonist, astressin (1 or 3 µg/rat) immediately before the injection of CRF dose-dependently prevented gastric stasis induced by ic CRF (the rate of gastric emptying was 28.1 ± 9.2%, n = 5, and 51.1 ± 10.3%, n = 6, respectively). The dose 10 µg ic produced similar full reversal of CRF inhibitory action as 3 µg (48.9 ± 8.1%, n = 5) (fig. 1). The antagonist by itself, either at doses 3 or 10 µg/rat injected ic, had no significant effect on basal gastric emptying (45.6 ± 3.4%, n = 5 and 45.8 ± 4.8%, n = 4, respectively).

Abdominal surgery-induced inhibition of gastric emptying. In rats maintained under enflurane anesthesia during 10 min and receiving ic injections with saline, the rate of gastric emptying at 3 hr post-anesthesia was 59.0 ± 5.2% (n = 5). Astressin injected ic at the dose of 3 µg/rat (n = 4) or 10 µg/rat (n = 3) did not influence basal gastric emptying (3 hr later) 54.5 ± 4.4 and 50.0 ± 2.7, respectively. Laparotomy and 1-min cecal manipulation performed under 10 min of enflurane anesthesia in rats receiving ic injections with vehicle reduced the rate of gastric emptying to 19.8 ± 5.5% at 3 hr postsurgery [n = 6, P < .01 vs ic vehicle plus enflurane alone; ANOVA: F(5,26) = 11.077, P < .0001] (fig. 2). Astressin injected ic at 1 and 3 µg/rat dose-dependently prevented abdominal surgery-induced gastric ileus (rate of gastric emptying was 34.1 ± 6.9%, n = 4, P > .05, and 50.2 ± 3.2%, n = 6, P < .001 vs. vehicle + surgery, respectively) (fig. 2). Astressin injected ic at 10 µg/rat had a similar reversal effect as 3 µg (46.1 ± 2.4%, n = 4, P < .01 vs. vehicle + surgery) (fig. 2).

Intracerebroventricular CRF-induced fecal pellet output. In rats injected with vehicles, no pellet output was observed during the next 60 min (n = 6). Astressin injected ic either at the dose of 3 or 10 µg did not increase pellet output during the same period (0 ± 0 and 0 ± 0 pellets/60 min respectively, n = 6 for each dose, P > .05 vs vehicle + vehicle). CRF (0.6 µg/rat) increased the total pellet output (nb/60 min) to 5 ± 1 [n = 6, P < .05 vs. vehicle + vehicle or astressin + vehicle, ANOVA: F(5,32) = 15.151, P < .0001] (fig. 3). Astressin injected ic at the doses of 3 or 10 µg/rat, 10 min before the administration of CRF, dose-dependently inhibited the response to CRF by 46 and 63%, respectively (3 ± 1 and 2 ± 1 respectively, n = 7 for each dose of astressin, P < .05 vs. vehicle + CRF) (fig. 3).

Psychological stress-(water-avoidance) induced fecal pellet output. In animals receiving icv injections with vehicle, water avoidance stress stimulated fecal pellet output (number/60 min, 11 ± 2, n = 6), although in animals, also injected with vehicle, but maintained in nonstressful condi-
tions, no pellet output was observed during the same period of time [number/60 min, 0 ± 0, n = 6, P < .001 vs. vehicle + stress, ANOVA: F(5,29)=32.4, P < .0001] (fig. 4). Astressin (10 μg/rat, icv) blocked water-avoidance stress-induced pellet output by 56% (number/60 min, 5 ± 1, n = 8, P < .01 vs. vehicle + stress), although the dose of 3 μg had no effect (number/60 min, 12 ± 1, n = 3, P > .05 vs. vehicle + stress). In animals not subjected to psychological stress, astressin, by itself, did not stimulate fecal pellet output (fig. 4).

Discussion

In the present study, we investigated the influence of the newly developed CRF antagonist, astressin, (Gulyas et al., 1995) injected into the CSF to antagonize ic/icv CRF- and stress-induced GI motor alterations. The sites of delivery of the peptides ic and icv were based on preferential sites of action of CRF to influence GI motor function, the dorsal vagal complex to inhibit gastric motility (Heymann-Mönnikes et al., 1991) and paraventricular nucleus of the hypothalamus for CRF-induced stimulation of colonic motor function (Mönnikes et al., 1993b). In agreement with the results of previous reports, we showed that CRF injected ic inhibits gastric emptying of a noncaloric liquid solution in rats (Sütó et al., 1994; Taché et al., 1987; 1991; Yoneda et al., 1992). The CRF antagonist, astressin injected ic blocked ic-CRF induced delay of gastric emptying in a dose dependent fashion. At 3 μg/rat, astressin injected ic completely prevented ic CRF-(0.6 μg/rat)-induced 66% inhibition of gastric emptying while at 1 μg/rat, a partial blockade (33%) was observed. Previous dose response studies using ic injection of other CRF antagonists (Hernandez et al., 1993; Rivier et al., 1984) showed that 50 μg of α-helical CRF9–41 and 20 μg of the D-Phe CRF12–41 analog were required to achieve maximal blockade (85% and 87% respectively) of ic CRF (0.6 μg)-induced 60–70% inhibition of gastric emptying of a similar noncaloric solution in rats (Sütó et al., 1994; Taché et al., 1993) (Table 1). Taken together the present and previous results suggest that astressin is more potent than α-helical CRF9–41 and D-Phe CRF12–41 to block ic CRF-induced delayed gastric emptying in conscious rats. However, no direct relative potency calculation can be derived since experiments were performed in different groups of animals and times. Several lines of evidence (Taché et al., 1993) indicate that gastric stasis induced...


TABLE 1

Comparison of central action of various CRF antagonists to block central CRF and stress-related alterations of GI motor function in conscious rats

<table>
<thead>
<tr>
<th>CRF antagonist</th>
<th>Blockade (%)</th>
<th>References</th>
<th>Dose (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Phe CRF&lt;sub&gt;12–41&lt;/sub&gt;</td>
<td>60</td>
<td>Hernandez et al., 1993</td>
<td>50</td>
</tr>
<tr>
<td>Astressin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60–84</td>
<td>Hernandez et al., 1993; Barquist et al., 1996</td>
<td>10</td>
</tr>
<tr>
<td>CRF&lt;sub&gt;12–41&lt;/sub&gt;</td>
<td>95</td>
<td>Current study</td>
<td>3</td>
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<sup>a</sup> Intracerebroventricular injection through a chronically implanted i.c.v. cannula.

Abdominal Surgery-Induced Gastric Ileus

A total of 60 to 70% inhibition of the 20-min rate of gastric emptying of a methyl-cellulose solution containing phenol red as marker, induced by a dose of 0.6 mg/rat of CRF injected i.c.

Psychological Stress-Induced Pellet Output

A total of 60 to 65% inhibition of the 20-min rate of gastric emptying of a methyl-cellulose solution containing phenol red as marker, measured 3 hr after the surgery (laparotomy 1 min cecal manipulation).

CRF-Induced Gastric Ileus

Sixty-min session of water-avoidance stress.

Intracerebroventricular injection through a chronically implanted i.c.v. cannula.

Motor effects including the induction of gastric stasis (Hockmann-Moennikes et al., 1992). In humans, surgical stress is known to have important GI motor effects including the induction of gastric stasis (Holking et al., 1992). In animal models, surgical stress also results in similar gastric motor alterations (Barquist et al., 1996; Dubois et al., 1973). In rats, abdominal surgery, consisting of laparotomy and handling of the cecum under short enflurane anesthesia, causes gastric ileus which persists for several hours. By contrast, 10 min exposure to enflurane alone or with laparotomy produces a significant inhibitory effect only during the first 30 min postsurgery which is no longer observed 3 hr later (Barquist et al., 1992b; 1996; Ruwart et al., 1979). Convergent anatomical and functional data support a role of brain CRF as key neurotransmitter in the coding of neurocircuitry involved in abdominal surgery-induced gastric ileus (Barquist et al., 1996; Bonaz et al., 1994; Tache et al., 1991). Firstly, this surgical procedure induced neuronal activation, as assessed by the presence of c-fos expression in brain nuclei involved in the control of autonomic functions and known to contain CRF immunoreactivity (Barquist et al., 1996; Bonaz et al., 1994; Zittel et al., 1993). In particular, the distribution of activated neurons in the paraventricular nucleus of the hypothalamus (PVN) 3 hr after abdominal surgery is congruent with the visualization of CRF-containing neurons in this area in colchicine pretreated rats (Barquist et al., 1996; Swanson et al., 1986). Moreover, it has been shown that various stressors, as well as abdominal surgery, increase hypothalamic CRF synthesis and release (Giuffre et al., 1988; Harbuz and Lightman, 1989; Plotsky and Vale, 1984). Secondly, we reported previously that abdominal surgery-induced gastric ileus measured at 30 min and 3 hr postoperatively is reverted by the prior ic injection of the CRF antagonists, α-helical CRF<sub>9–41</sub> and D-Phe CRF<sub>12–41</sub> (Barquist et al., 1996; Hernandez et al., 1993; Tache et al., 1991). However, doses of 50 μg/rat of α-helical CRF<sub>9–41</sub> and 20 μg/rat of D-Phe CRF<sub>12–41</sub> injected ic were necessary to produce a maximal 60–84% reversal of the gastric stasis at 3 h after abdominal surgery (Barquist et al., 1996; Hernandez et al., 1993). In the present study, astressin injected ic at 1 and 3 μg/rat reversed the effect of abdominal surgery by 56 and 93%, compared with animals exposed to enflurane anesthesia for 10 min and injected ic with the same dose of the antagonist but without surgery. By contrast, D-Phe CRF<sub>12–41</sub> injected ic at 3 μg was ineffective against abdominal surgery-induced delay of gastric emptying in our previous studies (Barquist et al., 1996). Based on the previous and present results, astressin injected ic appears to be more potent than ic D-Phe CRF<sub>12–41</sub> and α-helical CRF<sub>9–41</sub> at inhibiting postoperative gastric ileus as measured 3 hr after abdominal surgery (Table 1).

Central injection of CRF is well established to stimulate colonic motility, transit and fecal pellet output in conscious rats (Lenz et al., 1988a; Martinez and Buéno, 1991; Mönikes et al., 1992; 1993a; 1993b; 1994; Williams et al., 1987). Functional data suggests that central CRF-induced stimulation of colonic motility and transit are mediated by an action of the peptide in the PVN and/or the locus ceruleus complex (Bonaz et al., 1991).
and Taché, 1994; Mönningen et al., 1992; 1993b; 1994). CRF injected icv stimulates fecal pellet output in fed rats, as previously described (Williams et al., 1987). The CRF antagonist, α-helical CRF$_{9-41}$ injected icv at 50 μg/rat blocked water-avoidance and wrapping restraint stress-induced stimulation of fecal pellet output by 60% while a higher dose (100 μg) did not result in further attenuation of the colonic response (Bonaz and Taché, 1994; Williams et al., 1987) (Table 1). In the present study, astressin injected icv at the doses of 3 and 10 μg/rat blocked icv CRF-induced fecal pellets by 46% and 63% respectively. However, although astressin injected icv at 3 μg/rat was partially efficient in blocking bowel discharges induced by icv CRF, this dose of CRF antagonist did not modify water-avoidance stress-induced bowel discharges. At the icv dose of 10 μg, astressin blocked the response to water avoidance stress by 56%. The lower efficacy of icv astressin in blocking water avoidance stress-compared with icv CRF-induced bowel discharges may be due to a greater stimulatory colonic response induced by stress (11 ± 2 pellets) vs CRF (5 ± 1 pellets) and/or the involvement of multiple pathways in the response to stress, some of which may be unrelated to CRF or related to CRF in brain nuclei not accessible to the antagonist when administered icv.

Astrassin injected ic or icv at 3 or 10 μg exhibits no intrinsic agonist action since the basal rate of gastric emptying was not modified and there was no bowel discharge in fed rats. In addition, these results further strengthen previous evidence that central CRF is not involved in the basal regulation of GI motor function while playing an important role as a mediator of stress-related alterations of GI motor function (Taché et al., 1993).

Previous in vivo studies showed that astressin injected peripherally exhibited a higher duration of action and a 10 fold higher potency than D-Phe CRF$_{12-41}$ to block ACTH release induced by stress or adrenalectomy (Gulyas et al., 1995). Likewise, dose response curve in previous and present reports indicates that lower doses of astressin injected into the CSF are needed compared with D-Phe CRF$_{12-41}$ and α-helical CRF$_{9-41}$ to prevent CRF injected into the CSF- and stress-induced gastric stasis and bowel discharge suggesting a higher potency (Table 1). The enhanced potency of astressin injected into the CSF compared with the previous CRF antagonists is consistent with the higher affinity of the peptide to CRF receptors compared with previous CRF antagonists (Gulyas et al., 1995). In binding assay using cloned human CRF$_1$ receptors subtype, astressin displays a higher binding affinity (2 nM) compared with D-Phe CRF$_{12-41}$ (56 nM) or α-helical CRF$_{9-41}$ (17 nM) and, unlike previous CRF antagonists, astressin does not bind to the CRF binding protein (Gulyas et al., 1995). Preliminary evidence indicates that these peptide antagonists also bind with a similar affinity to the CRF$_2$ receptor subtypes than respectively displayed on CRF$_1$ receptor subtype (Lovenberg et al., 1995; Perrin et al., 1995). Therefore, the receptor subtype specificity involved in the central action of CRF to alter GI motor function cannot be inferred by using astressin or other CRF antagonists. However, sauvagine shows a higher affinity for CRF$_2$ receptor subtype and a similar affinity for CRF$_1$ receptor subtype compared with CRF (Lovenberg et al., 1995). Likewise, sauvagine is more potent than CRF itself to inhibit gastric emptying when injected into the CSF (Barquist et al., 1992a; Improma, 1991) suggesting a possible involvement of CRF$_2$ receptor subtype in CRF- or sauvagine-induced inhibition of gastric emptying.

In summary, the new CRF antagonist, astressin, injected into the CSF at low doses of 1–10 μg blocked ic CRF- and surgical stress-induced inhibition of gastric emptying and blunted icv CRF- and psychological stress-induced bowel discharges in conscious rats. These results further establish an important role of brain CRF in stress-related alterations of GI motor function and show that astressin may be a useful tool to dissect sites of action of CRF in stress-related GI motor disorders.

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