

Differential Actions of Peripheral Corticotropin-Releasing Factor (CRF), Urocortin II, and Urocortin III on Gastric Emptying and Colonic Transit in Mice: Role of CRF Receptor Subtypes 1 and 2

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ABSTRACT

Peripheral CRF inhibits gastric emptying and stimulates colonic motor function in rats. We investigated the role of CRF₁ and CRF₂ receptors in i.p. CRF-induced alterations of gut transit in conscious mice using selective CRF₁ and CRF₂ ligands injected i.p. Gastric emptying 2 h after ingestion of a solid chow meal and colonic transit (time to expel a bead inserted into the distal colon) were determined simultaneously. Rat/human (r/h)CRF, which has CRF₁ > CRF₂ binding affinity, decreased distal colonic transit time at lower doses (6–12 μg/kg) than those inhibiting gastric emptying (20–60 μg/kg). Ovine CRF, a preferential CRF₁ receptor agonist (6–60 μg/kg), reduced significantly the colonic transit time without altering gastric emptying, whereas the selective CRF₂ receptor agonists mouse urocortin II (20–60 μg/kg) and urocortin III (120 μg/kg) inhibited significantly gastric emptying without modifying colonic transit. The CRF₁/CRF₂ receptor antagonist, astressin (30–120 μg/kg),

dose dependently prevented r/hCRF (20 μg/kg)-induced inhibition of gastric emptying and reduction of colonic transit time. The selective CRF₁ receptor antagonists, NBI-27914 (C₁₈H₂₀Cl₄N₄C₇H₈O₃S) and CP-154,526 (butyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]ethylamine) (5–30 mg/kg), dose dependently blocked r/hCRF action on the colon without influencing the gastric response, whereas the CRF₂ receptor antagonist, antisauvagine-30 (30–100 μg/kg), dose dependently abolished r/hCRF-induced delayed gastric emptying and had no effect on colonic response. These data show that i.p. r/hCRF-induced opposite actions on upper and lower gut transit in conscious mice are mediated by different CRF receptor subtypes: the activation of CRF₁ receptors stimulates colonic propulsive activity, whereas activation of CRF₂ receptors inhibits gastric emptying.

The 41-amino acid peptide corticotropin-releasing factor (CRF) was isolated from ovine hypothalamus and structurally characterized in 1981, as a novel hypothalamic releasing factor stimulating the release of pituitary proopiomelanocortin peptides (Vale et al., 1981). Recently, in addition to CRF, three other mammalian CRF-related peptides, namely, urocortin (Ucn), urocortin II (Ucn II) and urocortin III (Ucn III), have been identified in humans and rodents (Donaldson et al., 1996; Vaughan et al., 1996; Lewis et al., 2001; Reyes et al., 2001). Rat urocortin (rUcn) is a 40-amino acid peptide which shares 45% homology with rat/human (r/h)CRF

(Vaughan et al., 1996). Human Ucn II and Ucn III have been discovered by sequence homology to r/hCRF from the Human Genome Database and the mouse orthologs have been cloned (Lewis et al., 2001; Reyes et al., 2001). Mouse (m)Ucn II and mUcn III display 34% and 26% sequence homology with r/hCRF and 42% and 18% identity with rUcn, respectively (Lewis et al., 2001; Reyes et al., 2001).

Two distinct seven-transmembrane domain G_s protein-coupled receptors, designated CRF₁ and CRF₂, each encoded by distinct genes, have been cloned in mammals (Perrin and Vale, 1999). Alternative splicing of the CRF₂ receptor leads to CRF_{2α}, CRF_{2β}, and CRF_{2γ} variants in humans, which differ in their amino terminus, whereas in mice only the CRF_{2β} form has been identified (Perrin and Vale, 1999). Although CRF₁ and CRF₂ receptors have considerable sequence similarity, they display distinct affinities for the four mammalian

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ABBREVIATIONS: CRF, corticotropin-releasing factor; DMSO, dimethyl sulfoxide; oCRF, ovine CRF; r/hCRF, rat/human CRF; Ucn, urocortin; mUcn II, mouse urocortin II; mUcn III, mouse urocortin III; rUcn, rat urocortin.

members of the CRF family characterized so far (Perrin and Vale, 1999; Lewis et al., 2001; Reyes et al., 2001). In vitro studies established that Ucn binds with high affinity to both CRF receptor subtypes, whereas r/hCRF exhibits higher affinity to CRF₁ than to CRF₂ receptors (Perrin and Vale, 1999). In contrast, human and mUcn II and mUcn III bind selectively to CRF₂ receptors, representing potential endogenous ligands for the receptor subtype 2 (Lewis et al., 2001; Reyes et al., 2001). In addition to their distinct pharmacological profiles, CRF₁ and CRF₂ receptors have different patterns of distribution in both the brain (Van Pett et al., 2000) and periphery (Baigent and Lowry, 2000; Muramatsu et al., 2000), indicating that they may subserve different biological functions.

Previous functional studies established that peripherally administered r/hCRF, rUcn, or the nonmammalian CRF-related peptides sauvagine and urotensin-I inhibit gastric emptying in rats, mice, and dogs (Pappas et al., 1985; Asakawa et al., 1999; Nozu et al., 1999; Wang et al., 2001). In contrast, r/hCRF and rUcn injected peripherally exert a stimulatory effect on colonic motor function as shown by the induction of spike burst activity in the proximal colon, acceleration of transit, and induction of defecation in rats (Williams et al., 1987; Maillot et al., 2000). The use of the nonselective CRF₁/CRF₂ receptor antagonists, α -helical CRF₉₋₄₁ and astressin (Gulyas et al., 1995), showed that peripheral r/hCRF-induced delayed gastric emptying in rats and mice and accelerated colonic motor function in rats are CRF receptor-mediated (Williams et al., 1987; Shelton et al., 1990; Martínez et al., 1999; Nozu et al., 1999; Maillot et al., 2000). However, the CRF receptor subtype(s) mediating alterations of gastrocolonic motor activity induced by peripheral CRF remains to be ascertained. We recently reported that the selective CRF₁ receptor antagonist, CP-154,526 (Schulz et al., 1996), injected subcutaneously, prevented i.p. r/hCRF-induced clustered spike burst activity in the proximal colon and defecation in rats, indicative of a CRF₁ receptor-mediated action (Maillot et al., 2000). In contrast, the CRF₁ receptor antagonists antalarmin and NBI-27914 (Schulz et al., 1997; Maciejewski-Lenoir et al., 2000) administered intravenously in rats did not influence intravenous r/hCRF- and rUcn-induced delay of gastric emptying of a liquid meal, suggesting a possible mediation through CRF₂ receptors of the latter response (Nozu et al., 1999).

Up to now, the lack of selective CRF₂ receptor agonists and antagonists has hampered the direct pharmacological assessment of CRF₂ receptors in the actions of CRF. Recently, the highly specific CRF_{2 β} antagonist antisauvagine-30 has been developed (Eckart et al., 2001; Higelin et al., 2001), and the novel endogenous CRF₂-related peptides, Ucn II and Ucn III, have selective in vitro binding affinity to CRF₂ receptors (Hsu and Hsueh, 2001; Lewis et al., 2001; Reyes et al., 2001).

The aim of the present study was to establish the CRF receptor subtypes mediating peripheral r/hCRF-induced opposite actions on the upper and lower gut transit in mice. We first assessed whether r/hCRF and ovine CRF (oCRF), which have preferential binding affinity to the CRF₁ receptor (Behan et al., 1996), and mUcn II and mUcn III, which exhibit selective affinity to the CRF₂ receptor (Lewis et al., 2001; Reyes et al., 2001), exert differential effects on postprandial gastric and colonic transit. In addition, we investigated the blockade of r/hCRF actions by the nonselective CRF₁/CRF₂

receptor antagonist, astressin (Gulyas et al., 1995), the CRF₁ receptor antagonists NBI-27914 and CP-154,526 (Schulz et al., 1997; Maciejewski-Lenoir et al., 2000), and the CRF₂ receptor antagonist antisauvagine-30 (Eckart et al., 2001; Higelin et al., 2001). Gastric and colonic transits were monitored using a method that we developed to measure simultaneously the gastric emptying of a solid meal and the distal colonic propulsion in conscious mice.

Materials and Methods

Animals

Adult male C57BL/6 mice (6–8 weeks of age; Harlan, Indianapolis, IN) were maintained on a 12-h light/dark cycle with controlled temperature (21–23°C) and humidity (30–35%). Animals were group-housed in direct bedding cages with free access to food (Purina Lab Chow) and tap water. Mice were deprived of food for 18 to 20 h, with free access to water before the experiments, which were conducted under the Veteran Affairs Animal Component of the Research Protocol number 99-092-05.

Experimental Compounds and Treatments

The following peptides were used: r/hCRF, oCRF, mUcn II, mUcn III, astressin (cyclo(30–33)-[D-Phe¹²,Nle^{21,38},Glu³⁰,Lys³³]r/hCRF_{12–41}) and antisauvagine-30 ([D-Phe¹¹,His¹²]sauvagine_{1–40}) (Salk Institute, Clayton Foundation Laboratories for Peptide Biology, La Jolla, CA). Peptides were synthesized as previously described (Gulyas et al., 1995; Reyes et al., 2001) and kept in powder form at –80°C. Immediately before use, peptides were weighed and dissolved in sterile saline except astressin and antisauvagine-30, which were dissolved in double-distilled water (pH ~7.6). NBI-27914 (tosylate salt; Neurocrine Biosciences, San Diego, CA) was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 5 mg/ml, and further dilutions were performed in saline solution. A dilution of DMSO in saline [80% DMSO/20% saline (v/v), pH ~6.5] served as the control vehicle. CP-154,526 (hydrochloride salt; Pfizer Inc., Groton, CT) was dissolved to a concentration of 10 mg/ml in a vehicle consisting of saline/cremophor EL/DMSO at a 90:5:5 ratio, as in our previous study (Maillot et al., 2000). A solution of saline/cremophor EL/DMSO at pH adjusted to ~5.1 (similar to pH of CP-154,526 at 30 mg/kg) was used as vehicle control. Intraperitoneal injections were performed in 0.1 ml. In vitro receptor selectivity of the different CRF receptor agonists and antagonists used in this study is indicated in Table 1.

Gastric and Distal Colonic Transit Measurements

Gastric emptying of a solid nutrient meal and distal colonic transit were simultaneously monitored in conscious mice by combining, with minor modifications, two techniques previously described (Yamada and Onoda, 1992; Barrachina et al., 1997). Fasted mice had free access to water and preweighed Purina chow for a 1-h period, then were briefly anesthetized with enflurane (1–2 min; Ethrane-Anaquest, Madison, WI) to insert a single 2-mm glass bead into the distal colon at 2 cm from the anus. Bead insertion was accomplished with a glass rod with a fire-polished end to avoid tissue damage. After bead insertion, mice were placed individually in their home cages without food and water. Mice regained consciousness within a 1- to 2-min period and thereafter showed normal behavior.

Distal colonic transit was determined to the nearest 0.1 min by monitoring the time required for the expulsion of the glass bead (bead latency). The percentage of gastric emptying of the ingested meal was assessed 2 h after the end of food exposure. Mice were euthanized by cervical dislocation followed by thoracotomy. The abdominal cavity was opened, the pylorus and cardia were clamped, and the stomach was removed. The stomach was weighed and opened, and the gastric content was washed out with tap water. The gastric wall was dried and weighed. The amount of food (grams)

TABLE 1
Inhibitory binding constant for CRF, CRF-related peptides, and CRF receptor antagonists used in this study

	K_i^a			References
	CRF ₁	CRF _{2α}	CRF _{2β}	
		<i>nM</i>		
CRF (rat/human)	2	44	30.7	Behan et al., 1996; Perrin et al., 1999
CRF (ovine)	1	184	162.4	Behan et al., 1996
Ucn II (mice)	>100	2.1	0.66	Lewis et al., 2001
Ucn III (mice)	>100	5.0	1.8	Lewis et al., 2001
Astressin	2.0	1.5	1.0	Perrin et al., 1999
Antisauvagine-30	370		0.3	Eckart et al., 2001
NBI-27914	1.9		>10 μ M	Maciejewski-Lenoir et al., 2000
CP-154,526	2.7	>10 μ M ^b		Schulz et al., 1996

^a See original references for experimental conditions.

^b No differentiation between α and β variants.

contained in the stomach was calculated as the difference between the total weight of the stomach with content and the weight of the stomach wall after the content was removed. The solid food ingested by each animal was determined by the difference between the weight of the Purina chow before feeding and the weight of the pellet and spill at the end of the 1-h feeding period. All weight measurements were recorded with an accuracy of two decimal points. The gastric emptying for the 2-h period was calculated according to the equation: % of gastric emptying = (1 - gastric content/food intake) \times 100.

Experimental Protocols

Effects of r/hCRF and CRF-Related Peptides on Gastric and Distal Colonic Transit. At the end of the 1-h feeding period, mice were briefly anesthetized with enflurane, and the bead inserted into the distal colon followed by the i.p. injection of either saline, r/hCRF (2, 6, 12, 20 or 60 μ g/kg; approximately 0.05–1.5 μ g/mouse), oCRF (6, 12, 20 or 60 μ g/kg), mUcn II (6, 12, 20 or 60 μ g/kg) or mUcn III (12, 60 or 120 μ g/kg). Animals were returned to their home cages without food or water and the bead expulsion time was monitored. Gastric emptying of the nutrient solid meal was determined 2 h after peptide or saline administration.

Effects of CRF Receptor Antagonists. Astressin (30, 60 or 120 μ g/kg), antisauvagine-30 (30, 50 or 100 μ g/kg), NBI-27914 (5, 10 or 20 mg/kg), or their respective vehicles were injected i.p. at 50 min and CP-154,526 (10 or 30 mg/kg) and its vehicle at 30 min after the start of the feeding period. At the end of the 1-h feeding period, mice were briefly anesthetized with enflurane for colonic bead insertion followed by the i.p. injection of r/hCRF (20 μ g/kg) or vehicle (0.1 ml). Thereafter, the time for colonic bead expulsion and gastric emptying of the solid meal were determined as described above. In each daily experiment, vehicle control and several peptide doses, with or without antagonists, were included and repeated in multiple days. The doses of the r/hCRF and CRF antagonists were selected based on our previous studies in rats and mice (Nozu et al., 1999; Maillot et al., 2000; Wang et al., 2001) and adjusted according to the results of preliminary data. To avoid circadian variations, all experiments were performed during the morning, finishing no later than 2:00 PM.

Statistical Analysis

Results are expressed as mean \pm S.E. Comparisons within multiple groups were performed using one-way analysis of variance followed by a Student-Newman-Keuls multiple comparison test. *P* values < 0.05 were considered statistically significant. ED₅₀ values for gastric emptying were determined by nonlinear regression to a sigmoidal equation with variable slope (Prism, version 2.0; Graph-Pad Software, San Diego, CA).

Results

Differential Actions of i.p. r/hCRF on Gastric Emptying and Distal Colonic Transit. In nontreated mice,

fasted for 18 to 20 h and re-fed for 1 h, the percentage of ingested food that had emptied from the stomach after 2 h was $43 \pm 7\%$ and the time for colonic bead expulsion was 14.6 ± 2.2 min ($n = 7$). Saline injected i.p. did not influence gastric emptying ($44 \pm 4\%$) or bead latency time (13.7 ± 0.6 min; $n = 7$). The i.p. injection of r/hCRF (2, 6, 12, 20, or 60 μ g/kg, $n = 6$ –12 for each dose) induced a dose-related reduction of gastric emptying to $36 \pm 5\%$, $32 \pm 7\%$, $29 \pm 8\%$, $9 \pm 3\%$ ($P < 0.05$ versus vehicle), and $2 \pm 2\%$ ($P < 0.05$ versus vehicle), respectively (Fig. 1); the ED₅₀ value was 10.7 μ g/kg (95% confidence interval, 5.6–20.4 μ g/kg; $r^2 = 0.989$). Simultaneously, r/hCRF at 2, 6, 12, 20, or 60 μ g/kg reduced the time latency for bead expulsion from the colon to 10.3 ± 1.4 min, 8.8 ± 1.0 min ($P < 0.05$), 7.0 ± 0.9 min ($P < 0.05$), 8.7 ± 0.7 min ($P < 0.05$), and 9.6 ± 1.4 min ($P < 0.05$), respectively (Fig. 1). The r/hCRF dose of 20 μ g/kg, which significantly accelerated distal colonic transit while simultaneously inhibiting gastric emptying of a solid meal, was selected in subsequent studies.

Effects of i.p. CRF-Related Peptides on Gastric Emptying and Distal Colonic Transit. The preferential CRF₁ receptor agonist, oCRF, injected i.p. at 6, 12, 20, or 60 μ g/kg ($n = 4$ –5 for each dose) induced a linear, dose-related, significant 35 to 54% shortening of bead latency expulsion time at doses ranging from 6 to 12 μ g/kg with a plateau of 51 to 55% reduction at doses from 12 to 60 μ g/kg (Fig. 1). In contrast, gastric emptying of the solid meal was not significantly modified at any dose, although there was a trend toward a reduction at the two highest doses (Fig. 1). The selective CRF₂ receptor agonist mUcn II (6, 12, 20, or 60 μ g/kg, i.p.; $n = 5$ for each dose) significantly reduced gastric emptying values to $13 \pm 6\%$, $7 \pm 7\%$, $0 \pm 0\%$, and $0 \pm 0\%$, respectively ($P < 0.05$ versus vehicle), with an ED₅₀ value of 4.1 μ g/kg (95% confidence interval: 2.3–7.1 μ g/kg; $r^2 = 0.998$). However, mUcn II, at all the doses tested, did not modify the time latency for bead expulsion from the distal colon (Fig. 1). The i.p. injection of mUcn III (12, 60, or 120 μ g/kg; $n = 3$ –4 for each dose) did not alter distal colonic transit while inhibiting gastric emptying of the solid meal only at the highest dose ($11 \pm 2\%$; $P < 0.05$ versus i.p. saline).

Effects of i.p. CRF Receptor Antagonists on i.p. r/hCRF Actions. In i.p. water-pretreated mice, r/hCRF (20 μ g/kg, i.p., $n = 7$) inhibited gastric emptying of the solid meal ($7.0 \pm 4.0\%$; $P < 0.05$ versus $42 \pm 6\%$ in water + saline) and shortened the bead latency to 6.7 ± 0.9 min compared with 12.0 ± 0.8 min in the i.p. water + saline group ($n = 7$; $P < 0.05$). Pretreatment with the CRF₁/CRF₂ receptor antago-

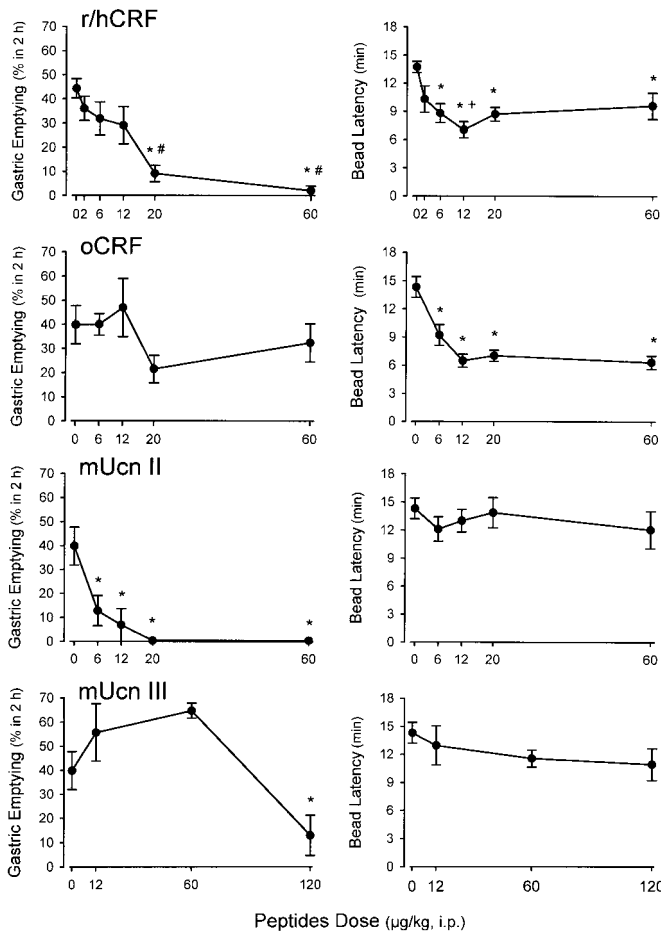


Fig. 1. Dose-related effects of r/hCRF, oCRF, mUcn II, and mUcn III injected i.p. on gastric emptying of a solid nutrient meal (left) and stimulation of distal colonic transit time (right) in conscious mice. Groups of fasted mice were given chow ad libitum for 1 h; then, under short anesthesia they were injected i.p. with either saline or peptide, and a glass bead was inserted into the distal colon 2 cm proximal from the anus. Gastric emptying of the ingested meal 2 h after peptide administration and the time for bead expulsion were monitored in the same animal. Data represent the mean ± S.E. of 6–12, 4–5, 5, and 3–4 mice per group for r/hCRF, oCRF, mUcn II, and mUcn III, respectively. r/hCRF: $F(5,39) = 10.1915$, $P = 0.0001$ for gastric emptying and $F(5,39) = 5.074$, $P = 0.001$ for colonic transit. Ovine CRF: $F(4,22) = 14.967$, $P < 0.01$ for colonic transit. Mouse Ucn II: $F(4,23) = 8.360$, $P < 0.001$ for gastric emptying. Mouse Ucn III: $F(3,14) = 4.96$, $P = 0.015$ for gastric emptying. *, $P < 0.05$ compared with vehicle-treated animals; #, $P < 0.05$ compared with r/hCRF at 2, 6, or 12 μg/kg; +, $P < 0.05$ compared with r/hCRF at 2 μg/kg.

nist, astressin (30, 60, and 120 μg/kg, i.p.) dose dependently prevented i.p. r/hCRF-induced inhibition of gastric emptying and the acceleration of distal colonic transit (Fig. 2). Complete significant blockade of i.p. r/hCRF actions on gastric and distal colonic transit was achieved by astressin at doses of 60 and 120 μg/kg, respectively (Fig. 2).

The selective CRF₂ receptor antagonist antisauvagine-30 (30, 50, and 100 μg/kg) dose dependently prevented the i.p. r/hCRF (20 μg/kg, $n = 6$)-induced inhibition of gastric emptying, whereas the concomitant reduction in the distal colonic transit time was not influenced (Fig. 2). Complete reversal of i.p. r/hCRF-induced inhibition of gastric emptying was observed at 50 μg/kg of antisauvagine-30 (Fig. 2).

Pretreatment with the selective CRF₁ receptor antagonist NBI-27914 (5, 10, or 20 mg/kg, i.p.) dose dependently blocked the r/hCRF-induced stimulation of distal colonic transit with-

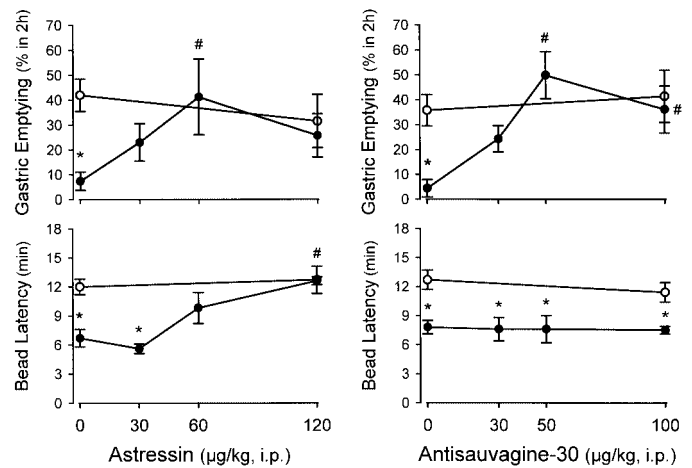


Fig. 2. Dose-related effects of the nonselective CRF₁/CRF₂ receptor antagonist, astressin, and the selective CRF₂ receptor antagonist, antisauvagine-30, on the inhibition of gastric emptying of a solid nutrient meal (top) and stimulation of distal colonic transit time (bottom) induced by peripheral r/hCRF in conscious mice. Protocols are the same as detailed in Fig. 1 except that astressin, antisauvagine-30, and vehicle (water) were injected i.p. 10 min before i.p. injection of r/hCRF or saline. Data represent the mean ± S.E. of five to seven animals per group. *, $P < 0.05$ versus vehicle + saline, astressin + saline, or antisauvagine-30 + saline; #, $P < 0.05$ versus CRF + vehicle. ○, vehicle (0.1 ml, i.p.); ●, r/hCRF (20 μg/kg, i.p.).

out influencing the gastric inhibitory response (Fig. 3). In vehicle (DMSO/saline) + saline-treated mice, bead latency was 13.5 ± 1.6 min ($n = 6$) and was reduced to 7.9 ± 0.8 min in the vehicle + r/hCRF group ($n = 8$; $P < 0.05$ versus vehicle + saline; Fig. 3). NBI-27914, at 10 and 20 mg/kg, dose dependently prevented the r/hCRF effect on the colon by 61% and 100% (bead latency; 11.3 ± 1.5 and 15.1 ± 1.8 min, respectively; $n = 7-8$ for each group; $P < 0.05$ versus vehicle + r/hCRF), whereas a lower dose (5 mg/kg) had no effect (bead latency: 7.6 ± 1.2 min, $n = 4$; $P > 0.05$ versus vehicle + r/hCRF; Fig. 3). In the same animals, r/hCRF-induced delay in gastric emptying ($11 \pm 4\%$; $P < 0.05$ versus vehicle

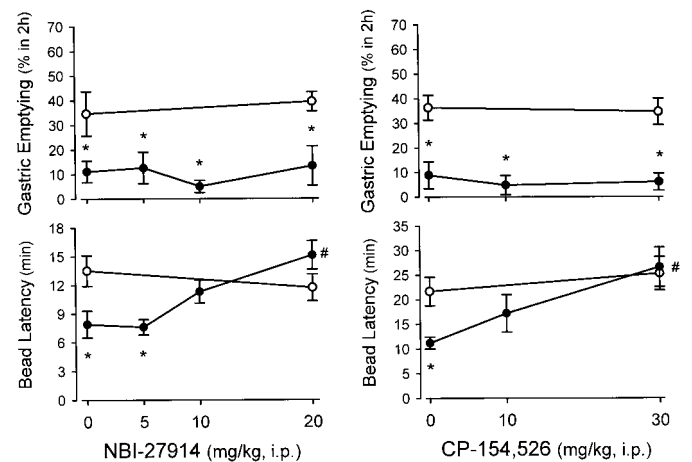


Fig. 3. Effects of the selective CRF₁ receptor antagonists NBI-27914 and CP-154,526 on the inhibition of gastric emptying of a solid nutrient meal (top) and stimulation of distal colonic transit time (bottom) induced by peripheral r/hCRF in conscious mice. Protocols are the same as in Fig. 2 except that CP-154,526 or its vehicle was injected i.p. 30 min before that of i.p. r/hCRF. Data represent the mean ± S.E. of four to eight animals per group. *, $P < 0.05$ versus vehicle + saline, NBI-27914 (20 mg/kg) + vehicle, or CP-154,526 (30 mg/kg) + saline; #, $P < 0.05$ versus vehicle + CRF. ○, vehicle (0.1 ml, i.p.); ●, r/hCRF (20 μg/kg, i.p.).

+ saline, $35 \pm 9\%$) was not influenced by NBI-27914 ($13 \pm 6\%$, $5 \pm 2\%$, and $13 \pm 8\%$, at the doses of 5, 10, and 20 mg/kg, respectively, $n = 4-8$ per group; Fig. 3).

Similar results were obtained with the selective CRF₁ receptor antagonist CP-154,526 (Fig. 3). In vehicle (saline/cremophor EL/DMSO, 90:5:5, pH ~5.1) + saline-treated mice, the bead latency time was 21.7 ± 2.9 min ($n = 7$). In vehicle-pretreated mice, r/hCRF (20 μ g/kg) reduced bead latency to 11.2 ± 1.2 min ($n = 9$; $P < 0.05$ versus vehicle + saline). CP-154,526 at 10 and 30 mg/kg reversed by 43% and 100%, respectively, the effects of r/hCRF on the colonic bead latency time (17.2 ± 3.8 min and 26.6 ± 4.0 min, $n = 6$ and 9; $P < 0.05$ versus vehicle + r/hCRF; Fig. 3). The gastric emptying of a solid meal in vehicle + saline-treated mice was $36 \pm 5\%$. R/hCRF reduced gastric emptying to $9 \pm 5\%$ ($n = 9$; $P < 0.05$ versus vehicle + saline). CP-154,526, either at the dose of 10 or 30 mg/kg, did not modify the r/hCRF-induced delay in gastric emptying ($5 \pm 4\%$ and $6 \pm 3\%$, respectively, $n = 6$ and 9; $P > 0.05$ versus vehicle + saline; Fig. 3). It is interesting to note that the control vehicle (saline/cremophor EL/DMSO, 90:5:5, pH ~5.1) used in this experiment resulted in higher basal values of colonic transit time compared with other vehicle groups (Fig. 3). However, r/hCRF had effects similar to those observed in other experimental conditions.

None of the CRF receptor antagonists, tested by themselves, influenced either the latency time of bead expulsion or the gastric emptying of the solid meal compared with their respective control vehicle-treated groups (Figs. 2 and 3).

Discussion

The present study shows that r/hCRF administered peripherally exerts opposite propulsive effects on the proximal (stomach) and distal (colon) segments of the gastrointestinal tract, which were simultaneously monitored in conscious mice. The i.p. injection of r/hCRF at 2, 6, 12, 20, or 60 μ g/kg resulted in a long-lasting (up to 2 h) dose-dependent 19 to 95% inhibition of gastric emptying of ingested chow and shortened the expulsion time of the bead inserted into the distal colon by 24, 36, 49, 36, and 30%, respectively. Consistent with our observations, a previous report showed that r/hCRF suppressed gastric emptying of a solid meal in mice up to 8 h after i.p. injection, although only a single high dose (400 μ g/kg) was tested in this study (Asakawa et al., 1999). The long-lasting effect of r/hCRF may be related to its half-life, which was estimated to be 151 min from elimination kinetics studies in mice (Martins et al., 1997). Likewise, CRF induces a long-lasting and dose-dependent inhibition of gastric emptying of a non-nutrient liquid solution when administered i.p. in mice (Sheldon et al., 1990) and i.v. in dogs and rats (Pappas et al., 1985; Taché et al., 1987; Williams et al., 1987; Nozu et al., 1999). The present data provide the first evidence in mice that peripheral injection of r/hCRF stimulates colonic transit. In rats, i.p. or i.v. injection of r/hCRF dose dependently accelerates ceco-colonic motility and transit, and induces defecation (Williams et al., 1987; Maillot et al., 2000). Taken together, these findings show convergent evidence across species that peripherally administered r/hCRF exerts an inhibitory effect on gastric transit of either a solid meal or noncaloric solution, while simultaneously stimulating colonic propulsive activity.

The alterations of gastric and colonic transit induced by i.p.

r/hCRF in mice are CRF receptor-mediated. The CRF₁/CRF₂ receptor antagonist astressin completely prevented both the 95% inhibition of gastric emptying of the solid meal and the 50% shortening of the distal colonic transit time induced by r/hCRF (20 μ g/kg) at an antagonist/agonist ratio of 3:1 and 6:1, respectively. In rats, astressin also completely antagonized r/hCRF-induced delayed gastric emptying of a non-nutrient solution and defecation (Martínez et al., 1999; Maillot et al., 2000).

Convergent evidence using selective CRF₂ receptor agonists and antagonists demonstrates that i.p. r/hCRF-induced delayed gastric emptying of a solid meal in mice is CRF₂ receptor-mediated. Mouse Ucn II and mUcn III injected i.p. inhibited gastric emptying of a solid meal, providing the first evidence that these selective CRF₂ receptor agonists are biologically active upon peripheral administration in mice. We found that mUcn II is more potent than mUcn III as shown by the 83% inhibition of gastric emptying at 12 μ g/kg, whereas a 10-fold higher dose is required for mUcn III to have a similar effect. The potency of mUcn II may have a bearing on the higher binding affinities and functional activity on CRF₂ receptors as shown in vitro on stably transfected cells (Lewis et al., 2001; Table 1). However, additional factors related to intrinsic pharmacodynamic and chemical properties of these peptides, as recently reported for r/hCRF, oCRF, and rUcn (Brauns et al., 2001), may also contribute to the observed differences in potencies. Consistent with the primary role of CRF₂ receptors, we also found that oCRF, which has preferential affinity to the CRF₁ receptor (Behan et al., 1998; Eckart et al., 2001; Table 1), did not delay gastric emptying.

The primary role of CRF₂ receptors on gastric emptying was further characterized by the use of selective CRF receptor subtype antagonists. The administration of the selective CRF₁ receptor antagonists NBI-27914 and CP-154,526 (Chen et al., 1996; Schulz et al., 1996) did not alter the i.p. r/hCRF-induced delayed gastric emptying of a solid meal in mice. In contrast, the newly developed, selective CRF₂ receptor antagonist, antisauvagine-30, injected i.p., completely prevented the i.p. r/hCRF-induced 97% inhibition of gastric emptying of a solid meal. Antisauvagine-30 has been previously used to ascertain the role of CRF₂ receptors in several behavioral tests and on peripheral rat Ucn-induced inhibition of gastric emptying and food intake in mice (Radulovic et al., 1999; Lu et al., 2000; Pelleymounter et al., 2000; Wang et al., 2001).

The present study also provides pharmacological evidence for a role of CRF₁ receptors in the stimulatory action of r/hCRF on distal colonic transit in mice. First, the preferential CRF₁ receptor agonist oCRF (Behan et al., 1996; Eckart et al., 2001) shortened significantly the distal colonic transit time by 50 to 55% at i.p. doses that did not influence gastric emptying. Second, r/hCRF significantly stimulated distal colonic transit at a lower dose (6 μ g/kg) than that required to significantly inhibit gastric emptying (20 μ g/kg). These results are consistent with r/hCRF displaying higher binding affinity for CRF₁ than for CRF₂ receptors (Behan et al., 1996; Vaughan et al., 1996; Perrin and Vale, 1999; Eckart et al., 2001; Table 1). Third, the selective CRF₂ receptor agonists mUcn II and mUcn III did not affect colonic transit when injected i.p. at doses that inhibited gastric emptying. Lastly, we showed that NBI-27914 and CP-154,526, injected periph-

erally, dose dependently prevented i.p. r/hCRF-induced shortening of the bead expulsion time, resulting in the complete normalization of the distal colonic transit time. In rats, we reported that CP-154,526, injected subcutaneously at a similar dose range, prevented peripheral r/hCRF-induced stimulation of colonic motor function (Maillot et al., 2000). Collectively these data show that CRF₁ receptors primarily mediate peripheral r/hCRF-induced stimulation of proximal and distal colonic motor activity in rodents.

These observations may have relevance to human pathophysiology. Intravenous injection of r/hCRF increased segmental contractions of sigmoid colon in healthy subjects and more so in patients with irritable bowel syndrome (Fukudo et al., 1998). In contrast, we reported that oCRF did not influence postprandial antral motility in healthy subjects (Mayer et al., 1992) when injected i.v. at a dose which significantly elevated serum cortisol levels, a response mediated by the activation of CRF₁ receptors (Turnbull and Rivier, 1997). These findings in humans are consistent with a CRF₁ receptor-mediated action of r/hCRF to stimulate colonic motility, whereas this receptor subtype does not influence gastric motor activity. The possible implications of CRF₁ receptors in the pathophysiology of irritable bowel syndrome have recently been reviewed (Heinrichs and Taché, 2001).

The exact sites at which peripherally injected r/hCRF induces a differential CRF receptor-mediated action on gastric and colonic transit need to be further elucidated. In vitro studies in rats established a direct action of r/hCRF in the stomach and the colon as shown by the reduction of spontaneous contractile activity through a tetrodotoxin-sensitive mechanism in longitudinal antral muscle strips (Raybould et al., 1990) and the increased mechanical and myoelectrical activity in isolated distal colonic preparations (Mancinelli et al., 1998; Maillot et al., 2000). Specific CRF binding has been reported in guinea pig cecal smooth muscles cells (Iwakiri et al., 1996), and CRF₁ and CRF₂ receptors have been found to be expressed in the lamina propria of the human colon (Murasu et al., 2000). However, the cellular distribution of CRF receptor subtypes in the stomach or colon is largely unknown, particularly at the level of the colonic myenteric nervous system where electrophysiological and functional studies indicate a possible direct excitatory action of r/hCRF (Hanani and Wood, 1992; Miampamba et al., 2002).

None of the nonselective and selective CRF receptor antagonists, at doses preventing exogenous CRF actions, influenced gastric emptying or distal colonic transit time on their own, indicating that CRF pathways do not modulate postprandial transit in mice. However, there are indications that peripheral administration of nonselective CRF receptor antagonists alleviates postoperative gastric ileus in rats and that activation of CRF receptors comes into play to activate colonic motility under stress (Martínez et al., 1999; Maillot et al., 2001).

In summary, we showed that r/hCRF injected i.p. dose dependently inhibited gastric emptying of a solid nutrient meal while stimulating distal colonic propulsion when monitored simultaneously in conscious mice. The newly identified selective ligands for the CRF₂ receptors mUcn II and, less potently, mUcn III, injected i.p., dose dependently inhibited gastric emptying while not influencing colonic transit. Conversely, the preferential CRF₁ receptor agonist oCRF increased distal colonic propulsion without altering gastric

emptying. The actions of r/hCRF are CRF receptor-mediated as shown by the blockade of gastric and colonic responses by the CRF₁/CRF₂ receptor antagonist astressin. In addition, the CRF₂ receptor antagonist antisauvagine-30 selectively prevented i.p. r/hCRF-induced delayed gastric emptying, whereas the CRF₁ receptor antagonists, NBI-27914 and CP-154,526, selectively inhibited the stimulating action of r/hCRF on colonic transit. Collectively these data support a differential role of CRF receptor subtypes 2 and 1 in mediating peripheral r/hCRF-induced gastric stasis and stimulation of colonic propulsive activity, respectively. These findings may provide new venues for selective drug targeting of these receptor subtypes under stress-related activation of CRF pathways, leading to disturbances of gut motor function.

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