Peripheral urocortin inhibits gastric emptying and food intake in mice: differential role of CRF receptor 2

LIXIN WANG,1 VICENTE MARTÍNEZ,1,2 JEAN E. RIVIER,3 AND YVETTE TACHÉ 1

1Center for Ulcer Research and Education: Digestive Diseases Research Center, Veterans Affairs Greater Los Angeles Healthcare System, Department of Medicine, Division of Digestive Diseases and Brain Research Institute, University of California at Los Angeles, Los Angeles 90073; 2Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, California 92037; and 3Cardenal Herrera University, Department of Basic Biomedical Sciences, 46113 Moncada, Valencia, Spain

Received 2 November 2000; accepted in final form 14 June 2001

Wang, Lixin, Vicente Martínez, Jean E. Rivier, and Yvette Taché. Peripheral urocortin inhibits gastric emptying and food intake in mice: differential role of CRF receptor 2. Am J Physiol Regulatory Integrative Comp Physiol 281: R1401–R1410, 2001.—Intraperitoneal urocortin inhibits gastric emptying and food intake in mice. We investigated corticotropin-releasing factor receptor (CRF-R) subtypes involved in intraperitoneal urocortin actions using selective CRF-R antagonists. Gastric emptying was measured 2 h after a chow meal, and food intake was measured hourly after an 18-h fast in mice. Urocortin (3 µg/kg ip) inhibited gastric emptying by 88%. The CRF-R1/CRF-R2 antagonist astressin B (30 µg/kg ip) and the selective CRF-R2 antagonist antisauvagine-30 (100 µg/kg ip) completely antagonized urocortin action, whereas the selective CRF-R1 antagonist CP-154,526 (10 µg/kg ip) had no effect. Urocortin (1–10 µg/kg ip) dose dependently decreased the 2-h cumulative food intake by 30–62%. Urocortin (3 µg/kg) induced hypophagia was completely antagonized by astressin B (30 µg/kg ip) and partially (35 and 31%) by antisauvagine-30 (100 or 200 µg/kg ip). The CRF-R1 antagonists CP-154,526 or DMP904 (10 µg/kg ip) had no effect. Capsaicin did not alter urocortin-inhibitory actions while blocking the satiety effect of intraperitoneal CCK. These data indicate that intraperitoneal urocortin-induced decrease in feeding is only partly mediated by CRF-R2, whereas urocortin action to delay gastric emptying of a meal involves primarily CRF-R2.

antisauvagine-30; astressin B; capsaicin; cholecystokinin; CP-154,526; DMP904; corticotropin-releasing factor

UROCORTIN IS A NEW MEMBER of the mammalian corticotropin-releasing factor (CRF) family that was characterized in 1995 from the rat brain and named after its 63% sequence homology to the fish urotensin-I ("uro") and to the mammalian CRF (45%, "cort") (55). Besides its specific distribution in the central nervous system (6), urocortin gene is expressed in peripheral tissues, including the gastrointestinal tract (18, 37). Currently, the actions of CRF and CRF-related peptides in mammals are mediated by two distinct seven-transmembrane domain G protein-coupled CRF receptors, the subtypes 1 and 2 (CRF-R1 and CRF-R2), which have heterogenous pharmacological profiles and tissue distribution (11, 13). Rat/human CRF binds with high affinity to CRF-R1 and with lower affinity to CRF-R2, whereas urocortin and the CRF-related peptides characterized from lower vertebrates, namely sauvagine and urotensin-I, display high affinity to both CRF-R1 and CRF-R2, including the splice variants CRF-R2α and CRF-R2β (14, 40, 41, 55).

Mapping studies in rats showed that CRF-R2β is found mainly in the brain, whereas CRF-R2α predominates in nonneuronal brain cells and peripheral tissues (2, 25, 40, 55). Consistent with the presence of CRF-R2β in the heart, immune cells, and gastrointestinal tract (25), peripherally administered urocortin exerts diverse cellular and biological effects on cardiovascular, immune, and gastrointestinal systems (9, 36, 52). In particular, we previously reported that urocortin injected intravenously exhibited greater potency than CRF to delay gastric emptying of a nonnutrient solution in rats (36). This action was blocked by the CRF-R1/CRF-R2 antagonist astressin, whereas the selective CRF-R1 antagonists NBI-27914 and antalarmin (31) had no effect (36). In addition, a recent study indicates that intraperitoneal urocortin-induced delayed gastric emptying in lean and ob/ob mice may contribute to the decrease in food intake (3). In the marsupial Sminthopsis crassicaudata, urocortin injected intraperitoneally was reported to be 50-fold more potent than CRF in decreasing food intake, and its action was not altered by antalarmin (21). These few observations provide indirect pharmacological evidence that peripheral administration of urocortin-induced inhibition of gastric emptying and reduction in food intake may be mediated by CRF-R2. However, less is known about the mechanisms involved in the inhibition of food intake induced by urocortin given peripherally (24) compared with centrally (8, 38, 53, 54).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Up to now, the lack of a selective CRF-R2 antagonist has restricted the direct assessment of CRF-R2-mediated actions of urocortin. Recently, the selective CRF-R2 antagonist antisauvagine-30 has been developed (50). In membrane-binding assays prepared from HEK293 cells stably expressing the CRF-Rs, antisauvagine-30 exhibits ~110-fold higher binding affinity for the murine CRF-R2β than the rat CRF-R1 (50) and binds exclusively to human CRF-2α but not to human CRF-R1 (20).

The objectives of the present study were twofold. First, it was to investigate the functional significance of CRF-R2 in the inhibition of gastric emptying and food intake induced by peripheral administration of urocortin in lean mice. We used the recently developed long-acting CRF-R antagonist astressin B, an isoform of astressin with high affinity to both CRF-R1 and CRF-R2 (49), the specific CRF-R2 antagonist antisauvagine-30 (20, 50), and the specific CRF-R1 antagonists CP-154,526 (51) and DMP904 (15). Second, we examined whether capsaicin-sensitive afferents may be part of the pathways underlying the inhibitory effects of peripheral urocortin on both gastric emptying and food intake. This is based on our previous report that intravenous injection of CRF and, more potently, urocortin induced Fos expression in specific autonomic nuclei in the rat brain (57) and that peripheral injection of CCK, which also induced Fos in these nuclei (7, 56), inhibits gastric emptying and food intake through capsaicin-sensitive afferents projecting to the nucleus of the solitary tract (5, 30, 46).

MATERIALS AND METHODS

Animals

Male C57BL/6 (20–25 g, 8- to 12-wk old; Harlan, San Diego, CA) were used. They were housed in group cages with free access to food (Purina Chow) and tap water and maintained under controlled conditions of illumination (light-dark cycle of 6:30 AM–6:30 PM), temperature (21–23°C), and humidity (30–35%). All experiments were performed in mice fasted for 18–20 h with free access to water. Experiments were performed between 9:00 AM and 4:00 PM and conducted under the Veterans Administration Animal Component of Research protocol number 99–070–04.

Drugs and Treatments

Rat urocortin, rat/human CRF, astressin B, and antisauvagine-30 were synthesized as previously described (49). Peptides were maintained in powder form at ~80°C and dissolved in pyrogen-free distilled water immediately before use. CP-154,526 (Pfizer, Groton, CT) was dissolved in 5% DMSO, 5% Cremaphor EL, and 90% saline (0.9% NaCl), and DMP904 (DuPont, Wilmington, DE) was dissolved in 10% DMSO, 5% Tween 80, and 85% water immediately before use. The pH of CP-154,526 solution was adjusted to the same as its vehicle. Capsaicin (8-methyl-N-vanillyl-6-nonenamide; Sigma Chemical, St. Louis, MO) was dissolved in 10% Tween 80, 10% ethanol, and 80% saline. CCK (sulfated CCK-8; Peninsula Labs, Belmont, CA) was stored at ~80°C in 1-μg/μl aliquots and further diluted in saline to the appropriate concentration immediately before use. All injections were performed intraperitoneally in a volume of 0.1 ml/mouse.

Measurement of Gastric Emptying

Gastric emptying of the nutrient solid meal was measured as described previously (5). Fasted mice were given preweighed Purina chow for 1 h, then urocortin (3 μg/kg), CRF (10 μg/kg), or water was injected intraperitoneally. Gastric emptying of the solid nutrient meal ingested during the 1-h refeeding period was determined by the difference between the total weight of the Purina chow before feeding and the weight of the pellets and spills at the end of the 1-h food exposure. The percentage of gastric emptying during the experimental periods was calculated according to the following equation: gastric emptying (%) = [1 – (wt weight of food recovered from the stomach/weight of food intake)] × 100.

Experimental Protocols

Effects of intraperitoneal CRF antagonists alone or with intraperitoneal urocortin on gastric emptying of a solid nutrient meal. Fasted mice were given preweighed Purina chow for 1 h, then urocortin (3 μg/kg), CRF (10 μg/kg), or water was injected intraperitoneally. Gastric emptying of the solid nutrient meal ingested during the 1-h refeeding period was determined at 1, 2, and 7 h after intraperitoneal injection of vehicle or urocortin and 2 h after CRF. Due to the absence of CRF action at 2 h, later time points were not investigated. In other experiments, astressin B (30 μg/kg), antisauvagine-30 (30, 100, or 200 μg/kg), or water was injected intraperitoneally, 10 min before that of urocortin (3 μg/kg) or water. The CRF-R1 antagonist CP-154,526 (10 mg/kg) or vehicle (5% DMSO, 5% Cremaphor EL, and 90% saline) was injected intraperitoneally 30 min before that of urocortin (3 μg/kg) or water. Urocortin was administered at the end of the 1-h refeeding period, and gastric emptying of ingested food was determined 2 h later. The regimen of CRF-R antagonists, CRF, and urocortin administration was based on previous dose-response studies in rats (27, 36).

Effects of intraperitoneal CRF-R antagonists alone or with urocortin on food intake. Mice, fasted for 18–20 h, received a single intraperitoneal injection of urocortin (1, 3, or 10 μg/kg), CRF (10 μg/kg), or water and were given free access to preweighed Purina chow. Food intake was measured at 30 min, 1, 2, 3, 4, and 7 h thereafter. Similar studies were performed in fasted mice injected intraperitoneally with astressin B (3, 10, 30, or 100 μg/kg) or antisauvagine-30 (30, 100, or 200 μg/kg), CP-154,526 (10 mg/kg), DMP904 (10 mg/kg), CP-154,526 (10 mg/kg) plus antisauvagine-30 (100 μg/kg), or the respective antagonist vehicles. Astressin B and antisauvagine-30 were injected 10 min before and CP-154,526 and DMP904 were injected 30 min before urocortin (3 μg/kg ip). Immediately after urocortin administration, preweighed Purina chow was given, and food intake was measured at 30 min, 1, 2, and 4 h later. Food intake was determined by measuring the difference between the preweighed standard chow and the weight of chow and spill at the end of each time point, as previously described in mice (5).
Effect of capsaicin pretreatment on urocortin-induced inhibition of gastric emptying and food intake. Two groups of mice were injected subcutaneously with capsaicin (50 mg/kg; two injections of 25 mg/kg at 12-h interval) or vehicle (two injections of 0.1 ml/mouse). Ten to 15 days later, capsaicin- and vehicle-pretreated mice fasted for 18–20 h and then had free access to preweighed Purina chow for 1 h. Thereafter, food was withdrawn, and mice received an intraperitoneal injection of either urocortin (3 μg/kg) or vehicle. Gastric emptying was determined 2 h after the intraperitoneal injection. In other groups, capsaicin- and vehicle-pretreated fasted mice were injected intraperitoneally with urocortin (3 μg/kg), CCK (10 μg/kg), or their respective vehicles (water and saline). Food intake was monitored for the following 4 h as described above.

The efficiency of capsaicin pretreatment was assessed immediately before euthanasia by the corneal chemosensory test that consisted of monitoring the wiping reflex to ocular instillation of a drop of 0.1% NH₂OH solution in one eye. None of the capsaicin-pretreated mice showed a wiping response, indicating the effective ablation of primary sensory afferents, as previously reported under the same conditions (5). Wiping reflex was present in vehicle-pretreated mice.

Statistical Analysis

Results are expressed as means ± SE and analyzed by one-way ANOVA followed by a multiple-comparison test (Tukey's test) of all pairs. Values of P < 0.05 are considered statistically significant.

RESULTS

Effects of CRF-R Antagonists Alone or With Urocortin on Gastric Emptying of a Solid Nutrient Meal

In mice fasted for 18–20 h that had access to standard Purina chow for 1 h, 56.6 ± 5.5, 80.5 ± 2.6, and 90.2 ± 1.5% of the ingested meal were emptied from the stomach at 2, 4, and 7 h, respectively, in the control groups injected intraperitoneally with vehicle (Fig. 1). When urocortin (3 μg/kg) was injected intraperitoneally at the end of the 1-h food exposure, only 6.6 ± 3.9% of the meal was emptied from the stomach after 2 h (Fig. 1). At 4 h, values increased to 62.1 ± 5.9%, although they remained significantly lower than those of intraperitoneal vehicle group. At 7 h, the inhibitory effect of intraperitoneal urocortin on gastric emptying was no longer observed (Fig. 1). CRF (10 μg/kg) injected intraperitoneally did not modify gastric emptying of the Purina chow meal at 2 h after the peptide administration (64.4 ± 6.1 vs. 56.6 ± 5.5% vehicle; P > 0.05, n = 5 for each group; Fig. 1). On the basis of this time course study, gastric emptying was assessed at 2 h after urocortin injection in all other experiments.

Astressin B (30 μg/kg ip) completely prevented urocortin (3 μg/kg ip)-induced inhibition of gastric emptying of the solid meal (49.4 ± 6.5 vs. 55.4 ± 8.6% in vehicle-pretreated mice; P > 0.05; Fig. 2A). Likewise, antisauvagine-30 injected intraperitoneally at 30 or 100 μg/kg dose dependently antagonized urocortin-induced inhibition of gastric emptying by 54 and 100%, respectively. Gastric emptying values were 40.9 ± 11.4 and 71.2 ± 6.9%, respectively, compared with 60.3 ± 9.5% in vehicle plus vehicle and 20.8 ± 5.1% in vehicle plus urocortin groups (Fig. 2B). By contrast, CP-154,526 (10 mg/kg ip) did not modify urocortin action (Fig. 2C). None of the individual CRF-R antagonists, including astressin B, antisauvagine-30, and CP-154,526, significantly modified basal gastric emptying of the solid meal (Fig. 2). Antisauvagine-30, at 100 μg/kg ip, showed a tendency to increase gastric emptying (78.3 ± 5.0%), although it did not reach statistical significance (Fig. 2B).

Effects of Intraperitoneal Urocortin and CRF on Food Intake in Fasted Mice

Urocortin (1, 3, or 10 μg/kg) injected intraperitoneally in fasted mice induced a time- and dose-related inhibition of food intake (Fig. 3). Cumulative chow intake was significantly reduced for 2, 4, and 7 h after intraperitoneal injection of urocortin at 1, 3, or 10 μg/kg, respectively, compared with intraperitoneal vehicle [F(4,35) = 13.34, P < 0.001; Fig. 3A]. Urocortin significantly reduced food intake within 30 min at all doses (Fig. 3A). The inhibition reached its maximum during the 1- to 2-h period postinjection that was maintained for the following hour only after urocortin at 10 μg/kg; thereafter, values were no longer significantly different from the vehicle group (Fig. 3B). The 2-h cumulative food intake was reduced in a dose-related manner from 0.66 ± 0.05 g (n = 8) in vehicle-injected mice to 0.46 ± 0.03 g (n = 9, P < 0.05), 0.33 ± 0.04 g (n = 9, P < 0.05), and 0.25 ± 0.07 g (n = 9, P < 0.05) by intraperitoneal urocortin at 1, 3, or 10 μg/kg, respectively (Fig. 3A); this corresponds to a 30 ± 5, 50 ± 6, and 62 ± 12% inhibition of the 2-h cumulative food intake, respectively. By contrast, CRF injected intraperitoneally at 10 μg/kg (n = 8) inhibited food intake by 35% only during the first 30 min postadmin-
Urocortin decreased the 2-h cumulative food intake to 0.21 ± 0.03 g (n = 6) in vehicle-pretreated mice compared with vehicle plus vehicle (0.53 ± 0.06 g; n = 6, P < 0.05) (Fig. 4A). Mice treated with urocortin did not show signs of illness. Astressin B (3, 10, 30, or 100 μg/kg ip) inhibited dose dependently urocortin-induced reduction of 2-h cumulative food intake by 34 ± 6, 25 ± 11, 73 ± 8, and 100 ± 29%, respectively (Fig. 4A). The 2-h cumulative food intake values were 0.32 ± 0.06 g (n = 6, P > 0.05), 0.29 ± 0.04 g (n = 9, P > 0.05), 0.45 ± 0.03 g (n = 6, P < 0.05), and 0.55 ± 0.09 g (n = 7, P < 0.05), respectively, compared with vehicle plus urocortin (0.21 ± 0.03 g; n = 6). Astressin B (30 or 100 μg/kg) injected intraperitoneally alone did not significantly influence the 2-h cumulative food consumption (Fig. 4A).

Antisauvagine-30 (100 or 200 μg/kg ip) resulted in a partial reversal of intraperitoneal urocortin-induced decrease in the 2-h cumulative food intake (35 ± 12 and 31 ± 24%, respectively), whereas 30 μg/kg had no effect. The 2-h cumulative food intake values were 0.36 ± 0.05 g (n = 9, P > 0.05), 0.34 ± 0.09 g (n = 7, P < 0.05 vs. urocortin).

On the basis of the robust food intake-reducing effect of urocortin at 3 μg/kg ip, this dose was selected in subsequent experiments. In addition, 2 h postadministration was chosen to represent food intake data (cumulated food intake, g/2 h).

Effects of CRF-R Antagonists on Intraperitoneal Urocortin-Induced Inhibition of Food Intake in Mice

Urocortin decreased the 2-h cumulative food intake to 0.21 ± 0.03 g (n = 6) in vehicle-pretreated mice compared with vehicle plus vehicle (0.53 ± 0.06 g; n = 6, P < 0.05) (Fig. 4A). Mice treated with urocortin did not show signs of illness. Astressin B (3, 10, 30, or 100 μg/kg ip) inhibited dose dependently urocortin-induced reduction of 2-h cumulative food intake by 34 ± 6, 25 ± 11, 73 ± 8, and 100 ± 29%, respectively (Fig. 4A). The 2-h cumulative food intake values were 0.32 ± 0.06 g (n = 6, P > 0.05), 0.29 ± 0.04 g (n = 9, P > 0.05), 0.45 ± 0.03 g (n = 6, P < 0.05), and 0.55 ± 0.09 g (n = 7, P < 0.05), respectively, compared with vehicle plus urocortin (0.21 ± 0.03 g; n = 6). Astressin B (30 or 100 μg/kg) injected intraperitoneally alone did not significantly influence the 2-h cumulative food consumption (Fig. 4A).

Antisauvagine-30 (100 or 200 μg/kg ip) resulted in a partial reversal of intraperitoneal urocortin-induced decrease in the 2-h cumulative food intake (35 ± 12 and 31 ± 24%, respectively), whereas 30 μg/kg had no effect. The 2-h cumulative food intake values were 0.36 ± 0.05 g (n = 9, P > 0.05), 0.34 ± 0.09 g (n = 7, P < 0.05 vs. urocortin).
Effects of Capsaicin Pretreatment on Urocortin-Induced Inhibition of Food Intake and Gastric Emptying

Pretreatment with capsaicin (10–15 days before) did not modify urocortin (3 μg/kg ip)-induced reduction of food intake in fasted mice (0.30 ± 0.06 g) compared with vehicle pretreatment plus urocortin (0.24 ± 0.06 g; \( F(3,14) = 10.561, P < 0.001 \) vs. vehicle plus vehicle group) (Fig. 6A). In vehicle-pretreated groups, CCK (10 μg/kg ip) inhibited the 2-h cumulative food intake to a similar extent (0.23 ± 0.02 g) as urocortin (Fig. 6). However, in contrast to urocortin, the satiety effect of CCK was no longer observed in capsaicin-pretreated mice (0.56 ± 0.04 g; \( P > 0.05 \) vs. vehicle 0.75 ± 0.08 g) (Fig. 6B). Capsaicin pretreatment by itself did not affect food intake significantly (Fig. 6).

Capsaicin pretreatment did not prevent urocortin (3 μg/kg ip)-induced inhibition of gastric emptying moni-

---

Fig. 4. Effect of CRF receptor antagonists astressin B (A) and anti-sauvagine-30 (B) on peripheral urocortin-induced reduction of food intake in mice. Mice, fasted for 18 to 20 h, were injected intraperitoneally with either urocortin or vehicle (0.1 ml/mouse); thereafter, chow was given ad libitum, and food intake was monitored for the next 4 h. Data represent cumulative food intake at 2-h post-urocortin administration (g/2 h) and are means ± SE, \( n = 5 \) to 13 in each group. * \( P < 0.05 \) vs. vehicle + vehicle; # \( P < 0.05 \) vs. astressin B at 3 and 10 μg/kg + urocortin.

Fig. 5. Effect of CP-154,526 alone (A) or with antisauvagine-30 (C) and DMP904 (B) on intraperitoneal urocortin-induced reduction of fasting-induced food intake in mice. Mice, fasted for 18 to 20 h, were injected intraperitoneally with CP-154,526 or DMP904 at 30 min or antisauvagine-30 at 10 min before intraperitoneal urocortin or vehicle; then chow pellets were given ad libitum, and food intake was monitored for the next 4 h. Data represent cumulative intake for 2 h after urocortin administration (g/2 h) and are means ± SE of the number of animals shown in the columns. * \( P < 0.05 \) vs. vehicle-treated group.
treated 2 h after a chow meal [vehicle plus urocortin: 12.6 ± 8.4%; capsaicin plus urocortin: 1.5 ± 1.5%; n = 3 for each group, P > 0.05, F(3,8) = 24.963, P < 0.001]. Capsaicin pretreatment by itself had no significant effect on gastric emptying of a solid meal (42.2 ± 5.3%; P > 0.05 vs. 59.5 ± 3.2% in vehicle-pretreated animals, n = 3 for each group).

**DISCUSSION**

In the present study, urocortin injected intraperitoneally inhibits gastric emptying of a solid meal in mice, whereas intraperitoneal CRF tested at a 3.3-fold higher dose than urocortin had no effect. Previous dose-response studies in mice (3) and rats (10, 36) showed a rank order of potency with intraperitoneal or intravenous rat urocortin > rat CRF to inhibit gastric emptying indicative of a CRF-R2-mediated response (40, 55). The use of selective CRF-R2 antagonists provides additional pharmacological support for a primary role of CRF-R2 in mediating intraperitoneal urocortin-induced inhibition of gastric emptying of a solid meal in mice. Antisauvagine-30 injected intraperitoneally at antagonist:agonist (wt:wt) ratios of 10:1 and 33:1 induced a dose-related 54–100% inhibition of intraperitoneal urocortin action. Antisauvagine-30 is a novel analog of sauvagine, devoid of intrinsic activity with high binding affinity for the recombinant mouse CRF-R2β (Kd = 1.4 nM) and human CRF-R2α (Kd = 0.8 nM) compared with the rat CRF-R1 (Kd = 153.6 nM) or human CRF-R1 (Kd = 1.3 μM) (20, 50). So far, antisauvagine-30 has been injected only centrally to establish the role of brain CRF-R2 in CRF-related modulation of behaviors (26, 38, 44). The present data showed that antisauvagine-30 is a relevant tool to assess the role of peripheral CRF-R2 in the regulation of visceral function. Likewise, preliminary studies with the recently developed selective and potent peptide CRF-R2 antagonist 338–086–15 (10 μg/kg ip) completely blocked urocortin (3 μg/kg ip)-inhibited gastric emptying in mice (48). This further supports the idea that urocortin-inhibitory effect on gastric emptying is mediated through CRF-R2.

The CRF-R1/CRF-R2 antagonist astressin B (49) also abolished urocortin-induced inhibition of gastric emptying at a 3.3-fold lower intraperitoneal antagonist:agonist ratio than that of antisauvagine-30. Astressin B is one of the most efficacious CRF-R1/CRF-R2 antagonists developed so far (49). Its efficiency (potency, duration of action, and bioavailability) results from the introduction of two CoMeLeu residues in position 27 and 40 of the astressin molecule (cyclo[30–33]-[DPhe12,Nle21,CoMeLeu27,Glu30,Lys33,Nle38,CoMeLeu40]Ac-hCRF(9–41)], which favors the bioactive conformation while preventing degradation (47). Previous in vivo studies showed that astressin B inhibits endogenous CRF-dependent ACTH secretion in adrenalectomized rats with a duration of action that extends beyond 6 h, whereas that of astressin lasts 90 min (47, 49). We also showed that astressin B exhibits a longer duration of action than astressin when injected intravenously to prevent intravenous CRF-induced decrease in intraluminal gastric pressure (35). The present observation provides additional evidence that astressin B is a potent CRF-R antagonist as established against intraperitoneal urocortin-induced delayed gastric emptying of a solid meal in mice.

By contrast, the selective CRF-R1 antagonist CP-154,526 injected peripherally did not influence urocortin-inhibitory action on gastric emptying. The regimen of CP-154,526 administration (10 mg/kg ip) was in the bioactive range established previously in rats, where we showed that CP-154,526 (6 mg/kg sc) completely blocked intraperitoneal CRF-induced stimulation of colonic motility (27). Moreover, other selective CRF-R1 antagonists NBI-27914 and antalarmin (16) did not block peripheral CRF- or urocortin-induced inhibition of gastric emptying in rats (36). Taken together, these data provide convergent evidence that urocortin action on gastric motor function is primarily mediated by CRF-R2. None of the CRF antagonists injected intraperitoneally significantly influenced gastric emptying, providing evidence that CRF-Rs are not involved in the basal regulation of gastric emptying of a solid meal in mice in agreement with previous reports in rats (28, 36). However, peripheral administration of astressin, unlike selective CRF-R1 antagonists, blocked postoperative gastric ileus in rats (28, 36), suggesting a possible relevance of CRF-R2 activation in the gastric motor response to viscerosomatic stress.

The mechanisms through which intraperitoneal urocortin induced a CRF-R2-mediated inhibition of gastric emptying are likely to be initiated in the periphery. Pharmacokinetic studies showed that CRF and urocortin are not transported into the brain (23, 29), and a reasonable inference can be made that the structurally CRF-related peptides astressin B and antisauvagine-30 may have a limited ability to cross the blood-brain barrier. Functional studies support this contention because astressin injected intravenously did not...
block intracisternal injection of CRF-induced delayed gastric transit while antagonizing intravenous CRF-inhibitory action (28). By contrast, CP-154,526 given peripherally has bioavailability to the central nervous system to antagonize exogenous or endogenous CRF in the brain (51). The unaltered urocortin action by intraperitoneal CP-154,526 supports the view that CRF-R1 in the brain and periphery is unlikely to be involved. Capsaicin failed to influence intraperitoneal urocortin-induced slowing of gastric emptying. Therefore, it is unlikely that intraperitoneal urocortin acts through capsaicin-sensitive afferents to delay gastric transit as reported for CCK and secretin (45,46). Although CRF-R2β is expressed in the rat visera including the heart and upper gastrointestinal tract (39), the location at which urocortin interacts with CRF-R2 to delay gastric emptying remains to be defined. Recent studies indicate that urocortin hyperpolarized isolated guinea pig stomach smooth muscle cells (42), suggesting a possible direct action on gastric muscle layers.

At the intraperitoneal dose at which urocortin inhibited gastric emptying, there was a decrease in feeding. Dose-response studies showed that low doses of urocortin (4–40 pmol/mouse ip) inhibited the 2-h cumulative feeding response to food deprivation by 30–62% in mice. The urocortin-inhibitory effect was observed during the 30-min to 3-h period postinjection, implying that urocortin displays a profile of a short-term satiety signal. These results are in agreement with previous reports showing that urocortin injected intraperitoneally suppressed cumulative food intake within 30 min in the fasted marsupial Sminthopsis crassicaudata (21) as well as in mice, although higher doses (0.03 to 3 nmol/mouse ip) were used (3). The inhibitory effect of intraperitoneal urocortin on food intake occurs via activation of CRF-Rs, because astressin B injected intraperitoneally blocked intraperitoneal urocortin-induced hypophagia by 73 and 100% at antagonist:agonist ratios of 10:1 and 33:1, respectively. By itself, astressin B did not alter the feeding response to fasting concurrent with the absence of changes in feeding pattern in CRF-R2 or CRF-R1 receptor-knockout mice (8,12). Previous dose-response studies showed that urocortin injected intraperitoneally is more potent than CRF in suppressing food intake in mice (3) as well as in marsupials (21). Likewise, in rats, sauavagine and urotensin-I injected subcutaneously exhibited a higher potency than CRF to suppress fasting-induced feeding (34). This shows a similar rank order of potency across species with urocortin, urotensin-I, and sauavagine > CRF. These observations should be indicative of a CRF-R2-mediated action. However, the selective CRF-R2 antagonist antisauvagine-30 attenuated urocortin-induced reduction of the 2-h cumulative food intake by only 35%. Such partial reversal cannot be related to a subeffective dose, because antisauvagine-30:urocortin at ratios of 33:1 and 66:1 resulted in a similar (35 and 31%, respectively) antagonism of urocortin-inhibitory effect on 2-h cumulative food intake. In addition, antisauvagine-30 at a ratio of 33:1 completely blocked intraperitoneal urocortin-induced inhibition of gastric emptying as monitored 2 h after treatment. The complete prevention of urocortin action on food intake by the CRF-R1/CRF-R2 antagonist astressin B and the partial blockade by the selective CRF-R2 antagonist may indicate an additional interaction with the CRF-R1 receptor. However, CP-154,526 did not modify urocortin action, and the partial reversal induced by antisauvagine-30 was not improved when both antisauvagine-30 and CP-154,526 were administered together. Likewise, the selective CRF-R1 antagonist DMP904 (19), shown to be more potent than CP-154,526 in a behavioral test (15), was ineffective in blocking urocortin action on food intake. A recent report indicates that the selective CRF-R1 antagonist antalarmin (31) injected intraperitoneally did not affect the anorectic effect of intraperitoneal urocortin or CRF in marsupials, and high doses resulted in a higher decrease in food intake when combined with CRF or urocortin (21). These data indicate that peripheral urocortin-induced decrease in food intake in food-deprived mice is CRF-R mediated, in part, through CRF-R2. Peripheral urocortin may activate a yet to be identified novel CRF-R or CRF-R1 or -2 subtype splice variant that can be blocked by astressin B and less efficiently by the selective CRF-R2 or CRF-R1 antagonist.

The differential antagonist actions of antisauvagine-30 on intraperitoneal urocortin-induced inhibition of gastric emptying and food intake emerged as an interesting finding in light of the previously suspected interrelationship between these two effects (3). Gastric distention acts as a satiety signal to reduce food intake (43), and the presence of food into the stomach could influence the degree of gastric fullness linking the satiating response with delayed gastric emptying (32). However, our results do not support the notion that the slowing of gastric emptying underlies the reduction in food intake induced by intraperitoneal injection of urocortin as suggested in a previous report (3). Indeed, the CRF-R2 antagonist administered at a dose that completely normalized the gastric emptying of a solid meal prevented the reduction of food intake only by 35%. These observations show that alterations of gastric emptying could not solely account for the urocortin hypophagic response. Moreover, the rapid onset of food intake suppression occurring within 30 min after the intraperitoneal injection of urocortin did not support the view that altered gastric emptying plays a major role in the reduction of ingestive behavior. We obtained evidence that capsaicin pretreatment did not alter the hypophagic action of intraperitoneal urocortin. The biological efficacy of capsaicin was established by the demonstration that CCK injected intraperitoneally no longer inhibits food intake in mice. Likewise, we previously reported that a similar capsaicin treatment abolished the synergistic hypophagic effect of CCK plus leptin injected intraperitoneally in mice (5). Therefore, peripherally administered urocortin did not communicate with the brain to reduce food intake via a neuronal system(s) whose afferent arm is composed of capsaicin-sensitive fibers. These findings are consistent with the demonstration that vagotomy
did not alter subcutaneous sauvagine-induced decreased eating response to food deprivation (34), whereas vagotomy abolished satiety cues produced by gastric distension in rats (17). Collectively, these results indicate that intraperitoneal urocortin may act as an early satiety signal through mechanisms largely independent of gastric emptying. In addition, urocortin and CCK administered peripherally, although sharing similar potent actions to inhibit gastric emptying of a solid meal and food intake in fasted mice, act through separate mechanisms.

In summary, urocortin administered peripherally inhibits gastric emptying of a solid meal and reduces the feeding response of fasted mice through activation of CRF-Rs. This was shown by the complete blockade of urocortin-inhibitory actions by intraperitoneal injection of astressin B, a long-acting CRF-R antagonist. The use of selective receptor antagonist to CRF-R1, CP-154,526, and to CRF-R2 antisauvagine-30 indicates that the inhibitory effects of intraperitoneal urocortin on gastric emptying are mediated by the activation of peripherally located CRF-R2 receptor. By contrast, the reduction of food intake following food deprivation is only partially antagonized by antisauvagine-30 and not altered by the CRF-R1 antagonists CP-154,256 and DMP904. Moreover, intraperitoneal urocortin-inhibitory actions on food intake and gastric emptying are not mediated by the activation of capsaicin-sensitive afferent fibers as those of intraperitoneal CCK. These results indicate that CRF-R2 receptor is differentially involved in the inhibitory effect of intraperitoneal urocortin on gastric emptying and food intake, and both actions are not interrelated.

Perspectives

Two unsolved issues arise from these observations. First, which CRF-R subtype(s) blocked by astressin B but not by CP-154,526 and DMP904 and partly by antisauvagine-30 are involved in mediating peripheral urocortin-induced inhibition of feeding response in fasted mice? The recent cloning of a novel CRF-R3 receptor gene in the catfish, in addition to CRF-R1 and CRF-R2 genes (1), raises the possibility that additional mammalian CRF-R gene(s) may also exist. This novel CRF-R subtype may have relevance in conveying the CRF-R-mediated actions of urocortin that are not antagonized by the selective CRF-R1 and CRF-R2 receptor antagonists. Second, what are the pathways outside of capsaicin-sensitive afferent fibers conveying the urocortin signals to the brain that decrease food intake? Although peripheral urocortin does not enter into the brain in mice, increased peripheral levels of leptin facilitate the entry of labeled urocortin into the brain parenchyma within 15 min with higher levels observed in the hypothalamus (23). Leptin and a recently hyperglycemia-assisted transport of urocortin from the periphery into the brain (22, 23) may open new possible mechanisms through which intraperitoneal urocortin signals the brain, resulting in anorexia. Other relevant future directions will be to establish the peripheral site at which activation of CRF-R2 by urocortin triggers alterations of gastric emptying of a solid meal. Also, it will be important to determine whether CRF-R2 activation by urocortin comes into play to induce gastric stasis under conditions of postoperative gastric ileus or immune challenges associated with delayed gastric emptying (4).

We thank Dr. E. D. Pagani (Central Research Division, Pfizer, Groton, CT) for the generous supply of CP-154,526 and Dr. P. J. Gilligan (DuPont Pharmaceutical, Wilmington, DE) for the generous supply of DMP904. P. Kirsh is acknowledged for assistance in the preparation of the manuscript.

The authors’ work was supported by the Medical Research Fund from the Veterans Affairs Merit Review (Y. Taché) and National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-41901 (Animal Core: Y. Taché; Pilot and Feasibility Study: L. Wang) and DK-26741 (J. Rivier).

REFERENCES


CRF RECEPTOR 2 ANTAGONIST BLOCKS UROCORTIN ACTIONS

50. Ruhmann A, Bonk I, Lin CR, Rosenfeld MG, and Spiess J. Structural requirements for peptidic antagonists of the cortico-


