Human Urocortin 2, a Corticotropin-Releasing Factor (CRF)₂ Agonist, and Ovine CRF, a CRF₁ Agonist, Differentially Alter Feeding and Motor Activity

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ABSTRACT

Two corticotropin-releasing factor (CRF) receptor families have been identified (CRF₁ and CRF₂). Whereas anxiogenic-like roles for the CRF₁ receptor have been identified, behavioral functions of the CRF₂ receptor remain obscure. Urocortin 2 (Ucn 2), a CRF-related peptide that selectively binds CRF2 receptors, was recently identified and recognized for its central anorectic properties. The present study tested the hypothesis that the anorexigenic mode of action of Ucn 2 differed from that of ovine CRF (oCRF), a preferential CRF₁ receptor agonist. The behavioral effects of intracerebroventricular administration of Ucn 2 were compared with those of oCRF in nondeprived male Wistar rats (n = 102). Ucn 2 reduced 6-h food and water intake at doses that did not induce visceral illness (0.1, 1, and 10 μ g), as indicated by kaolin intake. Ucn 2 retained its potent anorectic activity in rats receiving a highly palatable cafeteria diet, preferentially reducing intake of carbohydrate (CHO)-rich items while sparing intake of mixed-fat/CHO items. In contrast to Ucn 2, oCRF (10 µg) suppressed 6-h intake of cafeteria diet-fed rats without regard to macronutrient composition. Rather, oCRF most potently suppressed intake of preferred food items. Whereas oCRF had short-onset motor-activating effects, Ucn 2 had nondose-dependent, delayed-onset motor-suppressing effects. Thus, central infusion of a CRF₂ receptor agonist suppressed intake of both bland and palatable diets without inducing behavioral arousal or malaise, and the profile of anorexigenic effects gualitatively differed from those of a CRF1 receptor agonist. The results suggest the existence of distinct forms of CRF₁- and CRF₂-mediated anorexia.

The neuropeptide corticotropin-releasing factor (CRF) (Vale et al., 1981) is hypothesized to mediate behavioral, autonomic, and endocrine responses to stress (Zorrilla and Koob, 2004a). Two genes code for separate families of Gprotein-coupled CRF receptors (CRF₁ and CRF₂) with distinct distributions and pharmacological properties (Zorrilla and Koob, 2004a). Molecular and antagonist studies point to arousing and anxiogenic-like roles for the CRF1 receptor (Zorrilla and Koob, 2004b), but behavioral functions of the CRF₂ receptor remain obscure.

The characterization of urocortin 1 (Ucn 1) (Vaughan et al.,

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1995), a mammalian CRF paralog with greater affinity for the CRF₂ receptor than CRF, led to two hypotheses about the behavioral significance of the CRF₂ receptor. First, because i.c.v. Ucn 1 had more potent, efficacious, and prolonged anorectic effects than CRF (Spina et al., 1996), it was hypothesized that CRF₂ receptor activation suppressed feeding. Studies with a preferential CRF2 receptor antagonist and CRF₂ receptor-deficient mice supported this hypothesis (Zorrilla et al., 2003). Second, because i.c.v. Ucn 1 was less effective than CRF in stimulating motor activity in a familiar environment (Spina et al., 1996; Reyes et al., 2001), it was hypothesized that CRF2 receptor activation had motor suppressive effects. Selective agonists for the CRF₂ receptor had not been identified, however, precluding determination of whether CRF₂ receptor activation was sufficient for these effects.

Genes encoding two selective CRF₂ agonists—urocortin 2 (Ucn 2) and urocortin 3 (Ucn 3)—were recently cloned from

ABBREVIATIONS: CRF, corticotropin-releasing factor; Ucn 1, urocortin 1; Ucn 2, urocortin 2; Ucn 3, urocortin 3; oCRF, ovine CRF; CTA, conditioned taste aversion; hUcn 2, human Ucn 2; CHO, carbohydrate; MED, minimum effective dose.

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both mouse and human genomic libraries (Lewis et al., 2001; Reyes et al., 2001). Ucn 2, a putative 38-amino acid CRFrelated neuropeptide, is approximately 1000-fold more selective for the CRF₂ receptor than is Ucn 1 (Reyes et al., 2001). Conversely, ovine CRF (oCRF) shows almost 200-fold greater affinity for the CRF₁ than CRF₂ receptor (Behan et al., 1996). Neither peptide has affinity for the human CRF-binding protein (Sutton et al., 1995; Lewis et al., 2001). Thus, as relatively selective, direct receptor agonists, oCRF and Ucn 2 are powerful tools for discriminating the behavioral functions of the CRF₁ and CRF₂ receptors.

Centrally infused Ucn 2 is anorectic and, like several recognized appetite suppressants, activates interconnected brain regions known to participate in the regulation of autonomic and visceral functions related to energy balance (Inoue et al., 2003). Meal pattern analysis showed that Ucn 2 has satiation-like effects, reducing meal size and eating rate, without altering meal frequency. In the present study, the dose-related anorectic and antidipsogenic effects of Ucn 2, the highly selective CRF₂ receptor agonist, and oCRF, the moderately selective CRF₁ receptor agonist, were compared in nondeprived rats fed either a standard chow diet or a diet supplemented by highly palatable, energy-dense foods ("cafeteria diet"). The cafeteria diet is a well-validated procedure for inducing hyperphagia (30-100% caloric increase) (Rothwell and Stock, 1979; Rothwell and Stock, 1988). In contrast to models of drug-, lesion-, or deprivation-induced hyperphagia, the cafeteria diet models appetite characteristics that accompany overeating in humans. Using the diet, one also can observe shifts in food selection associated with the composition of or individual preferences for the food items (Rogers and Blundell, 1984; Prats et al., 1989; Esteve et al., 1994).

Potential aversive consequences that could account for ingestive effects also were examined. Although i.c.v. Ucn 2 does not share the anxiogenic-like effects of CRF (Valdez et al., 2002), it can induce a conditioned taste aversion (CTA) at a dose 100-fold higher than its minimal effective anorectic dose (Inoue et al., 2003). Ucn 2's anorectic effects are delayed, and the formation of a CTA depends on temporal proximity between the conditioned stimulus and the internal drug state. Therefore, results from a time-insensitive measure of malaise are needed to evaluate the potential role of malaise in Ucn 2-induced anorexia. Rats increase geophagia (e.g., kaolin clay intake), a form of pica behavior, in response to stimuli that elicit visceral illness (e.g., poisons, toxins, hypergravity, microgravity). Treatments that increase geophagia also can form a CTA, and antiemetics reduce clay intake. Accordingly, geophagia is a proposed rodent analog of emesis (Takeda et al., 1993). Unlike CTA testing, geophagia is an active, unconditioned behavior and is quantitatively dose-dependent. Therefore, we compared the ability of Ucn 2 and oCRF to increase kaolin intake. Motor activity also was monitored. LiCl, an aversive agent that induces gastrointestinal malaise, was used as a positive control.

Materials and Methods

Subjects

On arrival, adult (300-350 g) male Wistar rats (n = 102; Charles River Laboratories, Inc., Wilmington, MA) were group-housed in a 12-h/12-h reverse-lit (lights on 10:00 PM), humidity-controlled (60%), and temperature-controlled (22°C) vivarium with standard

rodent chow (Harlan Teklad LM-485 Diet 7012; Harlan, Indianapolis, IN) and water available ad libitum. Subjects were acclimated to the vivarium for 1 week prior to experiments and handled during the week prior to the start of testing. Surgical and experimental procedures adhered to the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication no. 85-23, revised 1996) and were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

Surgery

Rats that were to receive i.c.v. administration of human Ucn 2 (hUcn 2) or oCRF were implanted with indwelling cannulae directed unilaterally at the lateral ventricle. Anesthetized (halothane, 2–3% in oxygen) subjects were secured in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). Using sterile technique, a straight, stainless steel, 22-gauge guide cannula (Plastics One Inc., Roanoke, VA) was lowered above the lateral ventricle and anchored to the skull with screws and dental cement. With the tooth bar set 5.0 mm above interaural zero, the coordinates were A/P – 0.6 mm, M/L \pm 2.0 mm relative to bregma, and 3.2 mm ventral from the skull surface (Pellegrino et al., 1979). A dummy stylet (Plastics One) maintained patency. Subjects were allowed 1 week to recover from surgery.

Drugs and Injections

hUcn 2 and oCRF were synthesized manually using solid phase methodology, purified using high-performance liquid chromatography, and fully characterized using capillary zone electrophoresis, high-performance liquid chromatography, and mass spectrometry, as described previously (Reyes et al., 2001). Peptides were dissolved in sterile $0.5 \times$ phosphate-buffered saline (pH 7.4) immediately prior to testing and kept on ice. Peptides or vehicle were injected (i.c.v. 5 μ l) over 1 min with a Hamilton microsyringe using a 28-gauge stainless steel injector attached to PE 20 tubing. The injector, which projected 1.3 mm past the end of the cannula, was left in place for 1 min after infusion to allow diffusion. Placement was confirmed histologically. For pica assessment, sterile, isotonic (0.15 M) LiCl (Sigma-Aldrich, St. Louis, MO) or NaCl were administered intraperitoneally in a volume of 20 ml/kg.

Behavioral Tests

Chow, Cafeteria Diet, and Water Intake. Rats in the oCRF (n = 18) and hUcn 2 cohorts (n = 14) were randomly assigned to the chow or cafeteria diet conditions (n = 7–9/group). All received ad libitum chow and water for the duration of the study. One week prior to i.c.v. surgery, rats were single-housed. At this time, the following highly palatable, commercially available food items also were provided to cafeteria diet rats: rice cakes (Quaker Oats, Chicago, IL), cheese crackers (Keebler Company, Elmhurst, IL), mini marshmallows (Safeway, Pleasanton, CA), and cookies and cream milk chocolate (Hershey Foods Corporation, Hershey, PA). Items were chosen in pilot studies from an assortment of foods based on a combination of 1) the rats' preferences; 2) the homogeneity, macronutrient composition, unique orosensory characteristics, and energy density of the foods; and 3) the stability of the foods' hydration (weight) over a 6-h period. As shown in Table 1, supplemental

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Energy density and macronutrient composition of food items

| Food Item | | Macronutrient Composition | | |
|-----------------|----------------|---------------------------|-----|---------|
| | Energy Density | Carbohydrate | Fat | Protein |
| | kcal/g | % calories from | | |
| Chow | 3.75 | 65 | 13 | 21 |
| Marshmallows | 3.34 | > 99 | 0 | <1 |
| Rice cakes | 3.89 | 89 | 0 | 11 |
| Cheese crackers | 5.14 | 40 | 50 | 10 |
| Chocolate | 5.33 | 44 | 48 | 8 |

foods were uniformly low in protein. Two items, rice cakes and marshmallows, were composed almost exclusively of carbohydrates (CHOs), whereas two items, cheese crackers and chocolate, had mixed-fat/CHO content. After recovery from surgery, rats were reacclimated to the cafeteria diet for 1 week, receiving daily 6-h access to the supplemental items from 11:00 AM to 5:00 PM (i.e., starting 1 h after dark onset). This limited-access schedule induces hyperphagia on both a food weight and caloric basis, but avoids protein malnutrition because rats eat chow when cafeteria diet items are not available (Rothwell and Stock, 1988).

For testing, chow- and cafeteria-diet fed rats were pretreated at 10:30 AM (i.e., 30 min prior to preweighed food and water access) with hUcn 2 or oCRF (i.c.v. 0, 0.1, 1.0, or 10.0 μ g; for reference, 1 μ g of hUcn 2 = 241 pmol and 1 μ g of oCRF = 214 pmol) in a full Latin square design with 3 to 4 drug-free days between treatments. Peptide cohorts were tested identically on different days. Food, spillage, and water were postweighed after 6 h to determine intake. Each cafeteria diet rat's "preference" for food items was defined as a mean preference ratio (item intake/total intake on a weight basis) averaged from 3 drug-free baseline days.

Kaolin and Food Intake and Motor Activity. To compare the effects of hUcn 2 (n = 27) and oCRF (n = 27) on simultaneous intake of chow and kaolin clay, rats were first acclimated to ad libitum access to hardened kaolin pellets for 1 week in their home cages. To prepare kaolin, 1% acacia gum (w/w) (Sigma-Aldrich), kaolin (aluminum silicate; Sigma-Aldrich), and water (approximately 700 ml/kg kaolin) were heated to form a paste. The paste was extruded through a pastry sleeve to form uniform columns, dried thoroughly under a fume hood for several days, and broken into 1- to 1.5-inch pellets. The testing apparatus consisted of 16 wire mesh cages ($20 \times 25 \times 36$ cm), each equipped with two horizontal infrared photocell beams situated along the long axis of the cage, 2 cm above the floor and 16 cm from one another. On the 2 d prior to testing, rats were habituated to the test cages for 3 h with food and kaolin access to reduce the novelty of the environment. On the testing day, rats were acclimated to the test cages for an additional 90 min prior to drug treatment. White noise (70 db) was present during habituation and testing.

For testing, rats were pretreated 30 min prior to access to preweighed chow and kaolin with hUcn 2 or oCRF (i.c.v. 0, 0.1, 1.0, or 10.0 μ g) in a between-subjects design (n = 6-7/group). Peptide cohorts were tested identically on different days. To mimic the time frame of testing during ad libitum access, access was provided for 6 h starting 1 h after the onset of the dark cycle. Diaper pads were placed below the cages to collect spillage. Photocell counts were recorded automatically by a computer. To rule out that anorectic effects were secondary to antidipsogenic effects of the peptides, water was not provided during pica testing. The effects of isotonic LiCl (n = 8) were compared with those of NaCl (n = 8) as a positive control.

TABLE 2

Baseline 6-h diet composition of cafeteria diet- and chow-fed rats

Statistical Analyses

Baseline intake of chow- and cafeteria diet-fed rats was compared by Student's t test. Separate analyses of variance were used to determine drug effects on absolute changes in intake. Proportional changes in intake also were examined to standardize change according to each rat's intake under vehicle conditions, thereby accounting for any baseline differences. Statistics presented in the text reflect absolute caloric analyses, which were substantively identical to those from proportional analyses. For ad libitum intake studies, dose was a within-subject factor, and diet was a between-subject factor. To allow direct comparison of peptide effects on food intake within each diet condition, a two-way mixed analysis of variance also was performed with dose as a within-subject factor and peptide as a between-subject factor. For item analysis, dose and item macronutrient composition (i.e., CHO-rich versus fat/CHO-rich) were withinsubject factors. Stability of rats' baseline item preferences was calculated as an intraclass correlation (Shrout and Fleiss, 1979). Pearson correlations were used to examine the relation of baseline preference (percent total food weight) with drug effects on intake (percent caloric change). Differences between correlations of hUcn 2 and oCRF rats were evaluated using the Fisher r-to-z transform tion. For kaolin intake analysis, dose was a between-subject fact Time, as 1-h bins, also was a within-subject factor for motor activ analysis. Following significant omnibus F ratios, linear contrast were performed to determine the dose dependence of observed fects. Within-subject Dunnett's tests were used to compare dr effects to vehicle levels. Across peptides, Bonferroni-corrected (α (0.05/3) Student's t tests were used to compare proportionally sta dardized effects at each dose. The statistical packages used w SPSS 12.0 (SPSS Inc., Chicago, IL) and Instat 3.0 (GraphPad So ware Inc., San Diego, CA).

Results

Baseline Diet Composition

Table 2 shows baseline diet composition of chow- and cafeteria-diet fed animals. Baseline levels and patterns of intake did not differ between hUcn 2 and oCRF cohorts. Access to the cafeteria diet increased caloric intake almost 2-fold in both groups. Absolute caloric intake of CHO and fat increased approximately 2- and 3-fold, respectively. Protein intake decreased 20 to 30% but remained adequate. Thus, the self-selected cafeteria diet was proportionally higher in fat and lower in protein than the chow diet. Large individual differences in item preference were observed (r = 0.84-0.92for each food item, p < 0.001). Cafeteria diet rats typically

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| | hUcn 2 Cohort | | oCRF Cohort | |
|---------------------------------------|-----------------------------------|-----------------------|-----------------------------------|-----------------------|
| | Cafeteria Diet | Chow | Cafeteria Diet | Chow |
| Water intake (ml) | 18.1 ± 1.8 | 22.9 ± 1.9 | 14.8 ± 3.2 | 19.3 ± 1.8 |
| Food intake (kcal) | $94.2 \pm 8.6^{*}$ | 51.6 ± 2.2 | $102.7 \pm 22.8^{*}$ | 52.4 ± 3.2 |
| Macronutrient composition [kcal (%)] | | | | |
| Fat | $19.9 \pm 3.4^{*} (20 \pm 3)^{*}$ | $6.9 \pm 0.3 \ (13)$ | $24.0 \pm 5.0^{*} (24 \pm 2)^{*}$ | $7.0 \pm 0.4 (13)$ |
| Carbohydrate | $66.6 \pm 5.9^{*} (71 \pm 3)^{*}$ | $33.6 \pm 1.4 (65)$ | $70.4 \pm 16.4^{*} (68 \pm 2)$ | $34.0 \pm 2.1 (65)$ |
| Protein | $7.7 \pm 0.6^* (8 \pm 0.3)^*$ | $11.0 \pm 0.5 \ (21)$ | 8.3 ± 1.7 $(8 \pm 0.3)^*$ | $11.2 \pm 0.7 \ (21)$ |
| Palatable carbohydrate-rich items | | | | |
| Total mass intake (%) | 66 ± 5 | N/A | 57 ± 5 | N/A |
| Favorite food item | | | | |
| Total mass intake (%) | 44 ± 6 | N/A | 47 ± 3 | N/A |
| Rats that favored a carbohydrate item | 6/7 | N/A | 7/9 | N/A |

N/A, not applicable.

*p < 0.05 vs. chow comparison group (Student's t test). Values reflect mean \pm S.E.M., except for frequency of carbohydrate preference.

selected more supplemental CHO-rich food items than fat/ CHO-rich items on a weight basis (see Table 2).

Water Intake

Both hUcn 2 [F(3,36) = 7.85, p < 0.001) and oCRF [F(3,48) = 4.59, p < 0.01) significantly reduced 6-h water intake during the dark cycle. As shown in Fig. 1, however, lower doses of hUcn 2 than oCRF reduced intake both on an absolute and percent reduction basis [minimum effective dose (MED) of approximately 0.1 μ g for hUcn 2 versus 10.0 μ g for oCRF by both analyses]. Linear contrasts indicated that effects of hUcn 2 [F(1,12) = 25.47, p < 0.0001] and oCRF [F(1,16) = 11.99, p < 0.005] were dose-dependent. An overall effect of diet revealed that cafeteria diet rats drank less water than chow-fed rats [F(1,28) = 4.90, p < 0.05]. Nonsignificant dose \times diet interactions indicated that antidipsogenic effects of hUcn 2 [F(3,36) = 2.02, p > 0.10] and oCRF [F(3,48) = 0.74, p > 0.50] did not differ reliably between diet conditions.

Food Intake

Total Caloric Intake. The cafeteria diet increased caloric intake during testing in both the hUcn 2 [95% increase in vehicle-treated animals; F(1,12) = 28.95, p < 0.001] and oCRF cohorts [65% increase; F(1,16) = 12.71, p < 0.005]. Both hUcn 2 [F(3,36) = 8.71, p < 0.001] and oCRF [F(3,48) = 13.87, p < 0.001] reduced 6-h total caloric intake of chow- and cafeteria diet-fed rats. Linear contrasts indicated that these effects were dose-dependent [hUcn 2, F(1,12) = 25.03, p < 0.0001; CRF, F(1,16) = 29.96, p < 0.0001]. As shown in Fig. 2, lower doses of hUcn 2 than oCRF reduced chow intake [MED of 0.1 μ g for hUcn 2 versus 10.0 μ g for oCRF; dose \times peptide, F(3,42) = 3.58, p < 0.05]. In contrast, the anorectic

effects of oCRF and hUcn 2 on total caloric intake in cafeteria diet rats did not differ significantly from one another [see Fig. 2; dose \times peptide, F(3,42) = 0.57, p > 0.60]. This reflected that hUcn 2 less potently reduced total caloric intake in cafeteria diet rats than in chow diet rats.

Item Analysis by Macronutrient Composition. Results of item analysis according to macronutrient composition are shown in Fig. 3. Item selection did not differ according to macronutrient composition in either cohort (p > 0.20). hUcn 2 reduced intake of both CHO-rich and mixed-fat/CHO items, as indicated by a main effect of dose [F(3,18) = 6.16, p]< 0.005]. However, a significant macronutrient \times dose interaction [F(3,18) = 4.71, p = 0.01] reflected that hUcn 2 more potently reduced intake of the CHO-rich food items (MED = $0.1 \mu g$) than of the mixed-fat/CHO items (MED = 10 μg) both on an absolute caloric and percent reduction basis. The greater potency of hUcn 2 was observed against both rice cakes and marshmallows (data not shown). In contrast, oCRF comparably reduced CHO-rich and mixed-fat/CHO item intake [MED = 10.0 μ g; dose, F(3,24) = 11.96, p <0.001], as indicated by a nonsignificant macronutrient \times dose interaction [F(3,24) = 0.62, p > 0.60].

Item Analysis by Preference. The relation of baseline preference for food items to subsequent drug-induced changes also differed between hUcn 2 and oCRF. Greater baseline item preference was associated strongly with greater oCRF-induced anorexia (r = -0.57 and -0.74 for CHO-rich and fat/CHO items, respectively, at the 10- μ g dose). In contrast, greater baseline preference for fat/CHO items was associated with greater sparing of these items following hUcn 2 treatment (r = 0.36 to 0.60 across the effective dose range; p < 0.05 versus oCRF), and baseline preference for CHO items did not correlate systematically



Fig. 1. Effects of pretreatment with hUcn 2 or oCRF (i.c.v. 30 min before testing) on mean $(\pm S.E.M.)$ absolute (A and C) and percent (B and D) change in 6-h water intake in chow-fed (A and B) and cafeteria diet-fed (C and D) rats. Treatments were administered approximately 30 min into the rats' dark cycle in a full Latin square design. n = 7 to 9/group. *, p < 0.05 versus respective vehicle control (Dunnett's within-subject test); #, p < 0.05 versus 0 (Student's t test with Bonferroni correction); †, p < 0.05 versus oCRF group (Student's t test with Bonferroni correction).



Fig. 2. Effects of pretreatment with hUcn 2 or oCRF (i.c.v. 30 min before testing) on mean (±S.E.M.) absolute (A and C) and percent (B and D) change in 6-h caloric food intake in chow-fed (A and B) and cafeteria diet-fed (C and D) rats. Treatments were administered approximately 30 min into the rats' dark cycle in a full Latin square design. n = 7-9/group. *, p < 0.05 versus respective vehicle control (Dunnett's within-subject test); #, p < 0.05 versus 0 (Student's t test with Bonferroni correction); $\dagger, p < 0.05$ versus oCRF group (Student's t test with Bonferroni.

with the degree of hUcn 2-induced anorexia (r = 0.24 to -0.68, not significant).

Pica Testing

Kaolin and Chow Intake. During simultaneous access to food and kaolin, hUcn 2 reduced food intake [MED = 1.0 μ g; F(3,23) = 5.22, p < 0.01] (see Fig. 4). hUcn 2 did not significantly increase kaolin clay consumption [F(3,23) = 1.61, p > 0.20], although marginally increased clay intake was observed at the 10- μ g dose (p < 0.08). oCRF also significantly reduced food intake [MED = 10.0 μ g; F(3,23) = 5.41, p < 0.01) without significantly increasing kaolin intake [F(3,23) = 0.43, p > 0.70]. In contrast, the aversive positive control LiCl elicited an almost 4-fold increase in kaolin intake at a dose similarly anorectic to the highest doses of hUcn 2 and oCRF (see Fig. 4).

Motor Activity. As shown in Fig. 5, oCRF, hUcn 2, and LiCl differed in their effects on motor activity during access to food and kaolin. Ovine CRF [F(3,23) = 3.14, p < 0.05; linear contrast p < 0.01] but not hUcn 2 [dose, F(3,23) = 1.72, p > 0.15] dose-dependently increased motor activity. In fact, pair-wise comparisons indicated that 0.1 μ g of hUcn 2 reduced motor activity during the last 2 h of testing (see Fig. 5). Neither peptide exhibited a significant dose \times time interaction (p > 0.60). LiCl, in contrast to both oCRF and hUcn 2, selectively suppressed motor activity during early portions of the 6-h observation period, as indicated by a significant treatment \times time interaction [F(5,70) = 4.36, p < 0.005]. Activity levels varied across the observation period for all compounds, as evidenced by highly significant effects of time (p < 0.001).

The present study found that hUcn 2, a selective CRF_2 receptor agonist, and oCRF, a preferential CRF_1 receptor agonist, differentially alter feeding and motor activity. Ucn 2 potently and dose-dependently reduced 6-h food and water intake at doses that did not elicit visceral illness, as evidenced by kaolin clay intake. During self-selection of a highly palatable diet, hUcn 2 reduced intake of CHO-rich items and spared mixed-fat/CHO food items. In contrast, oCRF reduced intake of pre-ferred food items without regard to macronutrient composition.

Finally, whereas oCRF dose-dependently and acutely stimu-

Discussion



Fig. 3. Item analysis of effects of pretreatment (i.c.v. 30 min before testing) with hUcn 2 (left) or oCRF (right) on normalized mean (±S.E.M.) change from baseline 6-h caloric food intake of cafeteria-diet fed rats. Intake is differentiated between carbohydrate (CHO)-rich items and mixed-fat/CHO supplement food items according to the macronutrient composition data found in Table 1. Treatments were administered approximately 30 min into the rats' dark cycle in a full Latin square design. *, p < 0.05 versus 0 (Student's t test with Bonferroni correction); †, p < 0.05 versus fat-rich items (Student's paired t test with Bonferroni correction);



lated motor activity, hUcn 2 had nondose-dependent, delayedonset motor-suppressing effects. The findings support the hypotheses that CRF_1 and CRF_2 receptors differently regulate feeding and behavioral arousal in the rat.



Fig. 5. Effects of i.c.v. pretreatment (30 min before testing) with hUcn 2 (A), oCRF (B), or isotonic LiCl (C) (i.p. 0.15 M, 20 ml/kg) on motor activity during access to kaolin clay and chow. Motor activity is expressed as the incremental (left) and cumulative (right) mean number of photocell interruptions (\pm S.E.M.) during the 6-h observation period. Treatments were administered between-subject approximately 30 min into the rats' dark cycle. n = 6-8/group. *, p < 0.05 versus respective vehicle control (Dunnett's test).

Fig. 4. Effects of pretreatment (30 min before testing) with hUcn 2 or oCRF (i.c.v.) or isotonic LiCl (i.p. 0.15 M, 20 ml/kg) on simultaneous, 6-h intake (mean + SEM) of kaolin clay and chow food. Treatments were administered between-subject approximately 30 min into the rats' dark cycle. $n = 6-8/\text{group} \cdot *, p < 0.05$ versus respective vehicle control (Dunnett's test); $\dagger, p < 0.05$ versus kaolin intake of anorectic doses of hUcn 2 and oCRF (Student's t test).

In the present and previous (Reyes et al., 2001; Inoue et al., 2003) studies, central Ucn 2 infusion had potent, prolonged anorectic effects. Previously, antisauvagine-30, a preferential CRF₂ receptor antagonist and antisense knockdown of CRF_2 receptor expression, attenuated CRF- and Ucn 1-induced anorexia (Zorrilla et al., 2003). The findings suggest that brain CRF₂ receptor activation is sufficient to suppress feeding. Reductions in cumulative 6-h intake ranged from 25 to 60% for both relatively bland (chow) and highly palatable (rice cakes and marshmallows) low-fat/CHO-rich items. In contrast, intake of mixed-fat/CHO items was spared until the $10-\mu g$ dose, which tended to increase clay intake and previously promoted a CTA (Inoue et al., 2003), suggesting malaise. Because macronutrient composition is confounded with other food properties (e.g., energy density, texture), it is not clear whether hUcn 2 altered macronutrient preference per se, as opposed to preference for an associated food characteristic (Lawton and Blundell, 1993; Mok et al., 2000). Still, oCRF did not share the macronutrient-related anorexia of hUcn 2 but suppressed intake according to item preference. The different anorexigenic profiles suggest different mechanisms for the peptides' effects on feeding.

Time course and pharmacologic analyses also support the hypothesis that CRF_1 and CRF_2 receptors mediate distinct forms of anorexia (Zorrilla et al., 2003). Nonselective CRF receptor agonists produced prolonged, short-onset anorexia in wild-type mice; abbreviated, short-onset anorexia in CRF_2 receptor null mutant mice; and delayed-onset anorexia in CRF_1 receptor knockouts. Similarly, relative to CRF_1 receptor agonists, Ucn 2 induced delayed-onset anorexia in rats. Finally, Ucn 1, which activates both CRF receptors, had additive effects on food intake relative to subtype-selective agonists.

Both peptides suppressed drinking, with hypodipsic and anorectic effects corresponding in potency and magnitude. Hypodipsia following i.c.v. CRF/Ucn 1 similarly was reported in mice, rats, chickens, and sheep (Denbow et al., 1999; Weisinger et al., 2000; Inoue et al., 2003). The peptides' anorectic effects were not secondary to hypodipsia since they were retained in pica testing, during which water was not provided. However, the converse relation of hypodipsia to anorexia is less clear. Hypodipsia might reflect secondary decreases in prandial drinking. In rats, most water intake is prandial, and food-associated drinking is motivated by the quantity of food consumed (Fitzsimons and Le Magnen, 1969). This "secondary reduction" hypothesis is supported by pair-feeding studies in sheep, wherein vehicle-treated ewes provided with only the quantity of food consumed by Ucn 1-treated subjects mirrored their hypodipsia. Analogously, many "specific" anorectics, including fenfluramine and leptin, reduce prandial drinking. Alternatively, hypodipsia might reflect a primary effect on drinking, either as independent regulation of body fluid homeostasis (possibly via CRF/ urocortin signaling elements in the supraoptic nucleus) (Van Pett et al., 2000; Imaki et al., 2001; Reyes et al., 2001) or as nonspecific, but coordinated, suppression of diverse appetitive behaviors. A better understanding of the functional significance of CRF/Ucn-induced hypodipsia will increase understanding of the significance of CRF receptor-mediated anorexia.

Neither peptide increased kaolin clay intake, an unconditioned behavior reflecting malaise (Takeda et al., 1993). Given that i.c.v. r/hCRF and hUcn 2 induce a strong CTA at doses in the 0.5 to 5.0 (Krahn et al., 1988; Heinrichs et al., 1991; Benoit et al., 2000) and 10- μ g range, respectively (Inoue et al., 2003), the lack of effects on clay intake is noteworthy. Kaolin intake is putatively a more specific measure of visceral illness than CTAs, which might be formed in response to any novel state, be it aversive or appetitive (Hunt and Amit, 1987). Supporting the sensitivity of pica testing, LiCl increased clay intake 4-fold. Increased kaolin intake in response to noxious visceral stimuli also has been observed in mice (Santucci et al., 2000), rhesus monkeys (Knezevich, 1998), and humans (Vermeer and Ferrell, Jr., 1985; Grigsby et al., 1999) (c.f., Kaopectate).

Unlike oCRF, which dose-dependently increased motor activity in a familiar environment, hUcn 2 did not increase behavioral arousal. Rather, hUcn 2 nondose-dependently suppressed later motor activity. These findings replicate those of Valdez and colleagues (2002) using the identical photocell testing apparatus but differ slightly from those of Reves and colleagues (2001), who found no effect of mUcn 2 $(i.c.v. 1 \mu g)$ on home cage activity using radiotelemetry. Dose, peptide, or procedural differences may account for differences between studies. Clearly, however, i.c.v. Ucn 2 does not increase behavioral arousal, and the present study along with that of Valdez and colleagues (2002) suggests that it can mildly suppress motor activity. Similarly, i.c.v. Ucn 3, another selective CRF2 agonist, acutely suppressed motor activity (Valdez et al., 2003). Sedation does not account for hUcn 2's anorectic effects, however. The dose-response functions for its motor and feeding effects differed, and anorexia was item-specific under choice conditions.

Although oCRF appeared to be a less potent anorectic than hUcn 2, two caveats should be considered when interpreting this finding. First, potency differences may reflect different time courses of the peptides. The 6-h time point of measurement used in the present study, selected based on the time course of i.c.v. Ucn 2 anorexia, biases effects in favor of Ucn 2. At a shorter time point, CRF might have reduced feeding more potently than Ucn 2, as observed previously with 1-h, but not 6-h, chow intake (Reyes et al., 2001). Time course issues reflect not only the delayed onset of Ucn 2 anorexia but also the abbreviated nature of CRF-induced anorexia; previously, doses as high as 5 μ g of CRF markedly reduced cumulative food intake 30 to 60 min postinjection but not 3 to 6 h postinjection in nondeprived rats (Arase et al., 1989a,b). The putatively different time courses of the peptide's anorectic activity corresponds to the reviewed time courses of CRF₁ and CRF2 receptor-mediated anorexia and suggests different endogenous roles for CRF1 and CRF2 agonists in the regulation of food intake. Because cumulative anorexia in the present study persisted at 10- μ g doses of oCRF, it was possible to compare directly the qualitative anorexia produced by hUcn 2 and oCRF. Future studies could examine qualitatively the anorexia resulting from lower doses of CRF₁ receptor agonists at briefer posttreatment intervals.

Second, vehicle-treated rats in the oCRF cohorts ate less than those in the hUcn 2 cohorts. Reduced intake was evident on the initial treatment day of Latin square designs as well as in the pica study's between-subject design and was not evident on treatment-free days prior to or following test days (data not shown). Thus, the reduced intake of vehicletreated rats in CRF cohorts likely resulted from testing factors, perhaps from proximity to "stressed," CRF-treated conspecifics (Hotta et al., 1999), rather than from random sampling error, carryover, or conditioned drug effects. Several findings counter the hypothesis that the reduced vehicle baseline of oCRF cohorts artifactually accounts for the potency differences between hUcn 2 and oCRF. First, differences also were evident as proportional decreases relative to baseline and not only as absolute decreases in intake. Second, potency differences were observed under three separate testing conditions (chow diet, cafeteria diet, and kaolin/chow pica study), suggesting that they did not result from chance distortion of dose-response profiles. Third, under vehicle conditions that achieved similar levels of intake (e.g., oCRF cafeteria diet versus hUcn 2 chow diet), CRF also was less potent than hUcn 2, indicating that potency differences cannot be ascribed solely to baseline intake differences. Still, it remains possible that the relative potency of oCRF may be underestimated because of the consistently reduced baselines. This would especially be true if the reduction of intake in vehicle-treated rats resulted from conspecific stress, rather than from test procedures unrelated to oCRF treatment. Notwithstanding the caveats regarding relative potency, the qualitatively different patterns of anorexia produced by the peptides were consistent across different degrees of anorexia.

In summary, central infusion of a CRF_2 receptor agonist suppressed intake of bland and palatable diets without inducing behavioral arousal or malaise—anorexigenic effects that qualitatively differed from those of a CRF_1 receptor agonist. Ucn 2 preferentially reduced intake of CHO-rich food items and mildly suppressed motor activity. In contrast, oCRF suppressed preferred food intake and increased behavioral activation. The dissimilar behavioral consequences of hUcn 2 and oCRF administration are consistent with the dissimilar distribution and pharmacology of CRF_2 and CRF_1 receptors and support the hypothesis of distinct CRF_1 - and CRF_2 -mediated forms of anorexia.

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