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Peripheral activation of corticotropin-releasing factor receptor 2 inhibits food intake and alters meal structures in mice

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ABSTRACT

The orexigenic effect of urocortins (Ucns), namely Ucn 1, Ucn 2 and Ucn 3 through activation of corticotropin-releasing factor (CRF) receptors, has been well characterized after injection into the brain but not in the periphery. We examined the role of CRF receptor subtype 2 (CRF_2) in the regulation of food intake using intraperitoneal (ip) injection of Ucns and the selective CRF_2 antagonist, astressin$_2$-B, and CRF_2 knockout (−/−) mice. Meal structures were monitored using an automated episodic solid food intake monitoring system. Ucn 2 (3, 10 or 30 μg/kg, ip) induced a rapid in onset, long lasting and dose-dependent decrease (38%, 66% and 86%, respectively at 4 h) of cumulative food intake after an overnight fast in mice. Ucn 3 anorexic effect was 10-times less potent. Astressin$_2$-B (30 or 100 μg/kg) injected ip, but not intracerebroventricularly, blocked the inhibitory effect of ip Ucn 1 and Ucn 2 (10 μg/kg). Fasted $CRF_{2-/-}$ mice did not respond to ip Ucn 1 (10 μg/kg). Meal microstructure analysis of the 4-h re-feeding response to an overnight fast showed that Ucn 2 (10 μg/kg, ip) decreased meal size and duration, but increased meal frequency. In mice fed *ad libitum*, Ucn 2 (30 μg/kg) injected ip before the dark phase decreased the 4-h nocturnal meal size and duration without influencing meal frequency while the 10 μg/kg dose had no effect. These data indicate that Ucns, through peripheral CRF_2 receptor-mediated induction of satiation, inhibit the eating response to a fast more potently than the physiological nocturnal feeding in mice.

1. Introduction

Urocortins (Ucns), including Ucn 1, Ucn 2 and Ucn 3, belong to the mammalian corticotropin-releasing factor (CRF) family [16,28,36]. Ucn 1 displays a high affinity to both CRF receptor types 1 and 2 (CRF_1 and CRF_2), Ucn 2 binds with high affinity to CRF_2 but poorly to CRF_1 [8,13] and Ucn 3 is the most selective endogenous CRF_2 agonist but has lower binding affinity to CRF_2 than Ucn 2 [11,13,16]. The distribution of Ucns in the central nervous system is more restricted than that of CRF, while the peptides are widely expressed in the periphery, including the cardiovascular system, gastrointestinal tract, pancreas and endocrine glands in rodents [8]. Moreover, functional studies indicate that Ucns may have a more significant role in the periphery than in the brain, as indicated by the powerful effects of peripheral administration of Ucns on cardiovascular, gastrointestinal, reproductive and immune functions and energy balance [8].

In particular, convergent studies showed that an acute intraperitoneal (ip) injection of Ucn 1 was more potent than CRF, leptin or sulfated cholecystokinin-8 (CCK-8S) to induce a sustained inhibition of feeding response to a fast in lean mice [1,35,37]. Likewise, chronic subcutaneous infusion of Ucn 1 for three days suppressed daily food intake in mice [31]. In these studies, Ucn 1 injected peripherally displays a similar potency as intracerebroventricular (icv) injection [6,24,31]. We also previously demonstrated a synergistic interaction between ip injection of Ucn 1 and CCK-8S to reduce the feeding response to a fast and gastric emptying of a non-nutrient solution in mice [10]. Only a few studies have assessed the food intake alterations induced by other members of the urocortin family. Ucn 2, and to a smaller extent Ucn 3, inhibits food intake in the light phase after a fast and in the dark phase in mice [10,35]. So far, the specific involvement of peripheral CRF_2 in the peripherally

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administered Ucns-induced suppression of food intake is largely unexplored. Our previous study showed that selective CRF_1 antagonists did not alter the ip Ucn 1-induced suppression of food intake in fasted mice while the first generation of selective CRF_2 antagonist, antisauvagine-30 [30], resulted in a partial reversal, at a dose that completely suppressed ip Ucn 1-induced inhibition of gastric emptying [37]. Moreover, while meal pattern analysis is of primary importance to assess mechanisms regulating eating behavior [9], the underlying food intake microstructure induced by peripheral activation of CRF_2 receptors remains unknown.

In the present study, we first established the dose-related effects of Ucn 2 and Ucn 3 injected ip on the feeding response to an overnight fast in mice. The role of peripheral CRF_2 receptors in mediating ip Ucn 1 and Ucn 2 anorexigenic effects was investigated using peripheral or icv injection of the potent and long acting selective CRF_2 antagonist, astressin$_2$-B [29], and CRF_2 knockout (−/−) mice. To determine whether peripheral CRF_2 agonists may influence food intake through changes in gastric transit of a solid meal or behavior, the rate of gastric emptying of a standard chow meal as well as locomotor activity were assessed in ip Ucn 2-injected fasted/refed mice. Since recent studies indicate that $CRF_{2-/-}$ mice have increased dark phase meal size [33], we examined the influence of CRF_2 receptor activation by ip Ucn 2 on meal onset, size, duration and frequency using an automated continuous standard chow food intake monitoring system, a device recently developed for mice (BioDaq, Research Diets, Inc., New Brunswick, NJ). Both light phase food intake in overnight fasted mice and nocturnal feeding in freely fed mice were monitored and analyzed.

2. Methods

2.1. Animals

Adult male C57BL/6 (7–10 weeks-old, body weight 21–30 g, Harlan, San Diego, CA) and male $CRF_{2-/-}$ mice (Oregon Health & Science University, Portland, OR) were used. $CRF_{2-/-}$ mice and their littermates were generated as previously described [5] and backcrossed onto a C57BL/6J background for eight generations. The mice were group-housed (4/cage) and fed *ad libitum* with standard rodent chow (Prolab RMH 2500; PMI Nutrition International, Inc., Brentwood, MO, USA) and water under controlled temperature (21–23 °C) and light conditions (6:00 AM–6:00 PM). All experiments, except otherwise stated, started around 9:00 AM in mice fasted overnight. NIH guidelines were followed in all experimental procedures that were undertaken under the auspices of an OLAW Assurance of Compliance (A3002-01) and performed according to approved Animal Components of Research Protocols (IACUC Committee of the VA Greater Los Angeles Healthcare System, # 99-06-820 and 99-127-07).

2.2. Peptides

In initial binding studies, human Ucn 2, compared to mouse Ucn 2, and mouse Ucn 3, compared to human Ucn 3, were shown to have higher binding affinity to both CRF_2 receptor isoforms in membrane of mouse $CRF_{2\beta}$ stably transfected cells [16]. Therefore, the Ucn peptides used were human Ucn 2, mouse Ucn 3 and mouse Ucn 1, as well as the selective CRF_2 antagonist, astressin$_2$-B [29]. All peptides were synthesized as previously described [29] at the Clayton Foundation Laboratories (Salk Institute, La Jolla, CA). The peptides in powder form were stored at −80 °C, and dissolved in sterile distilled water immediately before use. CCK-8S (Bachem, Torrance, CA) was stored at −80 °C as stock solution (1 μg/μl saline) and diluted in saline before use.

2.3. Intracerebroventricular injections

The icv injections were performed as previously described [20]. Mice were acutely anaesthetized with isoflurane (Ethrane, Anaquest, Madison, WI), the head was carefully hand-restrained on a gauze and the injection site localized by visualizing an equilateral triangle between the eyes and the back of the head with the apex of the triangle being the injection site with the least resistance. The injection was performed manually using a 25 μl Hamilton syringe fitted with a 30 gauge needle that was shortened by adding a sleeve made from peristaltic pump tubing to obtain a needle length of 4 mm. After the procedure, which lasted approximately 1 min, mice recovered within 3–4 min and were monitored in their home cages afterwards.

2.4. Measures of food intake and gastric emptying of solid meal in fasted mice

Food intake of a solid nutrient meal was measured as detailed in our previous studies in mice [37]. Animals were housed singly during the overnight fast and at 9:00 AM, given pre-weighed normal rodent chow for the duration of the experimental period. Food intake was calculated as the difference between the food weights before and after the feeding period, corrected for spillage. Cumulative food intake was calculated by addition of the values at the different time periods. For food intake experiments, mice were used 3 times, by a Latin Square design, with a 6–7 days recovery period between the experiments.

In separate groups, gastric emptying was determined at 2 h after a 1-h re-feeding in mice fasted overnight as in our previous studies [20]. After the 1-h re-feeding period, food and water were removed and the treatments applied as appropriate. Mice were euthanized 2 h later by cervical dislocation and the stomach was removed and weighed. Then, the stomach was opened, its content washed out with tap water, and the gastric wall dried and weighed. The amount of food (g) retained in the stomach was quantified as the difference between the total weight of the stomach with the content and the weight of the gastric wall. The gastric emptying during the experimental period was calculated according to the following equation: gastric emptying (%) = (wet weight of content recovered from the stomach/weight of food intake during 1-h re-feeding) × 100.

2.5. Locomotor activity

Locomotor activity was measured using a similar method as that described previously [2]. Overnight fasted mice were placed in individual Plexiglas cages (10 × 15 × 25 cm) with the bottom divided into 15 equal squares and with access to water and pre-weighed food. Locomotor activity was monitored by visual examination of the total number of squares crossed by the animals during the observation time. Behavior was monitored by an investigator who was blinded to the treatments.

2.6. Automated monitoring of meal microstructures

The microstructure analysis of feeding behavior was conducted using the BioDAQ episodic Food Intake Monitor for mice (BioDAQ, Research Diets, Inc., New Brunswick, NJ), which allows continuous monitoring of meal patterns in undisturbed mice with minimal human interference as recently described [32]. Mice were accustomed for one week to single housing and to access the standard rodent diet (AIN-93M, Research Diets, Inc.) through the feeding hopper attached to a regular housing cage which contains environmental enrichment and bedding material. In these studies, we used the AIN-93M balanced rdent diet that causes less spillage to assure more accurate measurements. Water was provided *ad libi-*