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ORIGINAL ARTICLE

Centrally administered urocortin 3 inhibits food intake and gastric emptying in mice

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Abstract Urocortin 3 (Ucn3) is recognized as a member of the corticotropin-releasing factor (CRF) family, which plays an important role in regulating food intake. We investigated the effects of centrally administered Ucn3 on food intake and gastric emptying in mice. Intracerebroventricular (ICV) administration of Ucn3 (0.1–1 nmol per mouse) decreased food intake in a dose-dependent manner. The inhibitory effect of Ucn3 on food intake was less potent than that of centrally administered CRF and Urocortin 1. ICV administration of Ucn3 (0.1–1 nmol per mouse) decreased the gastric emptying rate in a dose-dependent manner. Ucn3 decreased food intake in high-fat diet-fed obese mice as well as in lean mice. These results indicated that Ucn3 influences feeding behavior and gut motility, and may be a promising therapeutic target in the treatment of eating and functional gastrointestinal disorders.

Keywords Urocortin 3 · Corticotropin-releasing factor · Food intake · Gastric emptying · Mice

Introduction

Corticotropin-releasing factor (CRF), which was isolated from ovine hypothalamus and structurally characterized in 1981 [1], is the primary mediator of stress responses. In recent years, urocortin 1 (Ucn1), urocortin 2 (Ucn2), and urocortin 3 (Ucn3) were cloned and recognized as members of the CRF family [2–4].

The CRF family peptides exert their effects through two receptor subtypes: CRF receptor 1 ($CRFR_1$) and CRF receptor 2 ($CRFR_2$) [5]. $CRFR_1$ mediates adrenocorticotropic hormone release, leading to anxiety-like behavior and stress-induced short-term anorexia [3, 6], whereas $CRFR_2$ mediates stress-coping responses, including anxiolytic behavior, and long-term anorexia [3]. CRF and Ucn1 have a high affinity for both receptors, but Ucn1 binds to $CRFR_2$ with a higher affinity than to $CRFR_1$ [2]; Ucn2 and Ucn3 bind specifically to $CRFR_2$ [3, 4].

Previous studies have shown that intracerebroventricular (ICV) administration of CRF induces anxiety and anorexia [7]. ICV administration of Ucn1 decreases food intake [8] and increases anxiogenic behavior [9]; Ucn1 is more potent than CRF in suppressing food intake [8]. Ucn2 has been implicated in the central regulation of food intake and autonomic functions [10]. It has been reported to exhibit cardiovascular physiologic actions and exert cardioprotective effects against ischemia–reperfusion injury [11, 12].

In 2001, Ucn3, a 38-amino acid peptide, was identified in humans and mice. Ucn3-expressing neurons were found predominantly within the medial amygdala, hypothalamic median preoptic nucleus, and rostral perifornical area lateral to the hypothalamic paraventricular nucleus [13]. Previous studies have shown that Ucn3 regulates cardiovascular and immune functions via the CRF receptors. ICV administration of Ucn3 increased blood pressure and heart rate [14]. In addition, peripherally administered Ucn3 influenced steroidogenesis and cell proliferation [15]. Fedeli et al. reported that ICV administration of Ucn3 inhibited food intake in food-restricted rats [16]. However,

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there are no reports on the effects of Ucn3 on gut motility, and no studies have compared the effects of ICV administration of Ucn3 on food intake with the effects of other CRF family peptides. In this study, we investigated the effects of Ucn3, in comparison with other CRF family peptides, on food intake and gastric emptying rate in mice.

Results

Effect of ICV administration of Ucn3 on the food intake

ICV administration of Ucn3 (0.1–1 nmol per mouse) significantly decreased food intake. This anorexigenic action was apparent 20 min (ACSF, 0.15 ± 0.03 g; 0.1 nmol, 0.00 ± 0.00 g, $P < 0.01$; 0.3 nmol, 0.05 ± 0.02 g, $P < 0.01$; 1 nmol, 0.02 ± 0.02 g, $P < 0.01$) after Ucn3 administration, and continued for 12 h (Fig. 1). Cumulative food intake was still significantly lower in the mice treated with 1 nmol Ucn3 24 h (ACSF, 6.57 ± 0.26 g; 0.1 nmol, 6.15 ± 0.17 g; 0.3 nmol, 5.64 ± 0.35 g; 1 nmol, 5.48 ± 0.37 g, $P < 0.05$) after administration. The change in body weight had significantly decreased in the 0.3 nmol and 1 nmol Ucn3-treated mice 24 h (ACSF, 3.62 ± 0.27 g; 0.1 nmol, 3.56 ± 0.20 g; 0.3 nmol, 2.84 ± 0.19 g, $P < 0.05$; 1 nmol, 2.54 ± 0.17 g, $P < 0.01$) after administration. Water intake was inhibited only by the highest dose (1 nmol) of Ucn3 24 h (ACSF, 10.28 ± 0.56 ml; 0.1 nmol, 9.80 ± 0.83 ml; 0.3 nmol, 7.34 ± 0.51 ml; 1 nmol, 7.26 ± 0.61 ml, $P < 0.01$) after administration. Ucn3 had no significant effect on cumulative food intake 48 h (ACSF, 12.04 ± 0.38 g; 0.1 nmol, 11.64 ± 0.21 g; 0.3 nmol, 10.77 ± 0.44 g; 1 nmol, 10.77 ± 0.50 g) after administration.

Food intake in feeding studies performed during the dark phase was inhibited only by the highest dose (1 nmol) of Ucn3 1 h (ACSF, 0.09 ± 0.03 g; 0.1 nmol, 0.04 ± 0.02 g; 0.3 nmol, 0.00 ± 0.00 g, $P < 0.05$; 1 nmol, 0.00 ± 0.00 g, $P < 0.05$; $n = 8$ per group), 12 h (ACSF, 2.42 ± 0.29 g; 0.1 nmol, 1.65 ± 0.35 g; 0.3 nmol, 0.89 ± 0.23 g, $P < 0.05$; 1 nmol, 0.83 ± 0.53 g, $P < 0.05$; $n = 8$ per group), and 24 h (ACSF, 2.90 ± 0.28 g; 0.1 nmol, 2.34 ± 0.32 g; 0.3 nmol, 1.73 ± 0.24 g; 1 nmol, 1.42 ± 0.55 g, $P < 0.05$; $n = 8$ per group) after ICV administration.

Effect of ICV administration of CRF family peptides on the food intake of fasted mice

ICV administration of the other CRF family peptides (0.1 nmol per mouse) significantly inhibited feeding behavior 1–2 h after administration (Fig. 2). CRF, Ucn1, Ucn2, and Ucn3 elicited this effect 20 min–2 h, 20 min–12 h, 1–2 h, and 1–2 h after administration, respectively. CRF and Ucn1 significantly decreased food intake 20 min (ACSF, 0.16 ± 0.02 g; CRF, 0.00 ± 0.00 g, $P < 0.01$; Ucn1, 0.01 ± 0.01 g, $P < 0.01$; Ucn2, 0.12 ± 0.07 g; Ucn3, 0.05 ± 0.03 g) after administration. Ucn1 decreased food intake more potently than the other CRF family peptides.

Effect of ICV administration of Ucn3 on gastric emptying

ICV administration of Ucn3 (0.1–1 nmol per mouse) significantly decreased the gastric emptying rate in a dose-dependent manner (Fig. 3).

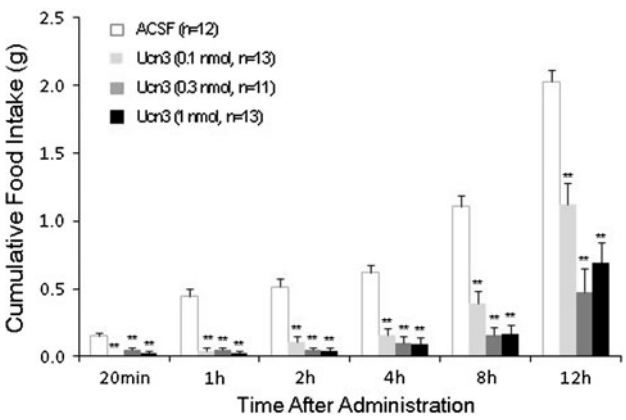

Fig. 1 Effects of ICV administration of Ucn3 (0.1–1 nmol per mouse) on cumulative food intake in food-deprived mice. Each *bar* represents the mean (SE), and *n* indicates the number of mice used. ** $P < 0.01$ as compared to the artificial cerebrospinal fluid (ACSF)-treated controls by Dunnett's test

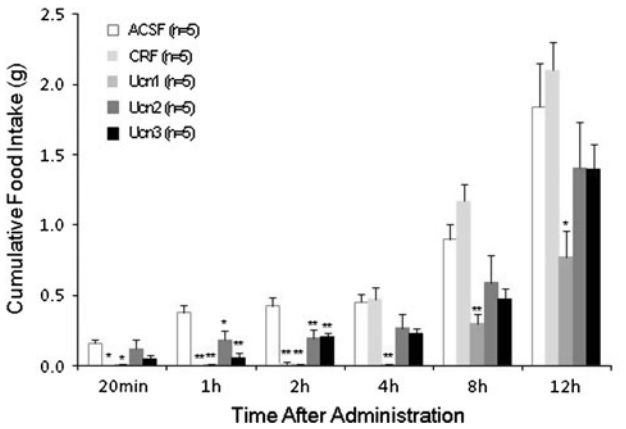

Fig. 2 Effects of ICV administration of 0.1 nmol per mouse CRF, Ucn1, Ucn2, and Ucn3 on cumulative food intake of food-deprived mice. Each *bar* represents the mean (SE), and *n* indicates the number of mice used. * $P < 0.05$, ** $P < 0.01$ as compared to the artificial cerebrospinal fluid (ACSF)-treated controls by Dunnett's test

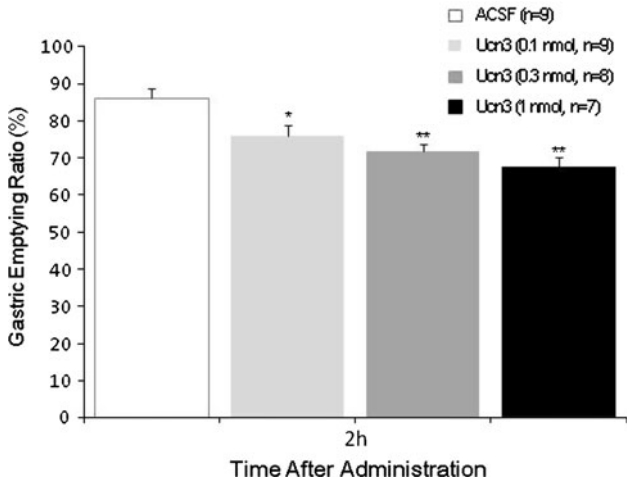

Fig. 3 Effects of ICV administration of Ucn3 (0.1–1 nmol per mouse) on the gastric emptying rate 2 h after administration. Each *bar* represents the mean (SE), and *n* indicates the number of mice used. * $P < 0.05$, ** $P < 0.01$ as compared to the artificial cerebrospinal fluid (ACSF)-treated controls by Dunnett's test

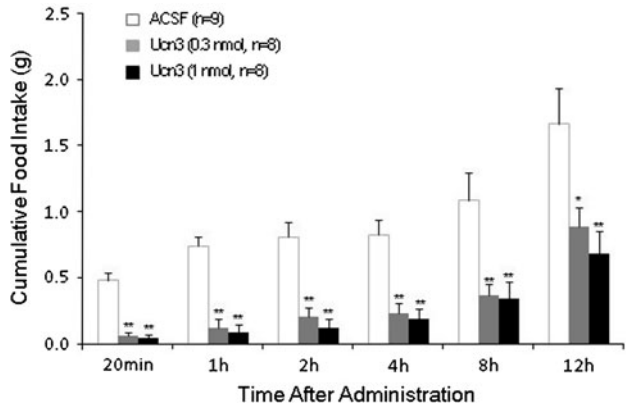

Fig. 4 Effects of ICV administration of Ucn3 (0.3–1 nmol per mouse) on cumulative food intake of food-deprived mice fed a high-fat diet for 1 month. Each *bar* represents the mean (SE), and *n* indicates the number of mice used. * $P < 0.05$, ** $P < 0.01$ as compared to the artificial cerebrospinal fluid (ACSF)-treated controls by Dunnett's test

Effect of ICV administration of Ucn3 on the food intake of mice fed high-fat diet for 1 month

ICV administration of Ucn3 (0.3–1 nmol per mouse) significantly decreased food intake in a dose-dependent manner. This anorexigenic action was apparent 20 min after ICV administration, and continued for 12 h (Fig. 4).

Discussion

Previous studies have reported that various peptides, including leptin [17] and ghrelin [18], influence feeding behavior. The CRF family peptides regulate feeding suppression [11], anxiety [12], and gut motility [19]. A previous study has shown that Ucn1 is more potent than CRF in suppressing food intake [8]. Recently, we reported that intraperitoneally administered Ucn1 decreases food intake and weight gain more potently than Ucn2 and Ucn3 in mice [20]. In this study, the intracerebroventricularly administered Ucn3 also exerted an inhibitory effect on food intake, although the potency for decreasing food intake differed with the experimental conditions. In addition, the effect of intracerebroventricularly administered Ucn3 on food intake was less potent than that of centrally administered CRF and Ucn1. Ucn1 had the most prolonged inhibitory effect on food intake. On the other hand, CRF induced short-term satiety compared with other peptides. The different binding affinity to $CRFR_1$ and $CRFR_2$ may be the cause of the different effects on feeding behavior.

Neuropeptides in the hypothalamus play a pivotal role in physiological mechanisms regulating food intake. Recent studies have shown that Ucn3 and thyrotropin-releasing hormone (TRH) are coexpressed in the neurons of the perifornical area and bed nucleus of the stria terminalis and that Ucn3/TRH fiber heavily innervated the hypothalamic ventromedial nucleus [21]. Other studies have reported the inhibitory effect of intracerebroventricularly administered TRH on food intake [22].

Previous studies have shown that feeding behavior is closely related to gut motility. Considerable evidence has accumulated to indicate that rapid gastric emptying is closely associated with overeating and obesity as is delayed gastric emptying to anorexia and cachexia [23, 24]. In addition, orexigenic peptides, including ghrelin [18], increase gastric motility/emptying, while anorexigenic peptides, including cholecystokinin [25], CRF, and Ucn1 [9], decrease it. Recently, Czimmer et al. reported that intracisternal administration of Ucn2 decreased gastric emptying in rats [26]. In our study, ICV administration of Ucn3 inhibited gastric emptying in mice.

It has been shown that $CRFR_2$ mRNA is highly expressed in the ventromedial nucleus of hypothalamus (VMH), classically referred to as the satiety center [27]. This finding indicates that the VMH is the possible site of action for central Ucn3 on food intake and gastric emptying.

It has been shown that the CRF family peptides play a major role in mediating behavioral responses to stressors via activation of the hypothalamic-pituitary-adrenal axis; they are involved in the pathogenesis of stress-mediated diseases and eating disorders, including anorexia and obesity [28–32]. In our study, Ucn3 decreased food intake in high-fat diet-fed obese mice as well as in lean mice. This finding indicates that Ucn3 is associated with the pathogenesis of obesity and is a potential target for the treatment

of metabolic diseases. Recently, Sharpe et al. showed that central administration of Ucn3 decreases limited-access ethanol intake in mice [33]. These results indicated that Ucn3 influences gut motility and emotional behaviors including feeding and ethanol intake.

Materials and methods

Animals and chemicals

Seven-week-old male C57BL/6 J mice were purchased from CLEA Japan, Inc. (Tokyo, Japan). They were individually housed in a controlled environment (22 ± 2°C, 50 ± 10% humidity, 12:12 h light:dark cycle with lights on at 07:00 AM) and had free access to laboratory chow. Mouse CRF, Ucn1, Ucn2, and Ucn3 were purchased from Phoenix Pharmaceuticals, Inc. (CA, USA). For ICV administration, the mice were anesthetized with sodium pentobarbital (100 mg/kg intraperitoneal administration) and placed in a stereotaxic instrument 7 days before the experiments. A hole was made in each mouse's skull using a needle inserted 1.0 mm lateral to the central suture and 0.5 mm posterior to the bregma. A 24-gauge cannula beveled at one end over a distance of 2.5 mm was implanted into the lateral ventricle for ICV administration. The location of the cannula tip was confirmed by administration of dye (Evans blue 0.5% and Zelatin 5%) and histologic examination of frozen brain sections. The cannula was fixed to the skull with dental cement and capped with silicon without an obtruder. A 27-gauge administration insert was attached to a microsyringe by PE-20 tubing. All experimental procedures were approved by the Laboratory Animal Committees of Kagoshima University Graduate School and were performed in accordance with the "Guidelines for the Care and Use of Laboratory Animals".

ICV administration in normal diet-fed mice

Before feeding tests, mice were deprived of food for 16 h with free access to water, or were given free access to food and water. The mice were fed a standard diet (CE-2, CLEA Japan, Inc., Tokyo, Japan). Just before administration, Ucn3 was diluted in 4 μl of artificial cerebrospinal fluid (ACSF: NaCl, 138.9 mM; KCl, 3.4 mM; $CaCl_2{\cdot}2H_2O$, 1.26 mM; $NaHCO_3$, 4.0 mM; $NaH_2PO_4{\cdot}2H_2O$, 0.6 mM; and glucose, 5.6 mM) for ICV administration except a comparison study. In a comparison study, CRF family peptides (CRF, Ucn1, Ucn2, and Ucn3) were diluted in 4 μl of ACSF containing 1% dimethylsulfoxide for ICV administration. Dark-phase feeding studies administrations were done immediately before lights-off (07:00 PM) for non-food-deprived mice. For food-deprived mice, ICV administration of the peptides was at 08:30 AM. Food intake was calculated by subtracting the weight of the uneaten food from the weight of the food provided after checking for spillage at 20 min and at 1, 2, 4, 8, 12, and 24 h after ICV administration. Body weight and water intake were measured at 0 and 24 h.

Gastric emptying

Before the experiments for gastric emptying, the mice were fasted for 16 h with free access to water. During the experiment, the fasted mice were given free access to preweighed pellets for 1 h. The mice in the test and control group were then administered Ucn3 (0.1–1 nmol per mouse) and ACSF, respectively. After ICV administration, the mice were fasted for 2 h. Food intake was calculated by subtracting the weight of the uneaten pellets from the weight of the pellets initially provided. Mice were euthanized by cervical dislocation 3 h after the start of the experiment. Immediately afterward, the stomach was exposed by laparotomy, quickly ligated at both the pylorus and cardia, and removed. The stomach contents were dried using a vacuum freeze-drying system (Labconco Corp., MO, USA) and weighed. Gastric emptying was calculated using the following formula: gastric emptying (%) = {1 − (dry weight of food recovered from the stomach/weight of food ingested)} × 100.

Acute ICV administration in high-fat diet-fed mice

The mice were fed a high-fat diet (60% kcal fat, 5.2 kcal/g; Diet D12492, Research Diets, Inc., NJ, USA) for 1 month. They were then fasted for 16 h before ICV administration of Ucn3 (0.3 and 1 nmol per mouse). Food intake was measured at 20 min and at 1, 2, 4, 8, and 12 h after administration.

Statistical analysis

Results are expressed as mean value ± standard error (SE). Comparisons with controls were performed by Dunnett's tests. *P* values less than 0.05 were considered to be statistically significant.

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