

# Effects of peripherally administered urocortin 3 on feeding behavior and gastric emptying in mice

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**Abstract.** Human and mouse urocortin 3 (Ucn3) were first identified in 2001. Ucn3 binds selectively to corticotropin-releasing factor receptor type 2 (CRF-R2). Previous studies have shown that centrally administered Ucn3 decreases food intake in rats. However, the role of Ucn3 in the regulation of gut motility remains to be determined. In the present study, we investigated the effects of peripherally administered Ucn3 on food intake and gastric emptying in mice. After intraperitoneal (i.p.) administration of Ucn3, food intake was measured in the light and dark phases, and the rate of gastric emptying was determined. We found that i.p. administration of Ucn3 significantly inhibited feeding behavior in mice, and significantly delayed gastric emptying 1-2 h after administration in a dose-dependent manner. These results suggest that Ucn3 contributes to the modulation of feeding behavior and gut motility. Thus, Ucn3 and CRF-R2 may be involved in the pathogenesis of functional gastrointestinal and eating disorders.

## Introduction

Corticotropin-releasing factor (CRF), a 41-amino acid peptide, was first isolated from the ovine hypothalamus in 1981 (1,2). CRF, acting through the hypothalamic-pituitary-adrenal axis, is a major mediator of the response to stress, and plays a key role in the regulation of anxiety-like behavior and energy

balance (3). The CRF system consists of two CRF receptor subtypes: CRF receptor type 1 (CRF-R1) and CRF receptor type 2 (CRF-R2) (3-5). CRF-R1 is implicated in anxiety behavior (3,6-8), while CRF-R2 is involved in feeding behavior and gut motility (3,9,10).

Rat urocortin 1 (Ucn1), a 40-amino acid peptide, was identified in the rat brain in 1995 (11). Ucn1 is structurally similar to CRF and binds to CRF-R1 and CRF-R2. Mouse urocortin 2 (Ucn2), a 38-amino acid peptide, was identified in the mouse brain in 2001 (12) and exhibits a more potent affinity for CRF-R2 than for CRF-R1. Human and mouse urocortin 3 (Ucn3) are 38-amino acid peptides that were also identified in 2001 (13,14). Ucn3 binds selectively to CRF-R2. Mouse Ucn3 mRNA expression has been detected in the hypothalamus, amygdalae, brainstem, small intestine and skin of mice (14). Previous studies have shown that centrally administered Ucn3 decreases food intake in rats (15). Recently, Czimmer *et al* showed that intracisternal administration of Ucn2 into conscious rats inhibited gastric emptying of a viscous non-caloric meal (16). However, the role of Ucn3 in the regulation of gut motility remains to be determined.

In this study, we investigated the effects of peripherally administered Ucn3 on food intake and gastric emptying in mice.

## Materials and methods

**Animals and chemicals.** Seven-week-old C57BL/6L male mice weighing 19-24 g (Clea Japan, Inc., Tokyo, Japan) were used in this study. The mice were individually housed in a regulated environment (23±1°C, 55±10% humidity, 12:12 h light:dark cycle, with lights on at 07:00 am). Food and water were available *ad libitum*, except when otherwise indicated.

Experiments were approved by the Laboratory Animal Committees of Kagoshima University Graduate School and were performed according to the Guidelines for the Care and Use of Laboratory Animals, which are standardized to the Japanese national guidelines.

Mouse Ucn3 (FW 4171.56; purity ≥95%) was purchased from Phoenix Pharmaceuticals, Inc. (CA, USA). Prior to the experiments, the mouse Ucn3 was diluted in 100 µl of physiological saline (Otsuka, Tokyo, Japan). An equal amount of physiological saline served as the control solution.

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**Abbreviations:** CRF, corticotropin-releasing factor; CRF-R1, CRF receptor type 1; CRF-R2, CRF receptor type 2; i.p., intraperitoneal(-ly); SE, standard error; Ucn1, urocortin 1; Ucn2, urocortin 2; Ucn3, urocortin 3

**Key words:** urocortin 3, feeding, gastric emptying, corticotropin-releasing factor

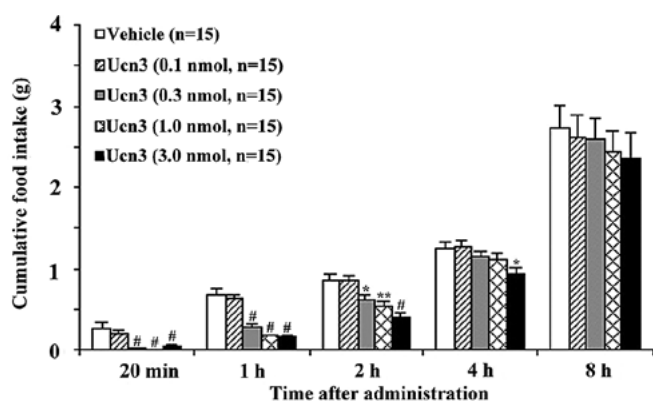


Figure 1. Inhibitory effect of i.p. administration of Ucn3 (0.1-3 nmol/mouse) on cumulative food intake in food-deprived mice in the light-phase feeding experiments. Error bars, mean  $\pm$  SE; n, number of mice used. \* $P$ <0.05, \*\* $P$ <0.01 and # $P$ <0.001, statistically significant difference compared to the controls.

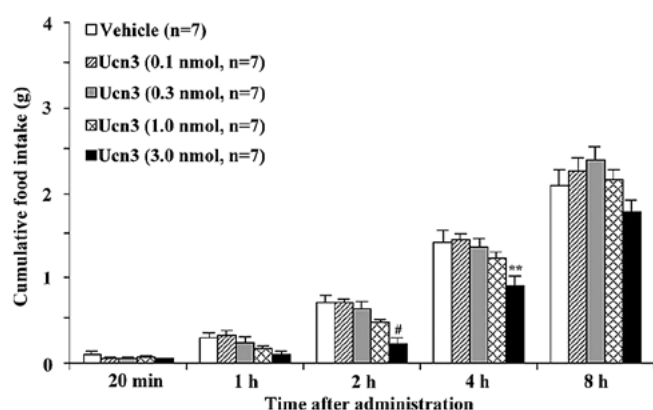


Figure 2. Inhibitory effect of i.p. administration of Ucn3 (0.1-3 nmol/mouse) on cumulative food intake in non-food-deprived mice in the dark-phase feeding experiments. Error bars, mean  $\pm$  SE; n, number of mice used. \*\* $P$ <0.01 and # $P$ <0.001, statistically significant difference compared to the controls.

**Food intake experiments.** Mice were provided with a standard diet (CE-2; Clea Japan, Inc.). Experiments were performed during the light and dark phases. Light-phase experiments were started at 08:30 am. Before the feeding tests were conducted, the mice were deprived of food for 16 h, but were allowed free access to water. After i.p. administration of Ucn3, the mice were allowed free access to a pre-weighed quantity of food. Food intake was calculated by subtracting the uneaten food from the initial quantity of food at 20 min, 1, 2, 4 and 8 h, taking into account food spillage. Dark-phase food intake experiments were started at 07:00 pm, and the mice were allowed free access to food and water before the experiments. After i.p. administration of Ucn3, food intake was calculated by subtracting the uneaten food from the initial quantity of food at 20 min, 1, 2, 4 and 8 h, taking into account food spillage. Cumulative food intake was calculated at 20 min, 1, 2, 4 and 8 h.

**Gastric emptying experiment.** Prior to the gastric emptying experiments, the mice were deprived of food for 16 h, but were allowed free access to water. The fasted mice were given free access to a pre-weighed quantity of food for 1 h. The mice were deprived of food again for 1 or 2 h after i.p. administration. Food intake was calculated by weighing the

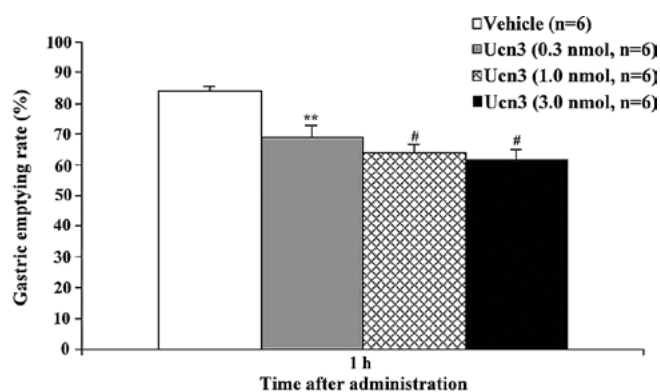


Figure 3. Inhibitory effect of i.p. administration of Ucn3 (0.1-3 nmol/mouse) on the gastric emptying rate 1 h after Ucn3 administration. Error bars, mean  $\pm$  SE; n, number of mice used. \*\* $P$ <0.01 and # $P$ <0.001, statistically significant difference compared to the controls.

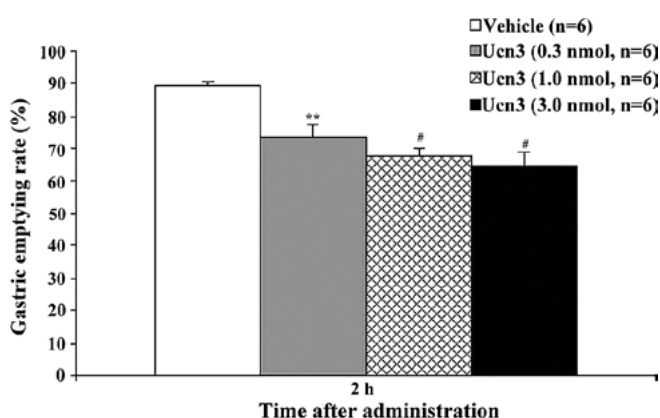


Figure 4. Inhibitory effect of i.p. administration of Ucn3 (0.1-3 nmol/mouse) on the gastric emptying rate 2 h after Ucn3 administration. Error bars, mean  $\pm$  SE; n, number of mice used. \*\* $P$ <0.01 and # $P$ <0.001, statistically significant difference compared to the controls.

amount of uneaten food as described above. The mice were sacrificed by cervical dislocation 2 or 3 h after the start of the experiments. Immediately after the mice were sacrificed, laparotomy was performed to expose the stomach, which was then quickly ligated at the pylorus and cardia, and removed. The contents were dried using a vacuum freeze-drying system (Model 7670500; Labconco Corp., MO, USA), and the dry content was weighed. Gastric emptying was calculated using the formula: Gastric emptying (%) = [1 - (dry weight of recovered food from the stomach/weight of food intake)]  $\times$  100.

**Statistical analysis.** The effects of different doses of Ucn3 were evaluated by an *a priori* Dunnett's test. Results from mice administered the specified individual doses of Ucn3 were compared to results from mice that received the saline control solution. For all comparisons,  $P$ -values <0.05 were considered to be statistically significant. Results are expressed as the mean  $\pm$  standard error (SE).

## Results

To investigate whether Ucn3 influences feeding behavior, we first examined the effects of an i.p. administration of Ucn3

(0.1-3 nmol/mouse) on food intake in food-deprived mice during the light-phase. In these mice, it was found that Ucn3 significantly inhibited feeding behavior in a dose-dependent manner (Fig. 1). Cumulative food intake significantly decreased from 20 min to 2 h after Ucn3 administration. We then investigated the effects of Ucn3 (0.1-3 nmol/mouse) on food intake in non-food-deprived mice during the dark-phase. It was found that Ucn3 significantly decreased food intake 2-4 h after its administration (Fig. 2). Lastly, we examined whether Ucn3 influences the gastric emptying rate. It was found that Ucn3 significantly delayed gastric emptying 1-2 h after its administration in a dose-dependent manner (Figs. 3 and 4).

## Discussion

To date, various hormones including leptin and ghrelin have been reported to be involved in feeding behavior. We previously reported that peripherally administered Ucn1 was more potent than CRF in the regulation of anorexigenic activity when the two were administered at the same doses (17). Ohata and Shibasaki found that intracerebroventricular administration of Ucn3 and Ucn2 decreased food intake in rats fed *ad libitum* (15). In our study, peripherally administered Ucn3 reduced food intake as effectively as the other CRF family peptides, and mediated anorexigenic activity in both the light- and dark-phase experiments.

It has been shown that gastrointestinal motility influences feeding behavior. Considerable evidence indicates that delayed gastric emptying is closely related to anorexia and cachexia, while rapid gastric emptying is found to be associated with overeating and obesity (18,19). We previously reported that Ucn1 more potently suppressed gastric emptying than did CRF when the two were administered at the same doses (17). Gourcerol *et al* reported that peripheral administration of Ucn2 delays gastric emptying in mice (20). In our study, peripherally administered Ucn3 inhibited gastric emptying as effectively as other CRF family peptides. Recently, Liu *et al* reported that Ucn1, 2 and 3 function as neuromodulators and influence the excitatory action of myenteric neurons (21). The induction of the excitatory action of myenteric neurons by Ucn1 was primarily mediated by CRF-R1, whereas its induction by Ucn2 and Ucn3 was mediated by CRF-R2 (21). Previous studies have shown that CRF-R2 is present in various peripheral tissues, including the gastrointestinal tract (22).

These findings and the results of the present study indicate that Ucn3 and other CRF family peptides are involved in the development of functional gastrointestinal and eating disorders. Ucn3 and CRF-R2 may therefore be promising targets for the treatment of various diseases.

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