ORIGINAL ARTICLE

Urocortin 1 administered into the hypothalamic supraoptic nucleus inhibits food intake in freely fed and food-deprived rats

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Abstract Peptides of the corticotropin-releasing hormone/Urocortin (CRH/Ucn) family are known to suppress appetite primarily via CRH$_2$ receptors. In the rat hypothalamic supraoptic nucleus (SON), synthesis of both Ucn1 and CRH$_2$ receptors has been reported, yet little is known about the effects of Ucn1 in the SON on feeding behaviour. We first established the dose-related effects of Ucn1 injected into the SON on the feeding response in both freely fed and 24-h food-deprived rats. A conditioned taste avoidance paradigm was performed to investigate possible generalised effects of local Ucn1 treatment. Administration of Ucn1 into the SON at doses equal to or higher than 0.5 µg significantly decreased food intake in both freely fed and food-deprived rats. The Ucn1-mediated suppression of food intake was delayed in freely fed as compared to food-deprived animals. Conditioning for taste aversion to saccharine appeared at 0.5 and 1 µg of Ucn1. Both the early and the delayed onset of anorexia observed after intra-SON injection of Ucn1 under fasting and fed conditions, respectively, suggest the possible involvement of different CRH receptor subtypes in the two conditions, while the conditioned taste aversion seems to be responsible for the initial latency to eat the first meal in these animals.

Keywords Urocortin 1 · Corticotropin-releasing hormone 2 receptor · Feeding microstructure · Conditioned taste aversion paradigm

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Introduction

Urocortin 1 (Ucn1), a member of corticotropin-releasing hormone (CRH) family of peptides, was first identified in the rat non-preganglionic Edinger-Westphal nucleus (npEW) (Vaughan et al. 1995). Two other members of this family, Ucn2 and Ucn3, were subsequently identified (Hauger et al. 2003). Research aimed at characterising the potential endogenous receptor(s) for the endogenous and synthetic peptides revealed a high affinity of Ucn1 to both CRH receptor subtypes 1 and 2 (CRH$_1$R and CRH$_2$R). Interestingly, the distribution of Ucn1-immunoreactivity corresponds to the distribution of CRH$_2$R in the rat brain, thereby indicating that Ucn1 may serve in addition to CRH as an endogenous CRH$_2$R ligand (Hauger et al. 2003). In contrast, Ucn2 and Ucn3 have a very low binding affinity to CRH$_1$R, but bind to CRH$_2$R with similar (Ucn2) or only minimally lower (Ucn3) affinity than Ucn1.

Subsequent studies revealed that intracerebroventricularly administered Ucn1 is more potent in suppressing appetite than CRH itself at doses as low as 0.01 µg were effective in decreasing food intake in both freely fed and food-deprived rats (Spina et al. 1996). Indeed, it has been reported that anorexia observed after central administration of Ucn1 is at least partly mediated by the interaction of the

synthetic peptide with the local CRH_2R receptors (Fekete and Zorrilla 2007). Also, a central injection of both Ucn2 and Ucn3 was found to suppress food intake in freely fed rats (Ohata and Shibasaki 2004).

The magnocellular, neuropeptidergic neurons constituting the hypothalamic supraoptic nucleus (SON) are well known for their involvement in functions of the body homoeostasis and reproduction including body fluid balance, parturition, and lactation (Engelmann et al. 2004). The investigation of the physiological relevance of the presence of both CRH_2R and Ucn1 in the SON has only recently started (Kozicz et al. 1998; Bittencourt et al. 1999; Spina et al. 2004). In this context, we found in a previous study that administration of Ucn1 into the SON significantly altered the open field behaviour of rats at doses lower than 0.01 μg via interaction with the CRH_2R (Fatima et al. 2010). The presence of several neuropeptides that modulate appetite has been well documented in the SON (Larsen et al. 1993; Wren et al. 2002). Also, an induction/suppression of appetite after intra-SON administration of their synthetic analogues or antagonists was observed, thereby suggesting an involvement of this nucleus in food intake regulation (Larsen et al. 1993; Wren et al. 2002). This is supported further by the observations that (1) 48 h of food deprivation causes a significant decrease in the number of Ucn1-immunoreactive neurons in the SON (Hara et al. 1997) and (2) that the neurons in the SON respond to peripheral feeding-relevant circulating signals including leptin (Isse et al. 1999).

Hence, considering the suggested role CRH_2R play in appetite regulation and energy balance (Fekete et al. 2007), it becomes a mandate to analyse the functional relevance of Ucn1 signalling via CRH_2R in the SON. Therefore, we characterised in the present study the dose-dependent effects of Ucn1 injected into SON on the feeding response in 24-h food-deprived rats. We then employed an automated monitoring of feeding microstructure to examine dose-related effects of Ucn1 administration into SON in freely fed rats. Finally, we used a conditioned taste aversion paradigm to explore the possibility of a generalised aversive effect that may contribute to possible hypophagic effects.

Methods

Animals

One hundred and twelve male Wistar rats (Harlan-Winkelmann, Germany), weighing 200 ± 20 g at the start of the experiments, were housed two per cage under controlled laboratory conditions and maintained at a 12:12 h reversed light–dark schedule (lights on at 6 p.m.), except

where otherwise specified. If not stated otherwise, animals had free access to standard rat food (ssniff Complete diet for rats and for mice maintenance ssniff R/MM-H, 10 mm, ssniff Spezialdiäten GmbH, D-59494, Soest, Germany) and tap water. They were allowed a 1-week period of acclimation to the animal room and handled three times a week before surgery.

Feeding behaviour was observed during the dark phase of the day–night cycle. All procedures were conducted in compliance with the EEC recommendations for the care and use of laboratory animals (86/609/CEE).

The procedure for surgery, drug preparation and administration has been described in detail elsewhere (Fatima et al. 2010).

Verification of cannula placement

Rats were killed at the end of the experiments with a terminal anaesthesia of isoflurane. Brains were removed from the skull and stored at −80 °C. For histological verification of the cannula placement, brains were sectioned in a cryostat, and every fourth coronal section (25 μm) was stained with cresyl violet. A cannula was considered to be incorrectly placed, if the injection site was outside of the SON area according to the rat brain atlas (Paxinos and Watson 1998). Approximately, 75 % of all cannulae hit the SON area. Only data of animals in which the cannula placement was found correctly located within the SON were included in the statistical analysis. According to the light microscopic investigations none of these animals showed a damage of SON.

24-h food deprivation-induced feeding

Rats were food deprived for 24 h prior to the experiment. Water was available throughout. On the day of the experiment, rats were separated and housed individually in cages without bedding 3–4 h before the beginning of the experiment; 10 min after bilateral intra-SON infusion (2 μl/2 min) of either vehicle or Ucn1, pre-measured water and pre-weighed food was provided. The remaining food, including spillage, and water were measured 30, 90 and 180 min after the start of food presentation. Food and water consumption was calculated for each of the above-mentioned time points.

Conditioned taste aversion (CTA)

Rats were water deprived for 23.5 h with access to fluids only 30 min per day throughout the duration of the experiment (12 days). After a stable water intake was established (7 days), rats had access to water on days 9 and 11, and saccharine solution (0.1 % aqueous solution) on days 8 and 10. Immediately after the end of the drinking

session of days 8 and 10, rats received a bilateral intra-SON injection of either vehicle or one of the doses of Ucn1. On day 12, all rats were given free access to both water and saccharine solution. The volume of liquid consumed from each bottle was recorded.

Automated monitoring of meal microstructures in freely fed rats

The microstructure analysis of feeding behaviour was conducted using the nose-poke operant chambers (Med associates Inc, St. Albans, VT, USA) for rodents. For this experiment, rats were kept in a regular 12:12 h light–dark regime (lights off at 6 p.m.). After the acclimation and handling period post-arrival, rats were trained for 1 week in the operant chambers to learn to make nose-poke responses and obtain palatable chow pellets (45 mg Noyes A/I precision pellets[TM], Res. Diets Inc. New Brunswick, NJ, USA) through the pellet dispenser. Rats had free access to food and water before and during the experiment. Intra-SON injections were performed just after the onset of the dark phase and the effects of the treatment on the feeding pattern of the animals were observed throughout the 12-h dark phase. Each animal received all doses tested, according to a Latin square design. Subsequent injections were performed with a minimal interval of 3–5 days in between. The latency to consume the first meal was defined as the time taken by the animal to make a first attempt to acquire food. Our meal structure analysis included the number of meals, total time spent eating, meal size, inter-meal interval, eating rate and satiety ratio. Based on Fekete et al. (2007), meals were defined as consecutive responses to food, which contained at least five food-directed responses. Meal duration was defined as the duration of consecutive responses to food. Meal sizes for eating were calculated as the average number of food-directed responses during meals. Inter-meal intervals were calculated as time difference between the end of one meal and the initiation of the next. Rates of eating were calculated by dividing each meal size with its respective duration. The satiety ratio was calculated as the average inter-meal interval divided by the average amount of food eaten.

Statistical analysis

Statistical analysis was performed using the SYSTAT software (v. 7.0 SPSS Inc., 1997). All values are expressed as mean ± SEM. A two-way repeated measure ANOVA (dose × time) was performed to assess the differences between the groups under 24-h food deprivation. Time course analysis was done assuming longitudinal data rather than time series modelling. Food intake for every half an hour interval up to 12-h post-SON infusion was calculated

for each animal. A multivariate ANOVA for repeated measures data was performed on the data. Latency to eat the first meal and meal microstructure were analysed by a one-way ANOVA followed by Dunnett's t test. Fluid type and dose effects in the CTA test were analysed by a two-way ANOVA. Post hoc analysis was performed using Bonferroni's multiple comparison. A p value less than 0.05 was considered to be significant.

Results

Effect of intra-SON administered Ucn1 on deprivation-induced feeding

The results of the two-way ANOVA revealed that both factors 'dose' [$F_{(5, 102)} = 3.2$; $p = 0.01$] and 'time' [$F_{(5, 102)} = 31.48$; $p < 0.001$] had significant effects on the feeding behaviour. There was no significant difference in the food intake in any of the groups 30 min after the presentation of food. However, 90 min post-food presentation, Ucn1 at a dose of 0.5 µg significantly reduced the food deprivation-stimulated feeding ($p < 0.01$). The reduction in food intake was apparent in all the groups receiving intra-SON Ucn1 compared to the controls at 90 min after food presentation (Fig. 1a). In fact, at this time point, animals treated with 0.001, 0.01 and 0.5 µg Ucn1 consumed significantly less food compared to the 30 min time point ($p < 0.05$). At 180 min after the presentation of food, none of the groups receiving intra-SON Ucn1 differed significantly from the controls. Furthermore, a two-way ANOVA showed that Ucn1 injection failed to produce significant differences in water intake compared to controls, at any of the time points measured (Fig. 1b).

Effect of intra-SON administered Ucn1 on preference for water solution

There was a significant effect of the factor 'dose' on preference of fluid intake in the CTA procedure [$F_{(3, 52)} = 14.44$; $p < 0.01$]. Similar to controls, rats treated with 0.1 µg Ucn1 showed neither a preference for water nor saccharine (Fig. 2). However, rats conditioned to associate saccharine consumption with a high dose of Ucn1 (0.5 and 1 µg) significantly reduced consumption of saccharine ($p < 0.001$). This suggests that high doses of Ucn1 have caused aversion to saccharine in these animals.

Effect of intra-SON administered Ucn1 on feeding in freely fed rats

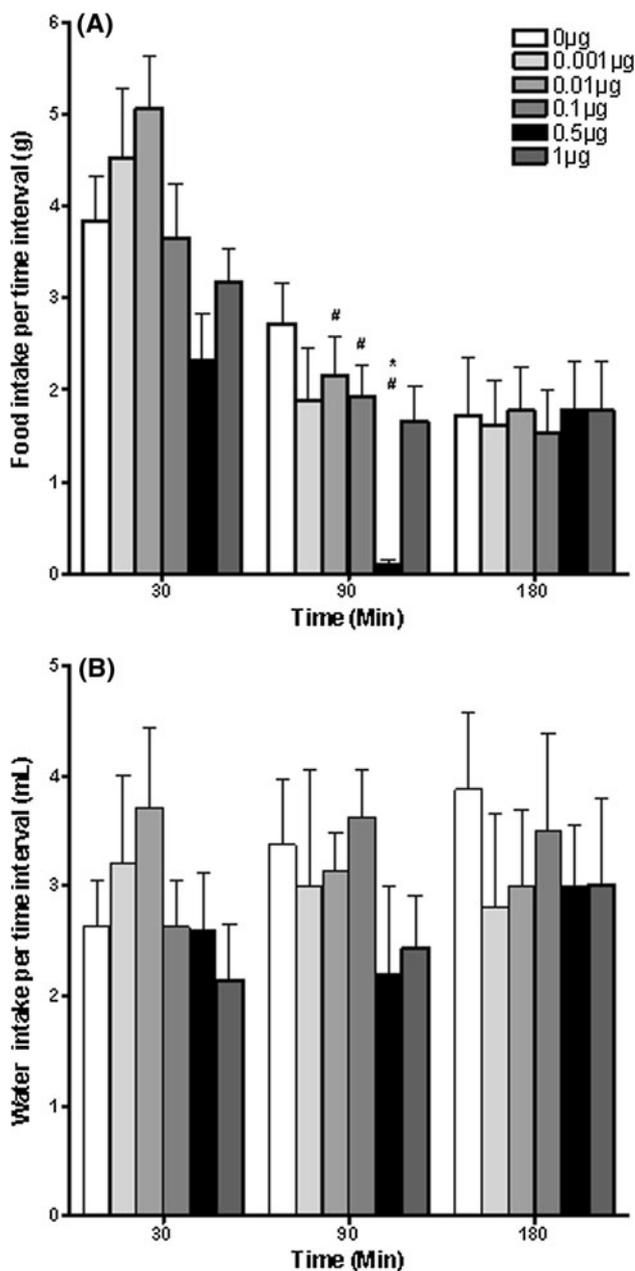1. Time course analysis of food intake: A time course of food intake observed in control and Ucn1-administered

Fig. 1 Effects of different doses of intra-SON Ucn1 on food and water intake in 24-h food-deprived rats. Data (mean + SEM) show the amount of food (**a**) and water (**b**) consumed during each time interval after bilateral SON injection. *$p < 0.05$ compared to control at the respective time interval; #$p < 0.05$ compared to interval '30 min', $n = 6$–10/group
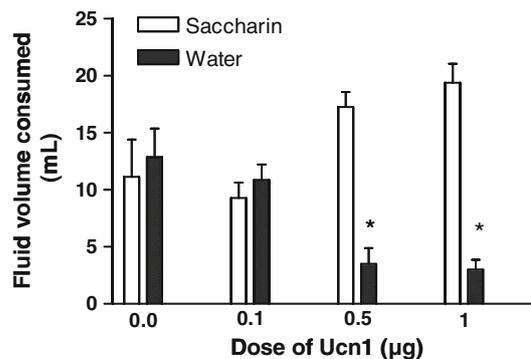

Fig. 2 Effects of different doses of intra-SON Ucn1 in the CTA paradigm. The data report the quantity of fluid intake, water and saccharine ingested by the rats on the last day of the multiple paring test conditioning procedure (mean + SEM). *$p < 0.05$ saccharine versus water, $n = 7$–10/group

$[F (5, 110) = 2.67; p < 0.05]$, whereas no significant differences compared to controls were found thereafter (Fig. 3).

2. Latency to eat the first meal: The latency to eat the first meal was similar in control animals and all doses of Ucn1 other than 1 μg (Fig. 4). In fact, at this dose, a significant increase in the time that passed between treatment and food consumption was measured (vehicle: 34.38 min; 1 μg: 89.55 min; $p < 0.01$).

3. Meal microstructure: The analysis of the meal microstructure during the 12-h dark phase did not reveal significant differences between any of the groups treated with Ucn1 and the control (Table 1).

Discussion

The main findings of the present study are: (1) administration of Ucn1 into SON at doses equal to or higher than 0.5 μg significantly decreased food intake in both freely fed and food-deprived rats; (2) we observed a CTA-like effect at the two highest doses of Ucn1 used; (3) in freely fed rats, 1 μg Ucn1 induced a delayed reduction of food intake as compared to food-deprived animals, without a significant disruption of the meal microstructure. Several studies confirmed that central and peripheral administration of synthetic Ucn1 suppressed appetite by interaction with CRH_2R (Spina et al. 1996; Wang et al. 2011). Defined hypothalamic structures including the paraventricular (Wang et al. 2011), ventromedial (Ohata and Shibasaki 2004) and arcuate nuclei (Sinnayah et al. 2003) were found to be involved in the anorexic action of Ucn1. Additionally, the lateral septum (Wang and Kotz 2002) and dorsal raphe (Weitemier and Ryabinin 2006) are non-hypothalamic brain areas thought to mediate Ucn1-induced anorexia.

groups mainly consisted of discrete bouts of food intake separated by non-feeding intervals. In freely fed rats, Ucn1 injection of doses lower than 0.5 μg failed to significantly affect the time course of food intake as compared to controls. On the contrary, Ucn1 treatment of 0.5 and 1 μg reduced feeding significantly around 4 h after injection. Administration of 1 μg Ucn1 reduced feeding significantly at 4.5-h post-injection
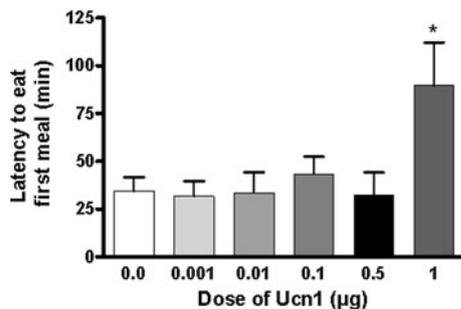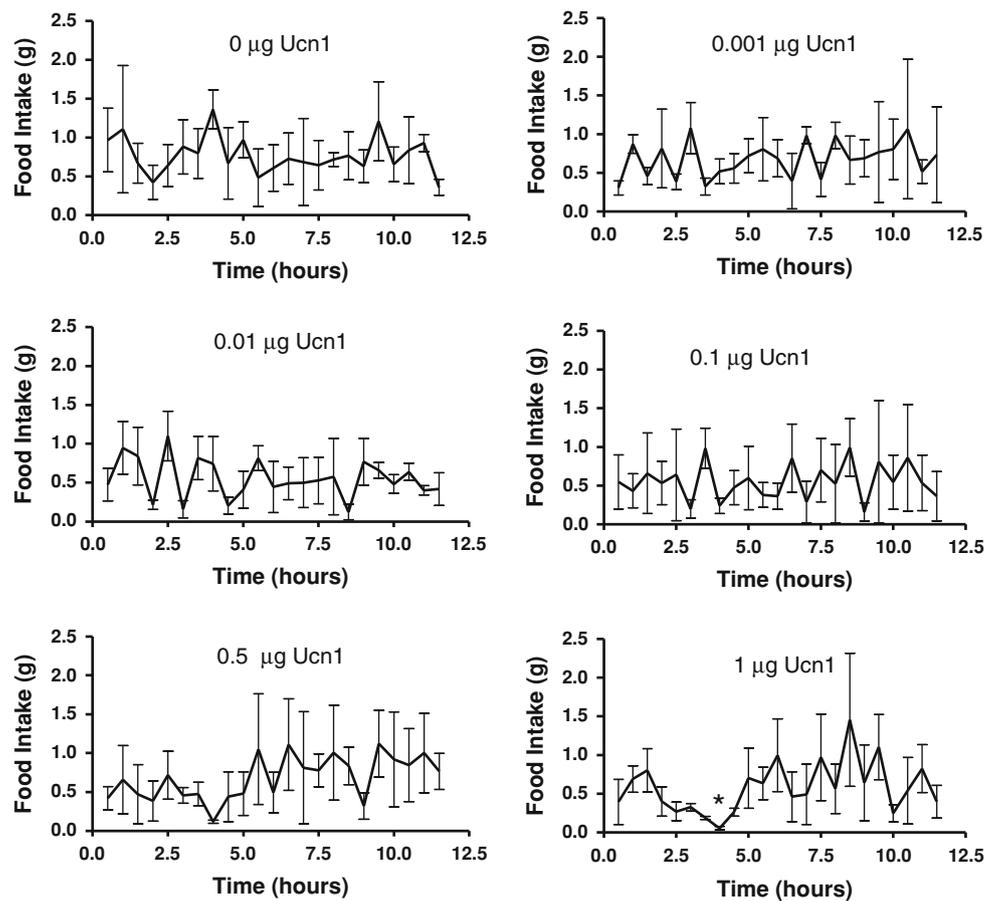
Fig. 4 Effect of intra-SON Ucn1 administration on latency to eat the first meal. Data are expressed as mean + SEM. *p < 0.05 compared to control, n = 9–15/group

Non-preganglionic Edinger-Westphal nucleus, which contains 80 % of Ucn1 present in the mammalian brain, has been implicated in food regulation as well (Weitemier and Ryabinin 2005; Xu et al. 2009, 2011). However, these reports question the anorexic effect of the centrally administered Ucn1 and suggest that the physiological role(s) of centrally injected Ucn1 highly depends on its concentration, its site of action and its binding with its receptor (Xu et al. 2011).

In our study, we observed a reduction in food intake after administration of 0.5 and 1 µg Ucn1 into the SON.

Interestingly at these doses, Ucn1 treatment also induced CTA-like effects (Fig. 2). The latter observation would plausibly explain the increased latency to eat the first meal after administration of 1 µg Ucn1, thereby suggesting a treatment-induced aversion or malaise. If solely aversion was responsible for the suppression of food intake, one would expect a similar timing of the onset of anorexia in all animals receiving a particular dose of Ucn1, irrespective of whether they were freely fed or food deprived. However, we observed a difference in the time of onset of anorexia between these two feeding conditions: the suppression of food intake in food-deprived animals was apparent at 90-min post-treatment of 0.5 µg of Ucn1 and vanished by 180 min. In contrast, in freely fed animals, the onset of anorexia is around 120 min, significantly peaking at 240 min and subsiding by 300 min. The differences in the onset of anorexia produced by Ucn1 under fed and fasting conditions seen in our study are consistent with the results of previous studies also using CRH-like peptides (Kihara et al. 2001; Fekete et al. 2007; Wang et al. 2011). These studies characterised the role of CRH-like peptides and contribution of CRHR subtypes in early and delayed onset of anorexia under fasting and fed conditions (Fekete et al. 2007; Wang et al. 2011). Interestingly, selective CRH$_2$R

Table 1 Effect intra-SON Ucn1 injection on 12-h feeding microstructure in freely fed rats

	0.0 μg	0.001 μg	0.01 μg	0.1 μg	0.5 μg	1 μg
Number of meals	11.75 ± 1.12	11.67 ± 1.26	8.455 ± 1.32	10.67 ± 1.14	9.571 ± 0.92	9.083 ± 0.87
Total meal time (min/12 h)	605.3 ± 16.59	588.3 ± 32.49	525 ± 57.5	600.6 ± 12.98	583.6 ± 19.57	534.6 ± 26.06
Meal duration (min)	106.9 ± 9.48	105.6 ± 10.08	77.27 ± 11.97	100.6 ± 10.49	86.93 ± 8.37	94.58 ± 8.31
Meal size (g)	1.32 ± 0.16	0.75 ± 0.20	1.06 ± 0.20	0.66 ± 0.15	1.13 ± 0.14	0.96 ± 0.18
Inter-meal interval (min)	54.91 ± 6.66	49.59 ± 5.55	70.99 ± 15.24	56.72 ± 7.71	77.43 ± 19.7	62.82 ± 6.92
Eating rate/12 h (mg/min)	3.19 ± 0.25	3.36 ± 0.30	3.79 ± 0.23	2.98 ± 0.29	3.36 ± 0.23	2.75 ± 0.24
Sateity ratio (min/g)	43.12 ± 8.68	38.96 ± 3.96	49.78 ± 12.05	51.96 ± 9.72	47.18 ± 6.77	58.33 ± 8.34

A statistical analysis failed to reveal significant differences between the groups for all parameters measured ($n = 9$–15/group)

ligands Ucn2 and Ucn3 have been also reported to produce delayed onset of anorectic effects (Ohata and Shibasaki 2004). The authors of these studies hypothesised that the central CRH$_2$R-induced delayed onset of anorexia resulted from a slow emptying of food from the gastrointestinal tract leading to progressive accrual of food in the stomach, hence prolonging gastric distension and reducing the subsequent food intake ability (Fekete et al. 2007). In contrast, it has been reported that intracerebroventricular administration of CRH$_1$R agonists elicited abbreviated, short-onset anorexia (Zorrilla et al. 2003) and disrupted fasted motor patterns of gastroduodenal motility, which were replaced by fed-like motor patterns (Kihara et al. 2001).

In situ hybridisation studies confirmed the presence of the CRH$_2$R mRNA in the SON (Van Pett et al. 2000). Interestingly, mRNA synthesis, not only for the CRH$_2$R, but also of the CRH$_1$R, can be triggered in the paraventricular nucleus of the hypothalamus (PVN) and SON by different stressors (Van Pett et al. 2000). Indeed, acute food deprivation was shown to be a stressor in different studies (Hara et al. 1997; Tobin et al. 2009). Furthermore, mRNA expression of CRH in the PVN and immunoreactivity of Ucn1 in the SON were reported to be altered by food deprivation (Brady et al. 1990; Hara et al. 1997). This may suggest that also in our study food deprivation induced an increase in CRH$_1$R expression, which may have added to the effects induced by Ucn1 signalling via local CRH$_2$R. This would explain the early onset and short-lasting anorexia triggered via activation of the CRH$_1$R seen in food-deprived rats, whereas freely fed animals responded with a delayed onset and prolonged anorexia due to gastrostasis. Recently, functional connections between SON and the stomach have been studied by Lu et al. (2011). The authors reported that microinjections of L-glutamate into SON inhibited gastric motility. In a different study, gastric distension induced c-Fos expression in the nucleus of the solitary tract (NTS), the area postrema, the PVN and the SON which could be blocked by truncal vagotomy (Mazda et al. 2004), implying that the SON receives information

about gastric volume changes via the vagal nerve. This is in line with findings about an activation of vasopressinergic and oxytocinergic neurons in the PVN after abdominal surgery (Barquist et al. 1992) and an involvement of vasopressin and oxytocin receptors at the level of dorsal motor nuclei of the vagus in modulating gastric motility (Lu et al. 2011). Ucn1 has been proposed to act in an autocrine and paracrine manner in the hypothalamus after dendritic and somatic release (Arima and Aguilera 2000). Hence, there may be a postprandial stimulation of Ucn1 neurons in the SON that triggers Ucn1 synthesis (possibly with other neuropeptides, including vasopressin and oxytocin, c.f. Spina et al. 2004) and controls gastric motility and feeding behaviour. Alternatively, hypothalamic Ucn1 signalling may induce neuronal activation of NTS via CRH$_2$R that leads to sympathetic nerve stimulation in the ventrolateral medulla, and thus an inhibition of ghrelin secretion and food intake (Yakabi et al. 2011). Further studies are needed to test these hypotheses.

Consistent with previous studies, we report here that 0.5 and 1 μg of synthetic Ucn1 administered into the SON produces CTA-like effect in water-deprived rats, increase the latency to eat the first meal in non-deprived animals and delays the onset of anorexia in freely fed as compared to fasted rats. Furthermore, our data support the possible involvement of different CRH receptor subtypes in early and delayed onset of anorexia under fed and fasted conditions.

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