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Urocortin I Inhibits the Effects of Ghrelin and Neuropeptide Y on Feeding and Energy Substrate Utilization

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Abstract

The corticotropin releasing hormone-related ligand, urocortin-I (UcnI), suppresses food intake when injected into multiple hypothalamic and extrahypothalamic areas. UcnI also alters energy substrate utilization, specifically via enhanced fat oxidation as reflected in reductions in respiratory quotient (RQ). In the present study we compared the feeding and metabolic effects of ghrelin and NPY following pretreatment with UcnI. Direct PVN injections of NPY (50 pmol) and ghrelin (50 pmol) were orexigenic while UcnI (10–40 pmol) reliably suppressed food intake. Both ghrelin and NPY increased RQ, indicating enhanced utilization of carbohydrates and the preservation of fat stores. UcnI alone suppressed RQ responses. PVN UcnI attenuated the effects of both ghrelin and NPY on food intake and energy substrate utilization. While ghrelin (5 pmol) potentiated the effect of NPY (25 pmol) on RQ and food intake, these responses were inhibited by pretreatment with UcnI (10 pmol). In conclusion, PVN NPY and ghrelin stimulate eating and promote carbohydrate oxidation while inhibiting fat utilization. These effects are blocked by UcnI which alone suppresses appetite and promotes fat oxidation. Overall these findings are consistent with a possible interactive role of PVN NPY, ghrelin and urocortin in the modulation of appetite and energy metabolism.

Keywords

Eating; Energy metabolism; Food intake; Ghrelin; Neuropeptide Y; Paraventricular nucleus; Respiratory quotient; Urocortin-I

I. Introduction

Urocortin-I (UcnI) is a potent anorexigenic peptide and belongs to the corticotropin-releasing hormone (CRH) family along with CRH, urotensin, urocortin II and urocortin III (Bamberger et al., 2007; Hauger et al., 2006; Tanaka et al., 2009). It is expressed in the rat central nervous system including the Edinger-Westphal nucleus, the hypothalamus, the dorsal raphe nuclei, motor brainstem nuclei and the substantia nigra (Boorse and Denver, 2006; Cepoi et al., 1999; Kozicz et al., 1998; Vaughan et al., 1995). The 40-amino acid peptide was first cloned from the rat midbrain exhibiting a 45% sequence homology to CRH and 63% sequence identity to urotensin (Latchman, 2002; Vaughan et al., 1995). The amino acid sequence of UcnI is highly conserved across species with rat UcnI showing a 95%

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homology with the human peptide (Bamberger et al., 2007; Donaldson et al., 1996; Zhao et al., 1998).

Prior investigations into the neurobiology of CRH receptor signaling have indicated that CRH and CRH-related peptides act on the central nervous system to alter energy balance and influence both food intake and sympathetically-mediated thermogenesis (Chen et al., 2010; Cottone et al., 2007; Heinrichs et al., 1992; Krahn et al., 1988; Neufeld-Cohen et al., 2010; Semjonous et al., 2009). Injections of UcnI into areas of the forebrain including hypothalamic nuclei and the lateral septum are anorexigenic (Ohata et al., 2000; Wang et al., 2001; Wang and Kotz, 2002), and in addition to suppressing food intake, fourth ventricular UcnI administration results in significant hyperglycemic responses (Daniels et al., 2004). We have previously reported that direct injections of UcnI into the hypothalamic paraventricular nucleus (PVN) suppress food consumption (Currie et al., 2001; Currie, 2003). Rats treated with PVN UcnI also exhibited alterations in fat metabolism as reflected in reductions in respiratory quotient (RQ). The effects of UcnI on food intake and energy metabolism are in contrast to those observed in response to PVN neuropeptide Y (NPY) and ghrelin. Both PVN NPY and ghrelin are potently orexigenic (Currie, 2003; Currie & Coscina, 1995; Currie et al., 2010; Leibowitz et al., 1988; Melis et al., 2002; Stanley et al., 1989; Wren et al., 2001) and both peptides elicit increases in RQ reflecting enhanced carbohydrate oxidation and the preservation of fat stores (Currie et al., 2005; Currie et al., 2010; Currie and Coscina, 1996).

In the current study we examined the effects of UcnI administration on the orexigenic and metabolic action of NPY and ghrelin within the hypothalamic PVN, a nucleus that plays a critical role in the homeostatic mechanisms regulating energy intake and metabolism (Billington et al., 1994; Currie and Coscina, 1995; Leibowitz et al., 1988; Madden and Morrison, 2009; Mano-Otagiri et al., 2009; Wang et al., 2007). One the basis of the above findings we hypothesized that UcnI would inhibit the feeding-stimulant action of both NPY and ghrelin, as well as their effects on substrate oxidation, through an action within the hypothalamic PVN. The findings of this study confirm that UcnI reliably inhibits food intake measured after either NPY or ghrelin microinjection. Further, the potentiation of NPY-induced eating by ghrelin is also attenuated by UcnI pretreatment. Similar effects were observed on respiratory quotient with UcnI reliably suppressing the enhanced oxidation of carbohydrates elicited by NPY and ghrelin.

2. Results

Figure 1 illustrates the effects of PVN UcnI and ghrelin microinjections on 2-h food intake at the onset of the nocturnal cycle. Two way repeated measures analysis of variance (ANOVA) indicated a significant UcnI × Ghrelin interaction ($F(3,21)=3.13$, $p<0.047$). Ghrelin reliably increased food intake compared to vehicle control while UcnI microinjection suppressed food intake at doses of 10 and 40 pmol. Importantly, UcnI pretreatment attenuated the orexigenic action of ghrelin and this effect was dose-dependent. The impact of PVN administration of UcnI on NPY-induced eating is shown in Figure 2. Two way repeated measures ANOVA confirmed a significant UcnI × NPY interaction ($F(3,21)=27.22$, $p<0.0001$). PVN NPY elicited a reliable increase in eating while 10 and 40 pmol of UcnI suppressed 2-h food intake. UcnI pretreatment suppressed the orexigenic action of NPY with both the 10 and 40 pmol doses completely blocking this effect. Figure 3 shows the combined effect of a low dose of NPY paired with a low dose of ghrelin. Here ghrelin significantly potentiated the orexigenic effect of NPY and this response was entirely reversed when rats were pretreated with UcnI ($F(7,21)=41.30$, $p<0.0001$).

A separate series of studies examined the effect of these peptides on PVN metabolic function. The dose-response effect of UcnI injected into the PVN is shown in Figure 4. Two way ANOVA confirmed that UcnI reliably decreased RQ at both the 10 and 40 pmol dose (Treatment × Time interaction, $F(48, 336)=87.4$, $p<0.0001$). Data are presented as mean RQ values over the initial 4 h of the dark period and are shown in 20-min intervals. The significant reduction in RQ represents a shift in energy utilization towards enhanced oxidation of fat.

The co-administration of UcnI and ghrelin is shown in Figure 5. The 50 pmol dose of ghrelin increased RQ within 40 min of testing and RQ values remained elevated over the 4-h test period. Pretreatment with 10 pmol of UcnI attenuated the RQ response. This was evident as a shift in the time course of the response to the right, but also as a reduction in the magnitude of the RQ effect. Two way ANOVA for repeated measures confirmed a reliable Treatment × Time interaction, ($F(48,336)=109.3$, $p<0.0001$).

UcnI also blocked the metabolic effects of NPY as illustrated in Figure 6. Specifically, two way ANOVA revealed a significant Treatment × Time interaction, ($F(48,336)=58.3$, $p<0.0001$). NPY (50 pmol) elicited a reliable increase in RQ within 20 min of PVN injection. Similar to its effect on ghrelin-treated rats, UcnI (10 pmol) attenuated the elevation in RQ observed after NPY treatment.

Finally, ANOVA also indicated that UcnI attenuated the metabolic effects of combined administration of ghrelin and NPY ($F(72,504)=17.9$, $P<0.001$). Specifically, as demonstrated in Figure 7, we observed that a low dose of ghrelin (5 pmol) potentiated the RQ response elicited by a low dose of NPY (25 pmol). That is, combined administration of both peptides evoked a significant increase in carbohydrate oxidation compared to NPY. The NPY condition was significantly elevated compared to vehicle control. The reliable increase in RQ, after combined ghrelin and NPY treatment, was observed within 20 min of treatment and was maintained throughout the 4-h test. UcnI pretreatment reversed RQ values to those comparable to NPY.

3. Discussion

In the present study UcnI reliably inhibited food intake when injected directly into the hypothalamic PVN and suppressed the eating-stimulant action of PVN NPY and ghrelin. The potentiation of NPY-induced eating by ghrelin was also attenuated by UcnI pretreatment. Similar effects were observed on respiratory quotient with UcnI reliably suppressing the enhanced oxidation of carbohydrates elicited by either NPY or ghrelin and by ghrelin/NPY co-administration. Since alterations in RQ were observed in rats under testing conditions where no food was available, the observed changes in RQ cannot be interpreted as simply the consequence of increases or decreases in food intake. That is, ghrelin, NPY and UCNI elicit changes in energy substrate utilization, independent of their effects on food intake.

Previous reports have implicated urocortin, ghrelin and NPY in the regulation of energy homeostasis. Urocortin-related peptides, as well as CRH, have been shown to act on the CNS to alter energy balance and inhibit food intake in several models of hyperphagia, including NPY-stimulated eating (Currie et al., 2001; Fekete et al., 2007; Heinrichs et al., 1992; Wang et al., 2001). These effects are in addition to other evidence showing that urocortins and CRH contribute to the integration of autonomic, neuroendocrine and behavioral responses to stress and aversive stimuli (Hauger et al., 2006; Pan and Kastin, 2008). UcnI binds with high affinity to CRH receptors and decreases food intake in food-deprived and free-feeding rodents (Currie et al., 2001; Spina et al., 1996; Tanaka et al.,

2009; Wang et al., 2001). The anorexigenic responses are mediated via hypothalamic and extrahypothalamic mechanisms (Currie et al., 2001; Kamdi et al., 2009; Ohata et al., 2000; Wang et al., 2001; Wang and Kotz, 2002). Moreover, UcnI and CRH are believed to suppress appetite through an action largely independent of ACTH activation, indicating a direct effect on CRH receptors (Heinrichs et al., 1992; Heinrichs and Richard, 1999; Pan and Kastin, 2008). CRH type 2 receptors are expressed in high concentrations within the PVN (Chalmers et al., 1995; Currie et al., 2001; Hauger et al., 2006), suggesting that the effects of UcnI on eating and energy metabolism, observed in the current study, are mediated by this receptor type.

In the present report UcnI also blocked the orexigenic and metabolic action of PVN NPY and ghrelin. NPY is a 36-amino acid peptide and is synthesized by neurons of the arcuate nucleus which in turn project to the PVN (Kolesnik et al., 2001). Injection of anti-NPY γ-globulin suppresses food intake while chronic PVN NPY administration induces rapid weight gain and enhanced body fat deposition (Raposinho et al., 2004; Shibasaki et al., 1993; Stanley et al., 1989). Consistent with an action on energy balance and metabolic function, NPY alters insulin secretion, decreases sympathetic nerve activity to brown adipose tissue, and stimulates the hypothalamo-pituitary adrenal axis (Dryden et al., 1994; Hanson and Dallman, 1995; Kuo et al., 2007; Wisialowski et al., 2000). Direct injections of NPY into the PVN stimulate ACTH secretion and alter the release of insulin, corticosterone and glucagon (Harfstrand et al., 1987; Leibowitz et al., 1988; Moltz and McDonald, 1985; Small et al., 1997). PVN, but not perifornical hypothalamic NPY injections, elicit hypothermia and as we confirm here, increase respiratory quotient (Currie and Coscina, 1995; Currie and Coscina, 1996; Semjonous et al., 2009). The increase in RQ elicited by NPY reflects an increase in carbohydrate oxidation in favor of fat storage.

The diversion of metabolism following PVN NPY treatment, toward carbohydrate oxidation and fat synthesis, is consistent with the metabolic effects of ghrelin, reported in the current study and others (Currie et al., 2005; Currie et al., 2010). Ghrelin, a 28-amino acid acetylated peptide, is an endogenous ligand for the growth hormone secretogue receptor (GHS-R) (Ghigo et al., 2005). There are currently two identified ghrelin receptor types, the GHS-R1a and GHS-R1b. The GHS-R1a is reported to bind ghrelin and GHS and mediate their respective actions while the 1b type is categorized as nonfunctional (Ghigo et al., 2005; Veldhuis and Bowers, 2010). Ventricular administration of ghrelin induces c-Fos expression in the PVN where GHS-R1a are localized (Lawrence et al., 2002).

Like NPY, central injections of ghrelin increase food intake and chronic treatment stimulates body weight gain and enhanced body fat mass deposition (Currie et al., 2005; Horvath et al., 2003; Tschop et al., 2000). In fact ghrelin has been reported to interact with a number of hypothalamic peptidergic systems including NPY/agouti-related peptide (AGRP) neurons of the arcuate nucleus (Veldhuis and Bowers, 2010). The expression of ghrelin peptide has been localized to a neuronal population adjacent to the third ventricle and along the medial axis of the hypothalamus, proximal to the PVN and arcuate (Cowley et al., 2003). These neurons send projections targeting hypothalamic circuits that play an integral role in the production of NPY, AGRP, proopiomelanocortin (POMC) and corticotropin releasing hormone (CRH) in the paraventricular and arcuate nuclei (Cowley et al., 2003; Veldhuis and Bowers, 2010). At the level of the arcuate and PVN, therefore, ghrelin may stimulate release of orexigenic transmitters controlling metabolic function.

Within the PVN, ghrelin's orexigenic action is comparable to NPY in magnitude. In addition, ghrelin elicits similar changes in nutrient partitioning. Indeed we report here that ghrelin potentiates the effects of NPY on food intake and RQ. These findings are consistent with an interaction of PVN ghrelin/NPY neuronal circuits in metabolic homoeostasis. Other

evidence supports NPY/ghrelin interactions. In humans, systemic administration of ghrelin enhances circulating NPY levels (Coiro et al., 2006). In rats, ghrelin increases hypothalamic NPY mRNA and eating elicited by ghrelin is attenuated by NPY Y1 receptor antagonism and antisera to NPY (Asakawa et al., 2003; Kamegai et al., 2001; Shintani et al., 2001; Tschop et al., 2002). Further, within the hypothalamus, NPY and ghrelin neurons synapse onto each other, suggesting autofeedback-dependent regulation (Hori et al., 2008). With respect to urocortin, human data confirm that UcnI stimulates plasma ATCH and cortisol while inhibiting plasma ghrelin (Davis et al., 2004). In rodents, UcnI inhibits gastric ghrelin secretion (Veldhuis and Bowers, 2010). Ghrelin receptor antagonism decreases activation of urocortin-containing neurons in brain whereas NPY has been reported to activate central UcnI neurons (Gaszner et al., 2007; Kaur and Ryabinin, 2010).

BAT activity is essential in the regulation of energy metabolism via altered energy expenditure and thermogenesis and all three peptides exert effects on brown adipose tissue (BAT). This is consistent with a role in metabolic function. Both ghrelin and NPY suppress activity of BAT through the inhibition of the sympathetic nervous system (Egawa et al., 1991; Mano-Otagiri et al., 2009). Very recent work has shown that the genetic suppression of ghrelin receptors activates BAT activity and decreases fat storage (Mano-Otagiri et al., 2010). NPY, ghrelin and UcnI alter gene expression for the brown fat thermogenic moiety, uncoupling protein (Billington et al., 1994; Kotz et al., 2005; Tsubone et al., 2005). These effects are, in turn, in agreement with the inhibitory role of NPY and ghrelin on fat expenditure as an energy substrate and the stimulatory action of UcnI on fat oxidation. Moreover, the impact of these peptide transmitters on substrate oxidation and appetite is consistent with their role in long-term weight control via hypothalamic homeostatic mechanisms.

While the above findings, including evidence from the present study, suggest that UcnI might modulate ghrelin/NPY interactions in the regulation of energy intake and metabolism, other transmitter systems appear to contribute to ghrelin/NPY regulatory control. For example, in one recent report, oxytocin was shown to inhibit the stimulatory effect of ghrelin on circulating NPY levels (Coiro et al., 2008). We have also reported that PVN 5-hydroxytryptamine and the 5-$HT_{2a/2c}$ agonist DOI, inhibit the eating and metabolic effects of both NPY and ghrelin, when microinjected into the PVN (Currie et al., 2010). Indeed, understanding the coordinated efforts of these systems is necessary in order to determine their physiological role in homeostatic regulation.

In conclusion, ghrelin and NPY alter appetite and energy metabolism, including the promotion of carbohydrate oxidation and the preservation of fat stores, by an action on hypothalamic PVN neurons mediating positive energy balance. PVN UcnI activation, in turn, inhibits the orexigenic and metabolic action of both neuropeptides. These findings are consistent with the hypothesis that UcnI may exert inhibitory control over PVN NPY and ghrelin systems, thereby promoting satiety, decreasing NPY and ghrelin-stimulated energy intake, and reducing the initial increase in carbohydrate oxidation resulting from enhanced NPY/ghrelin signaling at the onset of the nocturnal period.

4. Experimental Procedures

4.1. Animals

Adult male Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) were housed individually in polypropylene cages with ad libitum access to water and standard rat pellets (LabDiet, PMI Nutrition, Brentwood, MO, USA). The calculated metabolizable energy of the diet was 3.1 kcal/g with 59.7% of the calories provided by carbohydrates, 27.1% by protein and 13.2% by fat. The animal colony room was maintained on a 12 h light/dark cycle (lights on

at 0400 h) and at a temperature of 22 ± 2°C. All experiments were approved by the Institutional Animal Care and Use Committee of Reed College.

4.2. Apparatus

Oxygen consumption (O_2) and carbon dioxide (CO_2) production were measured using an Oxyscan open-circuit calorimetry system (AccuScan Instruments, Inc., Columbus, OH, USA). Atmospheric air was drawn into enclosed acrylic chambers at a rate of 1500 ml/min. O_2 and CO_2 analyzers measured gases sequentially across each chamber. The analyzers were calibrated before test sessions using primary gas standards of high purity (Polar Cryogenics, Portland, OR, USA). Concentrations of the gases were recorded in ml/kg body weight/min. Respiratory quotient (RQ) was calculated as the volume of CO_2 produced (VCO_2) divided by the volume of O_2 consumed (VO_2).

4.3. Stereotactic Surgery

When rats attained body weights of 275–300 g, approximately 2 weeks after arrival, they were anesthetized with pentobarbital sodium (50 mg/kg IP; Butler Schein, Dublin, OH, USA) and placed in a Kopf stereotaxic frame with the incisor bar set at 3.6 mm below the interaural line. A single incision was made along midline to expose bregma as well as the skull surface immediately anterior and posterior to bregma. Stereotactic coordinates for the guide cannula relative to bregma were AP −1.8 mm, L −0.3 mm, and V −4.5 mm (Paxinos and Watson, 2007). Unilateral guide cannulae (22-gauge; Plastics One, Roanoke, VA, USA) were implanted 4 mm dorsal to the PVN. The implant was secured with acrylic cement and three stainless steel screws embedded into the skull. A 28-gauge stainless-steel inner stylet was used to maintain cannula patency. All rats were administered 0.4 mg/kg SC of the analgesic buprenorphine (Butler Schein) immediately after surgery and again 12 h later. Behavioral and metabolic testing began after a postoperative recovery period of 14 days. During this time food intake and body weights were monitored, and rats received several mock injections in order to habituate them to the injection procedure.

4.4. Peptides

Urocortin-I, ghrelin and NPY (rat) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in sterile saline. Injections were administered in a volume of 0.2 μl into the PVN using a microinjector extending 4 mm beyond the permanent guide cannula. Doses were selected from previous reports examining effects on eating behavior and energy metabolism (Currie et al., 2001; Currie, 2003; Currie et al., 2005).

4.5. Experimental Design and Testing

The effects of PVN injections on food intake were examined at the onset of the dark cycle. For all tests, a within-subjects design was used with treatments administered in randomized order. A minimum of 4 days separated each test.

4.5.1. Feeding Tests—In feeding tests, food intake was measured 2 h after injections. Separate groups of rats were tested to determine whether UcnI would alter the orexigenic action of ghrelin, NPY and ghrelin co-injected with NPY. In one study (n=8), UcnI (2.5–40 pmol) was administered immediately prior to ghrelin (50 pmol) or vehicle. Under control conditions, rats received two vehicle injections. In a separate study (n=8), UcnI (2.5–40 pmol) was paired with NPY (50 pmol) or vehicle. Two vehicle injections were administered as the control condition and again, food intake was measured 2 h postinjection. A final feeding study was designed to determine if UcnI would block the combined the orexigenic action of NPY plus ghrelin. Rats (n=8) were injected with a low dose of NPY (25 pmol) paired with a low dose of ghrelin (5 pmol) to demonstrate that ghrelin potentiates NPY-

induced eating. These same rats were pretreated with UcnI (10 pmol) in order to assess whether UcnI would block any potentiated response. Again, all treatments were administered in randomized order. During testing, a stainless steel insert was placed on the floor of the home cage allowing food intake to be corrected for spillage. Rats were habituated to the insert for several weeks prior to testing.

4.5.2. Metabolic Testing—In metabolic testing, similar injection procedures were followed (n=8/group) in order to determine if UcnI would alter the effects of NPY and ghrelin on energy substrate utilization. Immediately after injections, rodents were placed in individual test chambers and O_2 consumption and CO_2 production were measured over a 4-h period. Food and water were not available at this time.

4.6. Histological and Statistical Analyses

Cannulae placements were confirmed via histological examination. Prior to brain extractions, rats were injected with black ink in a volume equal to that administered in microinjections. The black ink assists in localizing the site of injection as well as the spread of the injection. Forty μm coronal sections were cut through the hypothalamus using a cryostat and then stained with Cresyl violet. Sections were examined using light microscopy and viewed relative to the stereotaxic atlas of Paxinos and Watson (Paxinos and Watson, 2007). Accurate targeting of the PVN was confirmed by the presence of black ink in all rats tested. Moreover, given the microinjection volumes and concentrations of peptides used n the present study, it seems unlikely that our microinjections would spread to other brain areas, including the ventricles, thereby eliciting effects outside of the PVN. Indeed the doses of peptides used in our study fall within the subthreshold range when injected into the ventricles (Osto et al., 2007; Polidori et al., 2000; Spina et al., 1996).

Data were analyzed by separate one and two way analyses of variance (ANOVA) for repeated measures. Mean comparisons were evaluated using post hoc Tukey tests. The criterion for statistical significance was $p<0.05$. The Statistica software package was employed for all statistical analyses (Statsoft, Inc., Tulsa, OK, USA).

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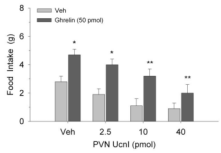

Fig. 1.

Food intake after ghrelin (50 pmol) and UcnI injection into the PVN over the initial 2 h of the dark period. While ghrelin reliably increased food intake, UcnI pretreatment, at doses of 10 and 40 pmol, attenuated ghrelin's orexigenic action. Values are represented as mean ± S.E.M. over 2 h. *P<0.05 compared to vehicle paired with vehicle. **P<0.05 compared to vehicle paired with ghrelin.

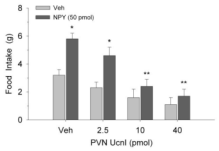

Fig. 2.

PVN injection of NPY (50 pmol) increased food intake over the 2-h test period beginning at the onset of the dark cycle. Doses of 10 and 40 pmol of UcnI reversed this effect. Values are represented as mean ± S.E.M. *P<0.05 compared to vehicle paired with vehicle. **P<0.05 compared to vehicle paired with NPY.

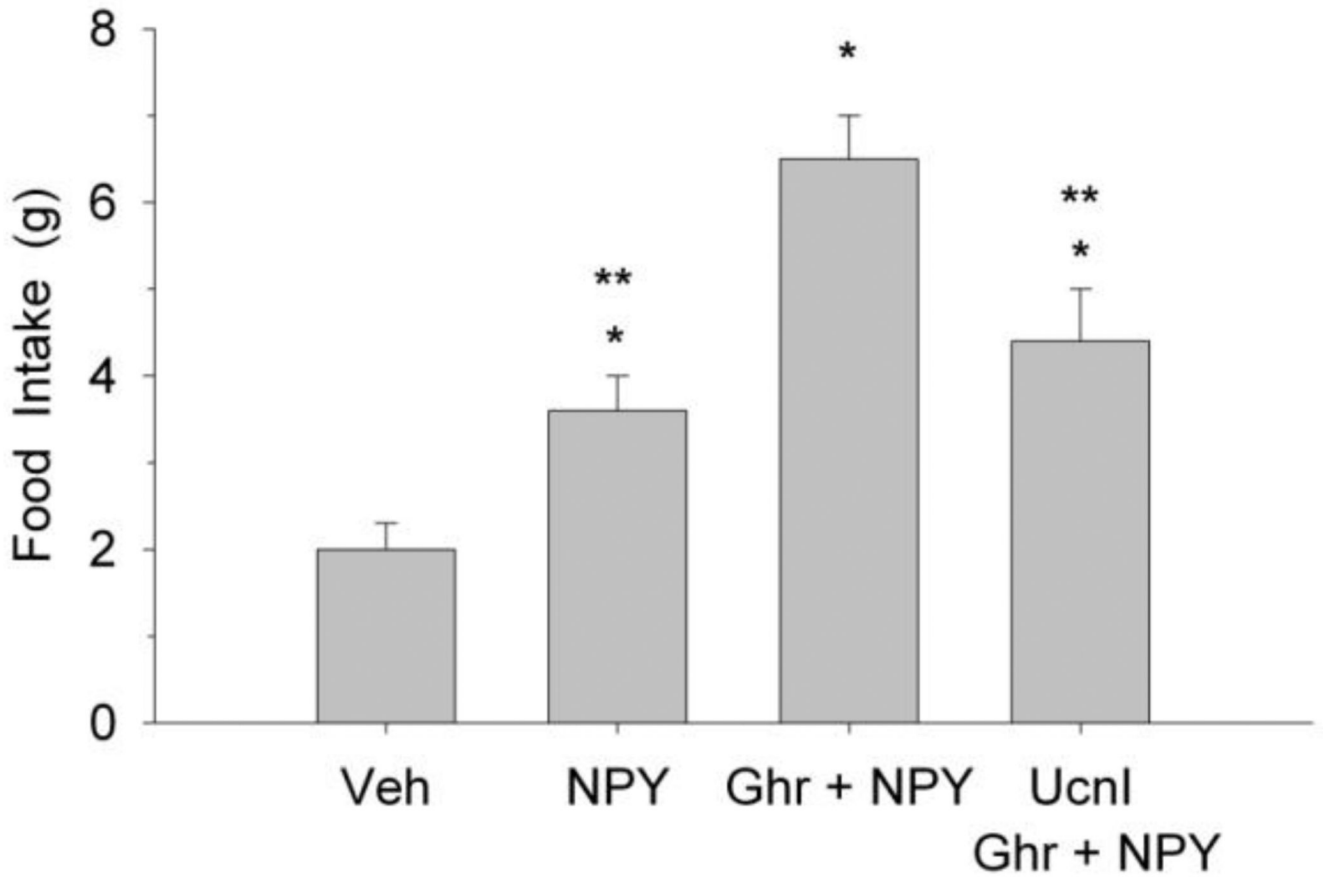

Fig. 3.

PVN injection of ghrelin (5 pmol) potentiated eating elicited by NPY (25 pmol). UcnI (10 pmol) pretreatment attenuated ghrelin's effects on NPY-stimulated eating. Values are represented as mean ± S.E.M. over 2 h. *P<0.05 compared to vehicle. **P<0.05 compared to ghrelin paired with NPY.

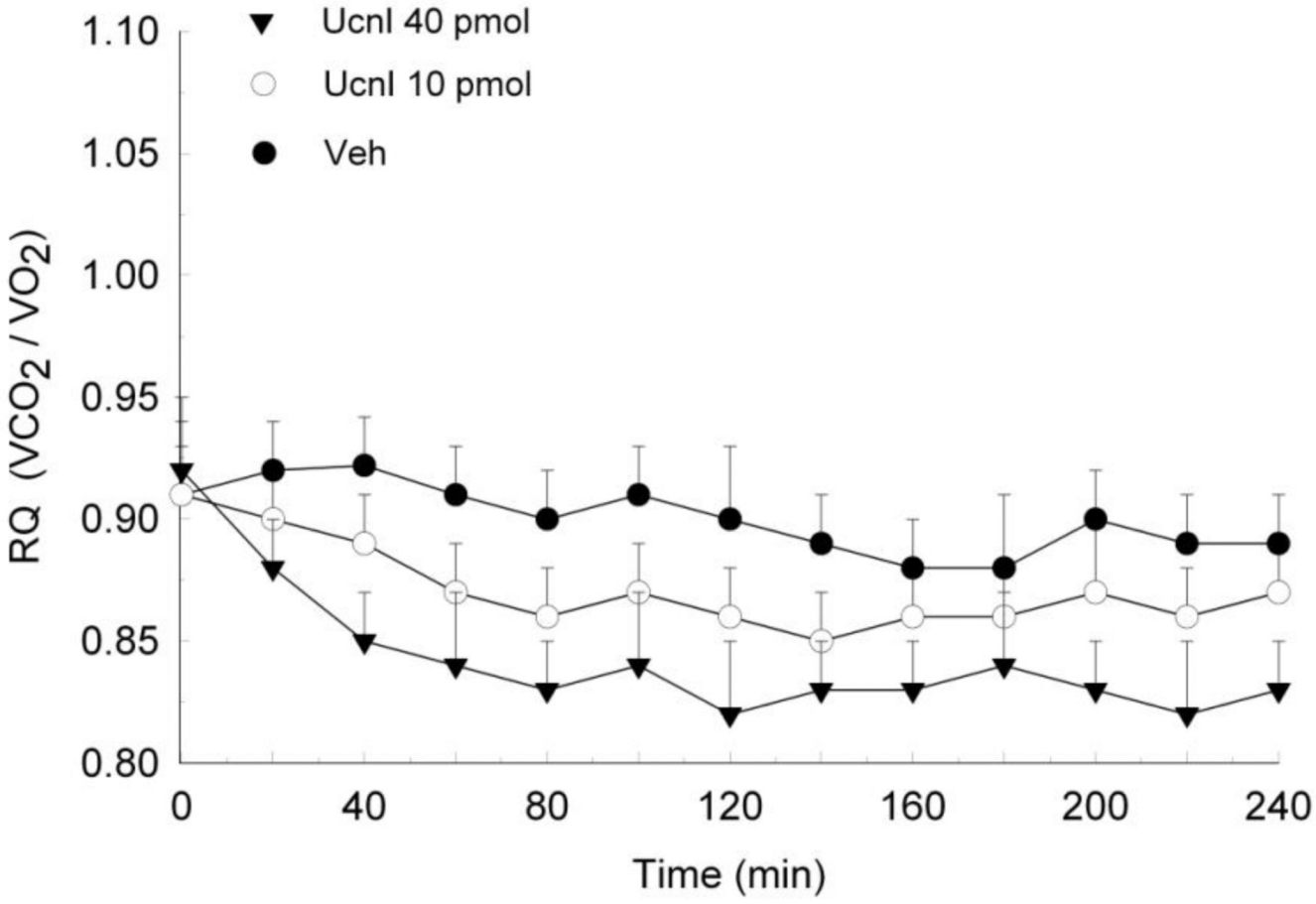

Fig. 4.

Energy substrate utilization (respiratory quotient; RQ) after UcnI injection into the PVN at the onset of the nocturnal cycle. Values are represented as mean ± S.E.M. measured over 4 h. UcnI dose-dependently decreased RQ within 40 min of treatment and the suppression in RQ was maintained over the remainder of the 4-h test.

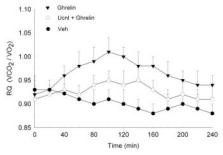

Fig. 5.

Respiratory quotient measured over the initial 4 h of the dark cycle. PVN injection of ghrelin (50 pmol) increased RQ within 40 min, reflecting enhanced carbohydrate oxidation. Pretreatment with UcnI (10 pmol) attenuated ghrelin's effect on substrate utilization throughout the entire 4-h test. Values are represented as mean ± S.E.M.

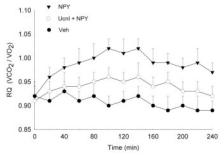

Fig. 6.

Effect of UcnI paired with NPY on RQ over 4 h following injection into the PVN. NPY (50 pmol) elicited a reliable increase in RQ within 20 min of testing. Enhanced carbohydrate oxidation observed after NPY administration was attenuated by UcnI (10 pmol) pretreatment and observed over the entire 4-h test period. Values are represented as mean ± S.E.M.

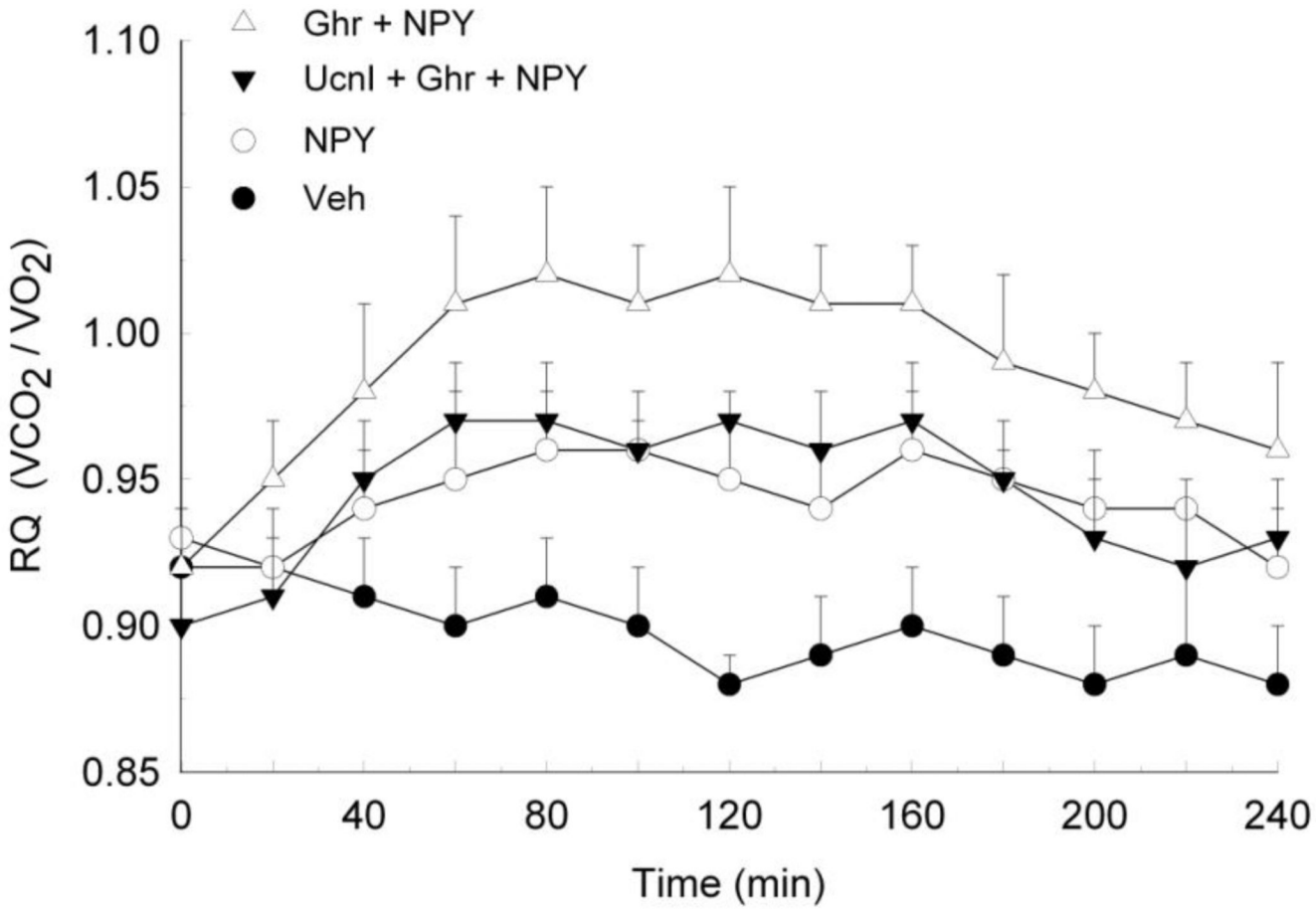

Fig. 7.

Injection of ghrelin (5 pmol) into the PVN potentiated the increase in RQ evoked by NPY (25 pmol) over the 4-h test period beginning at the onset of the dark cycle. This effect was reversed by UcnI (10 pmol) pretreatment. Values are represented as mean ± S.E.M.