Endocrine responses of ovariectomized ewes to i.c.v. infusion of urocortin

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Abstract

Urocortin is a novel corticotropin-releasing factor-like peptide, first isolated from the rat midbrain, which has anorexigenic properties, possibly associated with its involvement in the stress axis. Urocortin has been implicated in blood pressure regulation, ACTH release and feed intake, but its role as an integral component of the reproduction–nutrition axis has not been examined. The present experiment was designed to determine the effects of i.c.v. infusion of urocortin on feed intake and endocrine profiles of LH, GH, IGF-I, cortisol and leptin in ovariectomized ewes. Ewes were fitted with two laterocerebroventricular cannulae and urocortin was continuously infused in a linearly increasing manner from 0·001 µg/h on day 0, to a maximum of 31·6 µg/h on day 5. Blood samples were collected via jugular catheters at 10 min intervals for 4 h on day 1, 3 and 5, and assayed by RIA for LH, GH, IGF-I, cortisol and leptin. All ewes were allowed free access to feed and water, and feed intake was recorded daily. Urocortin-infused ewes responded with a significant decrease in feed intake beginning on day 1 (P<0·02) and were aphagic for the remainder of the experiment. Serum concentrations of LH were elevated in individual samples from urocortin-treated compared with saline-treated ewes on day 3 (treatment × day × sample, P=0·05), but were not different on day 1 or 5. Mean serum concentrations of GH increased (P<0·04) over days with urocortin treatment, although concentrations of IGF-I were not influenced by treatment (P>0·5). Serum concentrations of cortisol were markedly increased by urocortin treatment (P<0·001). Leptin tended to be influenced by treatment and day (P=0·08), with leptin levels tending to be elevated in urocortin-treated vs saline-treated ewes on day 5 (P=0·08). The ability of urocortin to decrease feed intake while increasing LH, GH, cortisol and leptin provides evidence that urocortin is not only an integral component of the stress axis, but possibly of the nutrition–reproduction axis in sheep.

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Introduction

Urocortin is a novel anorexigenic peptide with homology to corticotropin-releasing factor (CRF)-like proteins including CRF, urotensin I and sauvagine (Vaughan et al. 1995). Urocortin was first isolated from the Edinger–Westphal nucleus of the rat midbrain (Vaughan et al. 1995), and has since been detected in other animals including humans (Donaldson et al. 1995), Syrian hamsters (Robinson et al. 1999) and sheep (Cepoi et al. 1999). The physiological functions of urocortin and CRF are similar, most likely due to the cross-reactivity of these peptides for CRF receptors. i.c.v. infusion of CRF into rats activated the stress axis resulting in increased sympathetic activity, increased arousal, increased locomotor activity, decreased exploratory behavior, decreased parasympathetic outflow, and decreased appetite behavior (Spina et al. 1996). Similarly, i.v. or i.c.v. infusion of urocortin into rats resulted in dose-related decreases in meal sizes and frequency of food intake, and increased locomotor activity, but had no effect on exploratory behavior (Spina et al. 1996). In lean and ob/ob mice, urocortin dose-dependently decreased feed intake, body weight gain and gastric emptying. Urocortin was more potent than CRF, cholecystokinin-8 or leptin at producing these effects (Asakawa et al. 1999). In addition, due to its sympatho-mimetic actions, i.v. infusion of urocortin produced prolonged hypotension, and i.c.v. infusion also resulted in transient hypertension in the rat (Spina et al. 1996). Other cardiovascular changes that accompanied i.v. delivery of urocortin in the ovine included increased cardiac contractility, increased aortic flow, increased mean arterial pressure, increased heart rate, increased cardiac output and increased coronary blood flow, but no change in central venous pressure, total peripheral resistance or stroke volume (Parkes et al. 1997). Apart from the cardiovascular effects, ewes also exhibited a decrease in feed intake (Parkes et al. 1997). Collectively, the physiological effects of CRF and urocortin appear to overlap, with urocortin having more potent anorectic and cardiovascular effects.

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The objective of the present study was to determine the effect of i.c.v. infusion of urocortin on food intake and pituitary and adrenal hormone release, as well as compensatory or synergistic effects on concentrations of leptin and insulin-like growth factor (IGF-I). We hypothesized that urocortin would decrease both food intake and serum concentrations of luteinizing hormone (LH) and increase serum concentrations of growth hormone (GH) through modulation of the hypothalamic–pituitary–adrenal axis.

Materials and Methods

Cross-bred ewes, approximately 8 months of age and weighing 42 ± 5 kg, were randomly assigned to saline control (n=4) or urocortin (n=4) treatment groups. Ewes were housed in individual pens under ambient conditions with free access to water and a commercially blended pellet diet containing 16% crude protein beginning 2 weeks prior to the commencement of the study. According to procedures approved by the University of Missouri Animal Care and Use Committee, all ewes were bilaterally ovariectomized through a midventral celiotomy. After

![Figure 1: Feed intake among urocortin- and saline-infused ewes over days. Intake decreased (*P<0·02, **P<0·001) in urocortin-treated ewes by day 1, and urocortin-treated ewes were aphagic by day 3.](image1)

![Figure 2: Serum concentrations of LH in urocortin- and saline-infused ewes over days. Samples were taken at 10 min intervals on day 1, 3 and 5. Means for the sampling day are plotted after the individual samples. Asterisks indicate a significant difference between individual points in time. For means over days, means with different superscripts are different (P<0·05).](image2)
2 weeks of post-operative care, ewes were fitted with two lateral cerebroventricular cannulae. Under 1–3% halothane anesthesia, a 5 cm diameter skin flap was removed over the bregma, the periosteum was removed, and two 1·5 mm holes were drilled 1 cm caudal and 1 cm lateral to the bregma. Cannulae were then inserted to a depth of 35 mm, at which point retrograde flow of cerebrospinal fluid occurred and was used as a positive marker for correct placement into the laterocerebroventricles. Protective caps were then attached to the skull with dental acrylic to prevent movement of the cannulae. Ewes were allowed 1 week of recovery before silastic tubing was connected between the i.c.v. cannula and a computer-driven pump system designed to deliver the infusate (saline or rat urocortin (kindly provided by W Vale, Salk Institute, La Jolla, CA, USA) solubilized in saline). Coding regions of ovine and rat urocortin are 84% homologous and the ovine peptide encoded is predicted to be identical to the rat peptide (Cepoi et al. 1999). On day 0, the infusion was initiated and the infusate was continuously delivered in a linearly increasing manner. The infusion pump was programmed to deliver vehicle or urocortin beginning at 0·001 µg(µl)/h, and then increase 0·3 µg/h until a rate of 31·6 µg/h was attained on day 5. This linearly increasing method of delivery was utilized to ensure that animals were exposed to subphysiological through physiological dosages of urocortin over time. Neither physiological nor circulating concentrations of urocortin have previously been reported for the ovine, although dosages of urocortin that are capable of evoking an effect in the ovine have been reported. Thus the manner of urocortin delivery in this study allowed an investigation of urocortin-evoked effects over a continuous range of subphysiological through physiological dosages with intermittent blood sampling.

To assess feed intake, the amount of feed consumed by each ewe was measured at 24 h intervals. Each morning, the amount of feed remaining in each animal’s bin was weighed and recorded as an estimate of previous 24 h food intake and 4200 g fresh feed were replaced.

To facilitate repeated blood sample collection, ewes were fitted with indwelling jugular catheters on day 1. On day 1, 3 and 5, blood samples (4 ml) were collected every 10 min for 4 h. After collection of samples, blood

Figure 3 Serum concentrations of GH in urocortin- and saline-infused ewes over days. Samples were taken at 10 min intervals on day 1, 3 and 5. Means for the sampling day are plotted after the individual samples. For means over days, means with different superscripts are different (P<0·05). A day effect of GH was also present (P<0·055).
was allowed to clot at 4 °C overnight, and serum was harvested the following day. Serum concentrations of LH (McShane et al. 1993), GH (Powell & Keisler 1995), leptin (Delavaud et al. 2000) and IGF-I (Lamberson et al. 1995) were assessed using RIA procedures reported previously for use in our laboratory, and using a commercial kit for cortisol (Diagnostic Systems Laboratories, Webster, TX, USA). Intra-assay and inter-assay coefficients of variation for LH were 7 ± 1 and 5 ± 2%, and for GH were 4 ± 3 and 7 ± 1%. Intra-assay coefficients of variation for cortisol, leptin and IGF-I were 4 ± 1, 3 ± 1 and 7 ± 1% respectively.

At the completion of the experiment, ewes were killed in accordance with procedures approved by the United States Department of Agriculture. Placement of i.c.v. cannulae were confirmed by injection of India ink through the catheter and immediate removal and examination of the diencephalon and telencephalon for stain residue. Stain was located in the lateral cerebroventricles of all animals, confirming delivery of infusate into the laterocerebroventricular system.

Values for LH and GH were subjected to the CLUSTER method of pulse analysis (Veldhuis & Johnson 1986) for determination of number and amplitude of pulses per 4 h sampling period. All hormonal and feed-intake data were analyzed by repeated measures ANOVA using the General Linear Models procedures of SAS. All variables were tested using a statistical model that included treatment, ewe, sample and day. The error term ewe (treatment) was used to test for the effect of treatment, while ewe(treatment × day) was used to test for the effect of day and treatment × day. Differences between means were determined using the LSMEANS procedure of SAS with the PMIDF option (SAS 1985). Probability values of P<0·05 are considered significant.

Results

On day 0, feed intake did not differ between saline- and urocortin-infused ewes; however, within 24 h of starting the infusions (i.e. day 1), the urocortin-treated ewes
responded with a decrease ($P<0.02$) in feed intake relative to the saline-treated ewes. Feed intake continued to decrease in urocortin-treated ewes ($P<0.001$), and remained suppressed in urocortin-treated ewes for the duration of the study (Fig. 1).

Mean serum concentrations of LH were influenced by a treatment $\times$ day $\times$ sample interaction ($P=0.05$). On day 3, individual sample means were higher ($P<0.05$) in urocortin- vs saline-treated ewes, although no difference between sample means was detected on day 1 or 5 (Fig. 2). No differences were detected in LH pulse frequencies or amplitudes per sampling interval among treatment groups ($P>0.8$ and $P>0.7$ respectively).

Mean serum concentrations of GH increased ($P<0.04$) in urocortin-infused ewes compared with saline-infused ewes (Fig. 3), with the highest concentration occurring on day 5. A significant effect of day was also detected ($P<0.05$), with GH increasing in a stepwise manner over time. The number of GH pulses per sampling interval increased over days ($P<0.01$), but was not significantly different from saline-treated ewes on day 1 or 3 ($P>0.5$). Amplitude of GH pulses increased over days among urocortin-infused ewes and values were significantly greater than in saline-treated ewes on day 3 and 5 ($P<0.01$).

Serum concentrations of cortisol were significantly greater ($P<0.001$) among urocortin- vs saline-treated ewes only on day 5; notably long after urocortin’s effects on reducing feed intake were observed (Fig. 4).

Due to the similarity in leptin and urocortin in their anorexigenic properties, serum concentrations of leptin were assessed to determine if i.c.v. infusion of urocortin affected circulating concentrations of leptin. Serum leptin tended to be influenced by treatment and day (treatment $\times$ day, $P=0.08$), with serum leptin concentrations on day 5 tending to be greater in urocortin- than saline-treated ewes ($P<0.08$, Fig. 5).

IGF-I was assayed to determine if the increase in serum GH in urocortin-treated ewes was due to the lack of inhibitory feedback by IGF-I on GH-releasing hormone neurons or pituitary somatotrophs. IGF-I did not differ between urocortin- and saline-treated ewes (Fig. 6, $P>0.5$). There was a day effect, in which IGF-I increased over days within treatments ($P<0.008$).
Discussion

The present study examined the effect of centrally administered (via i.c.v. infusion) urocortin on LH, GH, IGF-I, cortisol, leptin and feed intake, as measures of urocortin’s involvement in the reproduction–nutrition–stress axes of ewes. Among urocortin-treated ewes, feed intake declined on day 1 and was negligible by day 3, illustrating that an i.c.v. dose of 12.4 µg or less of urocortin was capable of decreasing feed intake, while 25 µg or less completely abolished feed intake in the ewe. Of notable significance, the decrease in feed intake in the urocortin-treated ewes occurred prior to any changes in measured circulating hormone concentrations. Alterations in hormone levels did not differ until day 3, providing evidence that a low dose of urocortin can suppress feed intake without significantly affecting the secretion of these hormones. Chronic feed restriction alone negatively affects the release of LH and GH (reviewed by Keisler & Lucy 1996). Acute feed restriction also resulted in decreased LH secretion, but concomitantly increased serum concentrations of GH due most likely to decreased negative feedback from lower circulating IGF-I (Foster et al. 1989, Hodgkinson et al. 1991).

Despite the similarities between CRF, CRF-like peptides and urocortin, they all have individually unique actions. We observed that in ewes infused i.c.v. with urocortin, LH was increased on day 3 but not day 5. Cortisol negatively affects the hypothalamic–pituitary axis resulting in a decrease in LH release but not its synthesis (Li & Wagner 1983). Therefore, unlike cortisol, urocortin appears to have a stimulatory effect on the secretion of LH. As previously noted, the increase in LH on day 3 was attenuated by day 5. Interestingly, cortisol levels also increased during day 3 to day 5, and therefore may be the stimulus responsible for the attenuation in secretion of LH.

The cross-reactivity of urocortin and CRF for the CRF family of receptors may be responsible for their similar bioactivity. Both peptides bind to CRF-binding proteins, as well as CRF receptors 1, 2a and 2b (Behan et al. 1996),
and induce adrenocorticotropic release from the anterior pituitary through the activation of CRF-R1 (Asaba et al. 1998). Although both peptides bind CRF receptors, urocortin preferentially binds to CRF-R2a>CRF-R2b>CRF-R1, while CRF has highest affinity for CRF-R1 (Donaldson et al. 1995). The distribution of these receptors corresponds to the location of peptide, suggesting that urocortin is the endogenous ligand for CRF-R2a. CRF-R2a are primarily located peripherally in the duodenum, skeletal muscle and perivascular cells of the heart (Perrin et al. 1995), and centrally in the lateral septal nuclei and ventromedial hypothalamic nuclei (Lovenberg et al. 1995). Urocortin has been localized in the Edinger–Westphal nucleus, lateral superior olivary nucleus, lateral hypothalamus, supraoptic nuclei (Bittencourt et al. 1999) and pituitary (Oki et al. 1998). Neither CRF-R1 nor CRF mRNA is abundant in these areas and they tend to be absent in diencephalic nuclei, except for the paraventricular nucleus of the hypothalamus (Matthews et al. 1991). Therefore, the interaction of urocortin with CRF-R2a in the ventromedial and lateral hypothalamus (known ‘appetite centers’) may be responsible for the anorexia exhibited by animals treated with urocortin.

We hypothesized that GH would increase in urocortin-infused ewes due to lack of IGF-I negative feedback in response to the decrease in feed intake. GH was significantly elevated due to treatment, increasing in a stepwise manner over time in urocortin- but not saline-infused ewes. Since IGF-I concentrations were not different between treatment groups, the increase in GH over days may have been due to stimulation by urocortin and not due to a lack of IGF-I. Somatotrophs express CRF-R2 and stimulation of these receptors in human pituitary adenoma cells by urocortin resulted in an increase in GH secretion in 50% of cells (Murakami et al. 1997).

Cortisol, as well as other hormones such as insulin, stimulates the expression of leptin (Housencketh et al. 1998). Both leptin and urocortin suppress appetite and also appear to regulate the expression of the other. i.c.v. infusion of urocortin tended to increase serum concentrations of leptin. This response may be a direct action of urocortin due to entry into the blood stream, or more likely is secondary to the increase in cortisol secretion.

In summary, urocortin is an anorexigenic peptide that may serve as an integral component within the nutrition, reproductive and/or stress axes of ewes. Utilizing physiological to perhaps pharmacological levels of urocortin, appetitive and endocrinological responses were elicited in ewes following central urocortin administration.

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