The Role of Corticotropin-Releasing Factor and Urocortin in Brain Mechanisms Controlling Feed Intake of Sheep

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ABSTRACT : The aim of the present study was to determine whether brain corticotropin-releasing factor (CRF) and a new peptide, urocortin (UCN) have a direct action in brain mechanisms controlling feed, water and salt intake in sheep. We gave a continuous intracerebroventricular (ICV) infusion of the peptide at a small dose of 5 μg/0.2 ml/hr for 98.5 hrs from day 1 to day 5 in sheep not exposed to stress. Feed and water intake during ICV infusion of CRF or UCN decreased significantly compared to those during artificial cerebrospinal fluid (CSF) infusion. NaCl intake during infusion of CRF or UCN was the same as that during CSF infusion. Mean carotid arterial blood pressure (MAP) and heart rate during ICV infusion of CRF or UCN were not significantly different from that during CSF infusion. On the other hand, the plasma glucose concentration during ICV infusion of CRF or UCN tended to be higher than that during CSF infusion. These observations indicate that decreased feed intake induced by CRF and UCN infusion is not mediated by the activation of both the pituitary-adrenal axis and the sympathetic nervous system. The results suggested that brain CRF and UCN act directly in brain mechanisms controlling ingestive behavior to decrease feed and water intake, but do not alter salt intake in sheep. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 11 : 1529-1535*)

Key Words : CRF, Urocortin, Brain, Feed Intake, Sheep

INTRODUCTION

It is well known that when animals are exposed to stress, CRF plays a major role in integrating the endocrine and autonomic response to this stress through the activation of both the pituitary-adrenal axis and the sympathetic nervous system (Antoni, 1986; Menzaghi et al., 1993; Imai et al., 1995). When infused into the central nervous system CRF, like stress, produces anxiogenic-like and anorectic effects that are largely independent of the activation of adrenocorticotropic hormone (ACTH) and corticoids, suggesting a direct action on brain CRF receptors (Spina et al., 1996). Evidence shows that brain levels of CRF are elevated during restraint (immobilization) stress (Harbuz and Lightman, 1989; Imaki et al., 1992; Pich et al., 1993).

The CRF receptors, CRF-R1 and CRF-R2 are found in the limbic system, hypothalamus and brain stem as well as the hypophysis (Turnbull and River, 1997). On the other hand, a new peptide, UCN was isolated from a discrete rat midbrain region through cloning (Vaughan et al., 1995). The amino-acid sequence of this peptide is related to urotensin (63% sequence identity) and CRF (45% sequence identity). UCN has similar biological activities to urotensin and CRF. UCN is more potent than CRF at binding and activating CRF-R2 receptors (Vaughan et al., 1995).

It was reported that when ICV bolus injections of CRF and UCN were carried out in rats, plasma catecholamines concentration and MAP increased, anxiety appeared and feed intake decreased (Brown and Fisher, 1983; Spina et al., 1996; Moreau et al., 1997). The anxiogenic effects of CRF in rats are mediated by CRF-R1 receptors (Heinrichs et al., 1997). CRF and UCN, acting via CRF-R2 receptors, mediate changes in ingestive behavior (Smagin et al., 1998). Howevere, ICV administration of UCN was not carried out in ruminants.

The aim of the present study was to determine whether brain CRF and UCN have a direct action in brain mechanisms controlling feed, water and salt intake in ruminants. We gave a continuous ICV infusion of the peptide at a small dose of 5 μ g/0.2ml/hr for 98.5 hrs from day 1 to day 5 in sheep not exposed to stress.

MATERIALS AND METHODS

Animals

Twelve crossbred Merino ewes, 34-45 kg body weight, were used. The sheep were ovariectomized and had both carotid arteries exteriorized in skin loop. All animals were surgically prepared with a guide tube (17-gauge stainless needle, 34 mm long) implanted 6-10 mm above each lateral brain ventricle.

The sheep were maintained in metabolic cages, which allowed for the separate collection of urine, saliva and faeces. In addition, the cages contained two

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pedals. The animals were trained to press the left pedal to obtain 25 ml of 0.5 M NaCl (=12.5 mmol Na) and the right pedal to get 50 ml of water. All deliveries were consumed. The number of deliveries were counted and recorded continuously by computer. Typically, there was a minimum of 9 days between infusions.

We examined the effects of ICV infusion of peptides in sheep adapted to a 2 hrs, once a day feeding period. The sheep were fed dried alfalfa chaff (Na^+ 90-100 mmol, K^+ 250-400 mmol) once a day (12:00 to 14:00) before and during the experiment (RucKebusch and Malbert, 1986; Spina et al., 1996).

Intracerebroventricular infusion procedure

For intracerebroventricular (ICV) infusion, an obturator was removed from one of the guide tubes, and a LV (lateral ventricle) probe (20-gauge needle attached to a metal Luer-Lock cap) of the appropriate length was inserted through the guide tube into the lateral brain venricle. The probe was connected via a polyethylene cannula to a 10 ml syringe held in an infusion pump (Perfusor, Braun, Germany). Infusion experiment was of 98.5 hrs in duration, during which a continuous ICV infusion of the peptide was given at small doses of 5 μg/0.2ml/hr. The infusates used, rat Urocortin (4707.3 MW, Sigma, USA) and rat-human Corticotropin releasing factor (4758 MW, Auspep, Australia), were dissolved in artificial cerebrospinal fluid (CSF: 151 mM Na^+, 157.5 mM Cl^-, 2.8 mM K^+, 1.1 mM Ca^{2+}, 0.9 mM Mg^{2+} and 0.5 mM HPO_4^-). All animals received a control infusion of artificial CSF (0.2 ml/hr).

Blood pressure measurements and blood sampling

The mean arterial blood pressure was measured from the carotid artery via a heparin-saline filled 18 gauge needle and polyethylene tube hooked up to a COBE disposable transducer model 345-931-009. The pressure recording system was a JRACK (Australia) rack type RK8 pressure amplifier with a GRAPHTEC Thermal Arraycorder model WR7700. The blood samples were taken via this cannula from a carotid artery.

Experimental design

Five sheep were given an ICV infusion of a) Corticotropin releasing factor at 5 μg/hr, b) Urocortin at 5 μg/hr, and Artificial CSF for 98.5 hours. All infusions were at a rate of 0.2 ml/hr. The infusion started at 11:30 on day 1 and ended at 14:00 on day 5. Blood pressure was measured at 10:00 on day 1, 2, 3, 4 and 5 and a 10 ml blood sample was taken after that. In the text, baseline is prior to infusion. The feed, water and sodium chloride intake were also measured daily. Sheep were weighed once a week.

Chemical analysis

The Na^+, K^+, Cl^-, glucose and total protein of plasma were measured with a Beckman CX5 Clinical system. Osmolality was measured with a Digimatic osmometer (Advanced Instrument, Denmark).

The alfalfa chaff was ground by Willey milling machine (Type 40-525P, Ikemoto Rika Kougyou, Japan) and the chemical composition was analyzed (Kato, 1988). The digestible crude protein (DCP) and the total digestible nutrients (TDN) were calculated using the chemical composition and digestibility (table 1). The digestibility of the feed was determined using the in vivo method by the formula : the digestibility of feed (%) = (feed intake- feaces)/feed intake $\times$ 100.

Statistical analysis

The significant differences between CSF infusion and peptides infusion, and the differences between baseline and post infusion of peptides were analyzed using a paired t, test respectively.

Feed intake was calculated based upon dry matter content of the feed only. All data were expressed by means $\pm$ standard error (SE) of five animals.

RESULTS

ICV CRF infusion experiment

The results of feed, water and salt intake are shown in figure 1. All feed intake data were expressed on dry matter basis. Feed intake during ICV infusion of CSF (828$\pm$28.3 g/2hr) was the same as that of baseline values (780$\pm$23.4 g/2hr) and was not influenced by the continuous 98.5 hr infusion. On the other hand, feed intake during CRF infusion decreased significantly compared to that during CSF infusion.

Table 1. Chemical composition and nutritive values of alfalfa chaff

	Alfalfa chaff
Dry matter (%)	89.0$\pm$0.17
Chemical composition (% of DM)	
Organic matter	92.8$\pm$0.05
Crude protein	12.9$\pm$0.21
Crude fat	3.5$\pm$0.11
Nitrogen-free extracts	52.0$\pm$0.29
NDF[1]	45.6$\pm$0.21
ADF[2]	26.2$\pm$0.23
Nutritive values(% of DM)	
DCP[3]	9.2$\pm$0.13
TDN[4]	61.6$\pm$0.01

[1] NDF: Neutral degergent fiber.
[2] ADF: Acid dergent fiber.
[3] DCP: Digestible crude protein.
[4] TDN: Total digestible nutrients. Values are means$\pm$SE from five determinations.

While feed intake was depressed sharply on day 2 of the ICV CRF infusion, decreased feed intake levels attenuated slightly over days 3 to 5. Water intake during ICV infusion of CRF on day 2 decreased significantly compared to that duing CSF infusion. NaCl intake during ICV infusion of CRF was the same as that during CSF infusion.

MAP during ICV infusion of CSF was the same as that of baseline value and was not influenced by the continuous 98.5 hr infusion (figure 2). MAP and heart rate during ICV infusion of CRF were not significantly different from those during CSF infusion. MAP values during ICV CRF infusion on days 2 and 5 tended to be higher than that of the baseline. The plasma glucose concentration during ICV infusion of CRF tended to be higher than that during CSF infusion (figure 3).

The mean plasma concentrations of Na, K and Cl prior to beginning the infusion and during infusion of CSF were 144.5, 144.1; 4.3, 4.4; 110.7, 111.7 mmol/l, respectively.The mean plasma concentrations of Na, K and Cl prior to beginning the infusion and during infusion of CRF were 145.0, 144.8; 4.3, 4.2; 111.0, 112.7 mmol/l, respectively. The plasma osmolality during ICV infusion of CRF was not different from that during ICV CSF infusion (figure 2). On the other hand, the plasma total protein concentration during ICV infusion of CRF increased significantly compared to that during CSF infusion (figure 3).

The body weight of sheep after completion of ICV CRF infusion was not significantly different from that prior to beginning the infusion (figure 3). The dose of ICV CRF infusion in the present experiment did not induce anxiogenic-like effects. All animals remained calm during and after ICV CRF infusion.

ICV UCN Infusion Experiment

The results of feed, water and salt intake are shown in figure 4. Feed intake during ICV infusion of UCN decreased markedly compared to that during CSF infusion. A gradual decrease in feed intake was

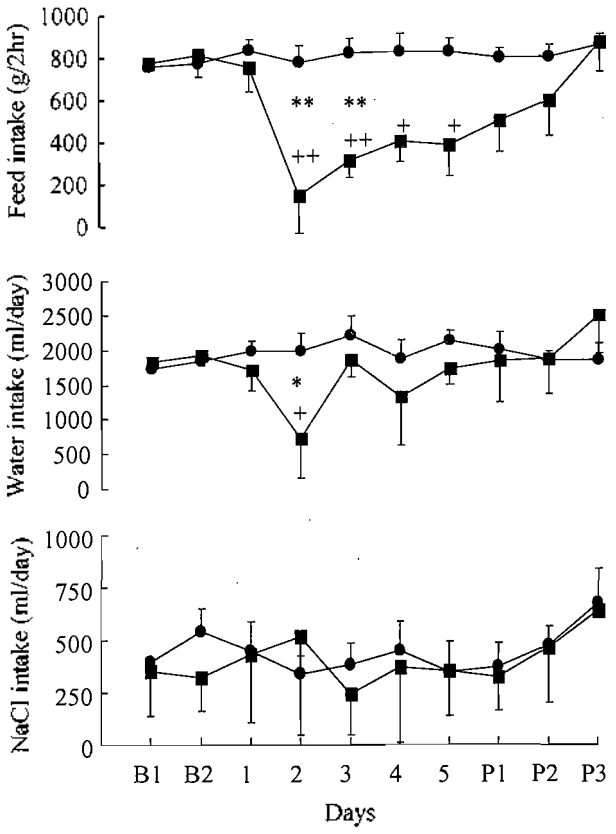

Figure 1. Effects of ICV infusion of CSF (0.2 ml/hr, ●) and CRF (5 μg/0.2ml/hr, ■) on feed, water and salt (0.5 M NaCl) intake. B, 1-5 and P represent prior to, during and post ICV CSF or CRF infusion, respectively. Each point represents the mean ±SE of 5 sheep. Statistical analysis is described in the text: *p<0.05, ** p<0.01 (vs. CSF); + p<0.05, ++ p<0.01 (vs. baseline).

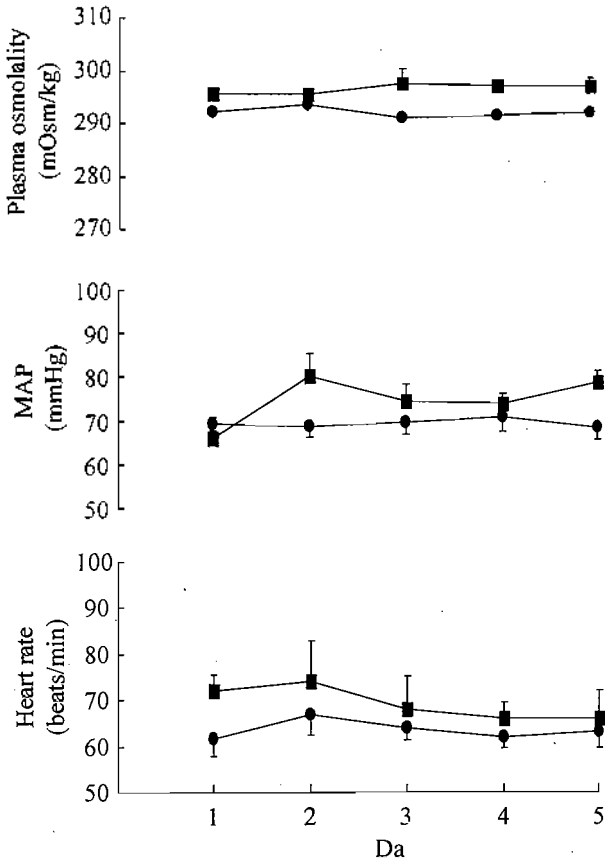

Figure 2. Effects of ICV infusion of CSF (0.2 ml/hr, ●) and CRF (5 μg/0.2 ml/hr, ■) on plasma osmolality, mean arterial blood pressure and heart rate. Each point represents the mean±SE of 5 sheep. Statistical analysis is described in the text: *p<0.05 (vs. CSF).

observed during ICV infusion of UCN. When the peptide infusions were stopped, feed intake gradually returned to baseline levels in the ICV UCN infusion experiment. Water intake during UCN infusion decreased markedly compared to that during CSF infusion. NaCl intake during infusion of UCN was the same as that during CSF infusion.

MAP and heart rate during ICV infusion of UCN were not different from those during CSF infusion (figure 5). The plasma glucose concentration during ICV infusion of UCN tended to be higher than that during CSF infusion (figure 6).

The mean plasma concentrations of Na, K and Cl prior to beginning the infusion and during infusion of CSF were 144.3, 144.0; 4.3, 4.4; 110.3, 110.3 mmol/l, respectively. The mean plasma concentrations of Na, K and Cl prior to beginning the infusion and during of UCN were 144.7, 143.2; 4.5, 4.4; 109.7, 109.7 mmol/l, respectively. The plasma osmolality during ICV UCN infusion was not different from that during ICV CSF infusion (figure 5). On the other hand, the plasma total protein concentration during ICV infusion of UCN increased significantly compared to that during CSF infusion (figure 6).

The body weight of the sheep after completion of ICV UCN infusion was not significantly different from that prior to beginning the infusion (figure 6). The anxiogenic-like effects were not observed during and after ICV infusion of UCN. All animals remained calm prior to, during and after ICV UCN infusion.

DISCUSSION

To clarify the biological activities of the peptides

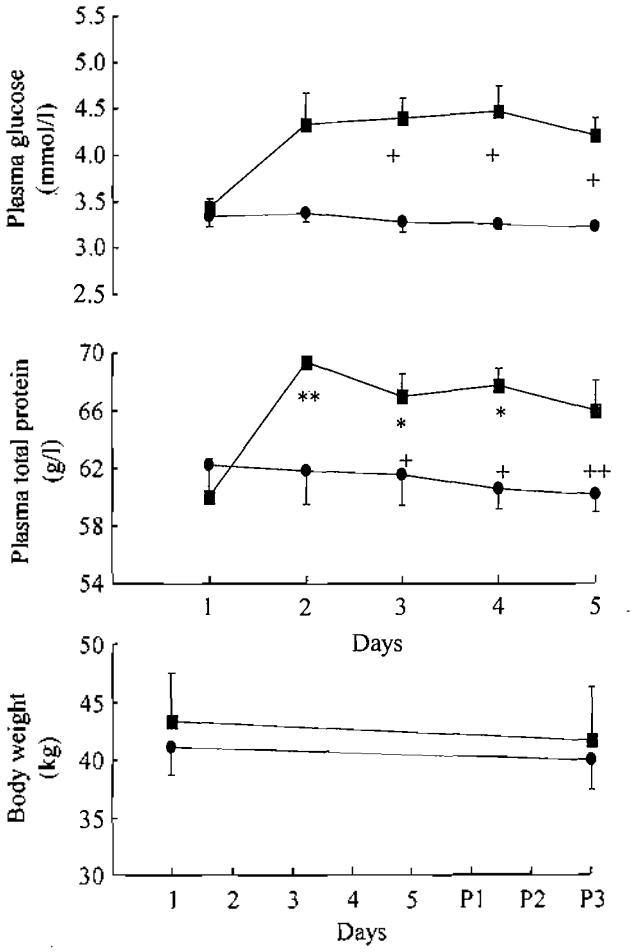

Figure 3. Effects of ICV infusion of CSF (0.2 ml/hr, ●) and CRF (5 μg/0.2ml/hr, ■) on plasma glucose concentration, plasma total protein concentration and body weight. Each point represents the mean ± SE of 5 sheep. Statistical analysis is described in the text: * p<0.05, ** p<0.01 (vs. CSF); + p<0.05, ++ p<0.01 (vs. baseline).

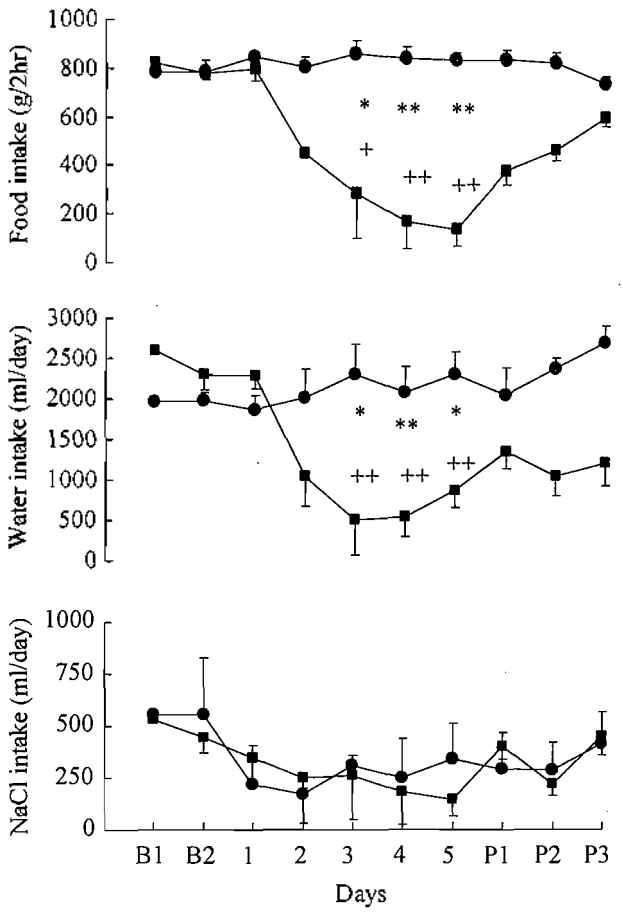

Figure 4. Effects of ICV infusion of CSF (0.2 ml/hr, ●) and UCN (5 μg/0.2ml/hr, ■) on feed, water and salt (0.5 M NaCl) intake. B, 1-5 and P represent prior to, during and post ICV CSF or UCN infusion, respectively. Each point represents the mean ± SE of 5 sheep. Statistical analysis is described in the text: * p<0.05, ** p<0.01 (vs. CSF); + p<0.05, ++ p<0.01 (vs. baseline).

in small animals, most studies have employed intravenous (IV) or ICV bolus injection of the peptide at a dose larger than the endogenous release. However, this method has not been successful in clarifying a biological activity of endogenous peptides in large animals due to the large size of the lateral ventricle and the rapid flow rate of cerebrospinal fluid (Okita et al., 1998). In order to elucidate the direct effect of endogenous brain CRF and UCN on brain mechanisms controlling feed, water and salt intake in sheep, we gave a continuous ICV infusion of the peptide at a small dose for long periods in sheep not exposed to stress. As a result, in this experiment, heart rate and MAP during ICV infusion of CRF or UCN were not significantly different from those levels prior to infusion (figure 2, 5). The anxiogenic-like effects during ICV infusion of CRF or UCN were not observed in this experiment. All animals remained calm during ICV infusion of CRF or UCN. The increases in plasma catecholamines concentration, heart rate and MAP caused by ICV infusion of CRF or UCN in rats (Brown and Fisher, 1983; Spina et al., 1996; Moreau et al., 1997), were not observed in this experiment. Therefore, these results indicate that the continuous ICV infusion of CRF or UCN at a small dose for long periods had no stressful effects on the sheep.

While feed intake was depressed markedly on day 2 of the ICV CRF infusion, depressed feed intake levels attenuated slightly over days 3 to 5 (figure 1). The reason for this result is unclear. On the other hand, feed intake during ICV UCN infusion gradually decreased throughout the entire infusion period (figure 4). The decrease in feed intake during ICV UCN infusion was larger than that during ICV CRF infusion. Within the rat brain, CRF-R1 mRNA is present in discrete regions, including neo-, olfactory, and hippocampal cortices, cerebellum, septum,

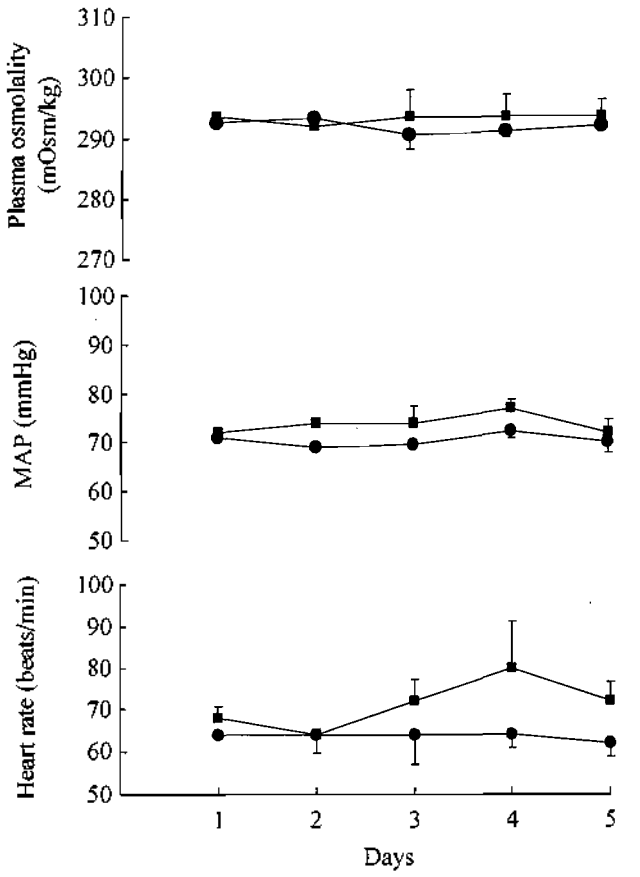

Figure 5. Effects of ICV infusion of CSF (0.2 ml/hr, ●) and UCN (5 μg/0.2ml/hr, ■) on plasma osmolality, mean arterial blood pressure and heart rate. Each point represents the mean ± SE of 5 sheep. Statistical analysis is described in the text: * $p<0.05$ (vs. CSF).

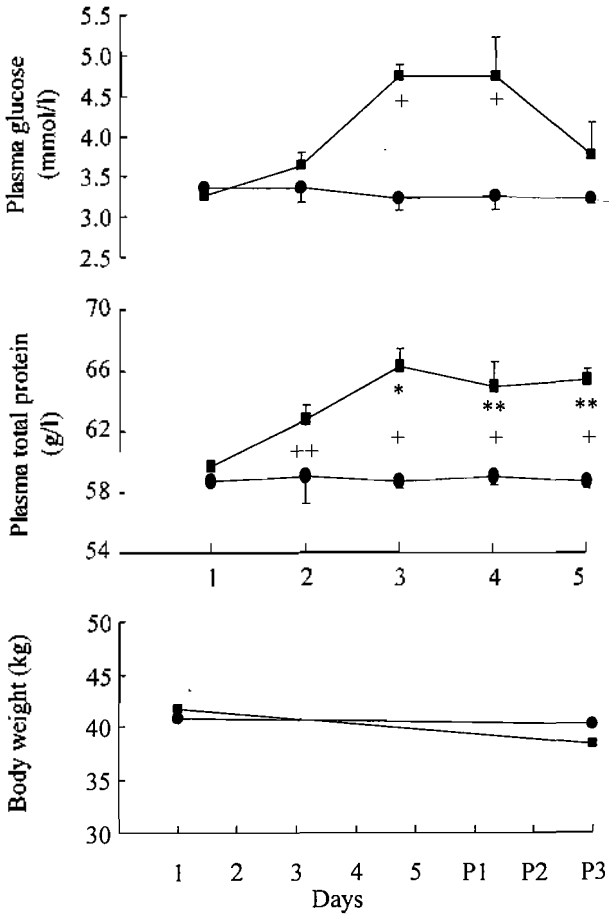

Figure 6. Effects of ICV infusion of CSF (0.2 ml/hr, ●) and UCN (5 μg/0.2ml/hr, ■) on plasma glucose concentration, plasma total protein concentration and body weight. Each point represents the mean ± SE of 5 sheep. Statistical analysis is described in the text: * $p<0.05$, ** $p<0.01$ (vs. CSF); + $p<0.05$, ++ $p<0.01$ (vs. baseline).

amygdala, and brainstem sensory relay structures, with only low levels being observed in thalamic and hypothalamic nuclei . In contrast, the expression of CRF-R2 mRNA within rat or mouse brain is more confined to subcortical structures: in lateral septal nucleus, ventromedial hypothalamic nucleus, paraventricular nucleus, olfactory bulb, amygdala, and the choroid plexus (Turnbull and River, 1997). These nuclei are related to the central control of feed and water intake (Vaughan et al., 1995). CRF and UCN, acting via CRF-R2 receptors, mediate changes in ingestive behavior (Smagin et al., 1998). A physiological role of CRF has been shown in experiments where a CRF antagonist prevents the reduction in feed intake caused by restraint stress (Krahn et al., 1986). UCN binds CRF-R2 receptor with 10 to 40-fold greater affinity than CRF (Spina et al., 1996). On the other hand, the location of these receptors in the brain sheep is unclear. Unlike ICV infusion, intravenous infusion of CRF did not alter feed intake in sheep (Ruckebusch and Malbert, 1986). From these reports, the results in this experiment may be explained by the fact that UCN binds CRF-R2 receptors in feeding and drinking centers with greater affinity than CRF. In this experiment, the decrease in feed intake caused by restraint stress in rats (Krahn et al., 1986; Shimizu et al., 1989) is mimicked by the direct action of CRF or UCN in the brain of sheep not exposed to stress. When the peptide infusions were stopped, feed intake returned to baseline levels in the ICV CRF of UCN infusion experiment (figure 1, 4). The results indicate that ICV infused CRF or UCN acted physiologically in brain mechanisms controlling feed intake in sheep.

Brain CRF and UCN have some influences in the activation of the pituitary-adrenal axis. In this experiment, the plasma glucose concentration during ICV infusion of CRF or UCN tended to be higher than that during CSF infusion (figure 3, 6). An ICV bolus injection of CRF in rats evoked increases in plasma adrenocorticotropic hormone (ACTH) levels (Turnbull and River, 1997). Thirty minutes after a bolus intravenous injection (100 μg) of CRF or UCN, plasma ACTH and cortisol levels increased in sheep (Parkes et al., 1997). Both CRF and UCN acting via CRF-R1 or CRF-R2 receptors (Smagin et al., 1998), cause release of ACTH from anterior pituitary cells and increase secretion of adrenocortical hormones (Hotta et al., 1991; Smagin et al., 1998). The small increases in plasma glucose levels in this experiment might have been due to a slight increase in the release of ACTH and cortisol (May and Bednarik, 1995). However, it is difficult to consider that the peripheral actions of these peptides decrease feed and water intake in sheep. The reason for this is that the changes in plasma glucose concentration does not affect feed intake in ruminants (Baile and Mayer, 1969). Therefore, these results show that the influence of CRF or UCN on feed intake is independent of its ability to cause the secretion of ACTH and the subsequent secretion of the adrenocorticoid hormones.

Blair-West et al. (1995) indicated that subcutaneous infusion of ACTH for 7 days in mice increased Na intake. However, Na intake during CRF or UCN infusion remained unchanged in this experiment (figure 1, 4). Therefore, it is concluded that brain CRF and UCN are not involved in the controlling mechanism for Na intake in sheep.

Approximately 90% of total plasma osmotic pressure is contributed by plasma Na and Cl, while about 0.01% is contributed by plasma total protein. The plasma osmolality during ICV infusion of CRF or UCN was equal to that during CSF infusion due to the fact that concentrations of plasma Na, K and Cl remained unchanged following infusion. On the other hand, plasma total protein concentration during ICV infusion of CRF or UCN significantly increased in compared to that during CSF infusion (figures 3 and 6). This result might be explained by the decrease in water intake during ICV infusion of CRF or UCN.

In conclusion, the results in this experiments suggested that brain CRF and UCN act directly in brain mechanisms controlling ingestive behaviors to decrease feed and water intake, but do not alter salt intake in sheep.

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