The inhibitory effect of hormones associated with stress on Na appetite of sheep

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Stress is a large stimulus of Na appetite in rabbits, rats, and mice. This study investigated the influence of some peptides implicated in stress, i.e., adrenocorticotropin (ACTH), corticotropin-releasing factor (CRF), and the recently discovered member of the CRF family, urocortin, on the ingestive behavior of sheep. Intracerebroventricular infusion of these peptides over 4 days decreased the need-free Na intake of Na-repleted sheep. Intracerebroventricular infusion of urocortin, however, did not alter Na intake of Na-depleted sheep. Systemic infusion of ACTH increased, whereas systemic infusion of either urocortin or CRF decreased, Na intake of Na-repleted sheep. The increase in Na intake caused by the peripheral infusion of ACTH was blocked by concurrent i.v. infusion of urocortin, substantiating the inhibitory role of this peptide on Na appetite. Central administration of all peptides and i.v. administration of urocortin or urocortin and ACTH combined decreased food intake. Water intake was not directly influenced by the peptides. Rather, decreased water intake, when observed, was secondary to decreased food intake, as determined by pair-feeding experiments. Whereas systemic infusion of ACTH mimics the increase in Na intake observed in several different stressful situations, CRF and urocortin actually inhibit Na intake, indicating a direct central action overriding any effect of these peptides on ACTH release. Indeed, the inhibition of Na intake by urocortin occurred despite its stimulation of ACTH release and the subsequent increase in peripheral level of cortisol. Thus it would appear that hormones associated with stress have both excitatory and inhibitory influences on Na intake. Presumably, other physiological processes entrained by stress also will be important in determining the quantitative outcome on Na appetite.

Stress changes physiological functions by altering neuroendocrine and neural systems (1–5). Stress can cause an increase in Na intake (6–9) and, in some instances, a decrease in food intake (10, 11). Corticotropin-releasing factor (CRF), a 41-aa peptide originally isolated from the ovine hypothalamus (12), is one factor involved in the initiation of the various behavioral and physiological responses to stress (1, 2, 4, 5). The influence of CRF can be mediated by its stimulation of both the sympathetic nervous system and the pituitary-adrenal axis (1, 2, 5, 13). Urocortin, a recently discovered member of the CRF family (i.e., with 45% homology to CRF), binds with high affinity to CRF receptors (14) and may contribute to responses previously attributed to CRF. However, although evidence shows that brain levels of CRF are elevated during stress (5, 15, 16), this is still to be shown for urocortin. Both urocortin and CRF, acting via CRF-R1 or CRF-R2 receptors (17, 18), cause release of adrenocorticotropin (ACTH) from anterior pituitary cells and increase secretion of adrenocortical hormones (17–20). The anxiogenic effects of CRF are mediated by CRF-R1 receptors (21). CRF and urocortin, acting via CRF-R2 receptors, mediate changes in ingestive behavior (18).

In rats, urocortin and CRF are found in brain areas known to be important in body fluid and electrolyte homeostasis, e.g., supraoptic nucleus, paraventricular nucleus of the hypothalamus, septal area, bed nucleus of the stria terminalis, and organum vasculosum of the lamina terminalis (14, 22, 23), and there is some evidence consistent with a role for these peptides in the behavioral control of body water and Na homeostasis. A direct stimulatory action of ovine CRF on Na intake of rabbits (20) and mice (9) has been reported. Thus far, however, an action of urocortin on Na intake has not been reported.

Consistent with an increase in Na intake caused by stress, peripheral administration of ACTH causes a large increase in Na intake in several different species including sheep (24), rabbits (25), rats (26), and mice (9, 27). In sheep and rats, this appetite appears to be an effect of the ACTH on adrenal gland hormones because it can be prevented by adrenalectomy (24, 26). On the other hand, ACTH can cause an increase in Na intake in the adrenalectomized wild rabbit (25).

Although it is clear that stress or the hormones released in response to stress can stimulate Na appetite, there is no data, at present, that chronic central administration of CRF or urocortin can influence the Na or water intake of sheep. Thus, the aim of the present studies was to determine the influence of chronic intracerebroventricular (ICV) and systemic infusion of urocortin, CRF, and ACTH on Na and water intakes of Na-repleted sheep. The effect of ICV infusion of urocortin on the Na appetite of Na-depleted sheep, an appetite mediated by both circulating levels of angiotensin II (28, 29) and cerebral Na concentration (30), also was evaluated. The systemic administration of the peptides, at the same dosage as given ICV, was used to evaluate the possibility that the changes observed during ICV administration were mediated by the peptides influence on peripheral mechanisms, i.e., because of escape of the peptide into the circulation. In addition, the interaction of systemically administered urocortin, the most potent of the peptides in the CRF family, and systemically administered ACTH, a peptide known to enhance Na appetite (24), was evaluated. Changes in food intake and body weight also were determined. The effect of decreased food intake, reflecting that observed during ICV administration of urocortin, on Na and water intakes and body weight was determined by pair-feeding.

Methods

Animals. Fourteen crossbred Merino ewes were used. Before experimentation all animals were surgically prepared under general anesthesia: a guide tube was implanted above each lateral brain ventricle (29, 30); the sheep were oophorectomized; both carotid arteries were exteriorized in skin loops. In addition, seven of the sheep had a permanent unilateral parotid fistula (31) so that $NaHCO_3$-rich saliva was continually lost.

Abbreviations: CRF, corticotropin-releasing factor; ACTH, adrenocorticotropin; aCSF, artificial cerebrospinal fluid; ICV, intracerebroventricular.

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The sheep were maintained in metabolism cages that allowed for the separate collection of urine and saliva. In addition, the cages were fitted with pedals that they were trained to press. One pedal delivered Na to a drinking cup (sheep without a parotid fistula: Na delivery = 25 ml of 0.5 M NaCl; sheep with a parotid fistula: Na delivery = 15 ml of 0.6 M $NaHCO_3$). A second pedal delivered 50 ml of water to a second drinking cup. Sheep without a parotid fistula had continuous access to water and Na (Na-repleted sheep). Sheep with a parotid fistula had continuous access to water but only 2-h daily (1200–1400 h) access to Na (Na-depleted sheep) after a tone sounded. All deliveries were consumed. The number of deliveries was counted and recorded continuously by computer.

The sheep were trained to consume their daily ration of food (oaten-lucerne chaff, Na content 90–100 mmol/kg, K content 200–300 mmol/kg) in a restricted daily time period. Na-repleted sheep ate in a 2-h period beginning at 1200 h and Na-depleted sheep in a 3-h period beginning at 1400 h. Ten grams of KH_2PO_4 was added daily to the chaff of Na-depleted sheep to replace phosphate lost in saliva.

In the experiments described below, each sheep had 1–9 infusions. Where described, body weight was measured before and at the end of the experimental period. Typically, 14–21 days elapsed between successive experiments.

Infusion and Sampling Procedures. Infusions started at 11:30 h on day 1, except in experiment 3 where they started at 17:00 h. Intakes of food, water, and Na were measured daily during a baseline period (3 days), an infusion period (4 days), and a postinfusion period (7 days).

All infusions were delivered via a polyethylene cannula connected to a 10-ml syringe held in an infusion pump (Perfusor, Braun, Melsungen, Germany) at a rate of 0.2 ml/h. In Na-repleted sheep, a blood sample was obtained (10 ml from a carotid artery) at 1000 h on day 1, before the start of infusion, and on the subsequent 4 days of infusion.

For ICV infusion, a probe (20-gauge needle + Luer-Lok cap) was inserted through the guide tube into a lateral brain ventricle and locked down. The infusates used were: rat urocortin [molecular weight (MW) = 4707.3, synthesized by J. Rivier, The Salk Institute]; human CRF (MW = 4758, Auspep, Melbourne, Australia); ACTH (1–24, MW = 2934, CIBA); and angiotensin II (MW = 1046, Auspep). They were dissolved in artificial cerebrospinal fluid (aCSF; [Na] = 151 mM, [Cl] = 157.5 mM, [K] = 2.8 mM, [Ca] = 1.1 mM, [Mg] = 0.9 mM, and [HPO_4] = 0.5 mM) and were infused at 5 μg/h. Using decrease in food intake of Na-repleted sheep as a marker of activity, the dose of 5 μg/h was chosen based on the results observed during preliminary experiments with ICV infusion of urocortin (doses ranging from 1 to 10 μg/h). Na- and water-repleted sheep were infused ICV with urocortin (n = 5), CRF (n = 5), ACTH (n = 5), or aCSF (n = 7). Na-depleted sheep (n = 7) were infused ICV with urocortin or aCSF; during the last 30 min of urocortin infusion, the sheep (n = 6) were infused concurrently with angiotensin II (3.8 μg/h, a potent dipsogenic stimulus; ref. 30). Intake of water during this period was compared with the water intake of Na-depleted sheep infused with angiotensin II under baseline conditions (n = 6). In addition, Na and water intakes were measured during a baseline period (3 days) and during a 4-day period where the animal's food intake was limited to the same amount of food as that eaten during the ICV infusion of urocortin (pair-feeding condition; n = five Na-repleted sheep, n = seven Na-depleted sheep).

For i.v. infusion, 1–2 days before the experiment, a polyethylene cannula was inserted into the jugular vein (10–12 cm heartward). The infusates of urocortin, CRF, and ACTH were dissolved in normal saline and infused at 5 μg/h. Na- and water-repleted sheep (n = 5) were infused i.v. with normal saline, urocortin, CRF, ACTH, or urocortin (5 μg/h) and ACTH (5 μg/h) combined.

Statistical Analysis. A two-way ANOVA, repeated measures on one variable (e.g., days), and either repeated (see Figs. 2 and 3) or independent (see Fig. 1) measures and subsequent least significant difference tests (Statistica, Statsoft, Tulsa, OK) were used to compare the baseline value to the value(s) obtained during or after each of the treatments or to compare various treatment values. The mean of the values obtained on the 3 days before each infusion for each animal was used in determining the baseline value (no change in food or water intake occurred during the first 90 min of infusion and these values have been included in the baseline value, i.e., third day of baseline period). Analysis of plasma glucose concentration (baseline, average preinfusion value for each animal; infusion, average 4-day infusion value for each animal) and cortisol concentration (see Fig. 4; baseline, average preinfusion value; infusion, average of values obtained on days 2 and 4 of infusion), and change in average daily intake [(100 $\times$ (mean intake during the infusion period $-$ mean intake during the baseline period)/(mean intake during the baseline period)] was done by a one-way ANOVA and subsequent least significant difference test. A t test was used to compare water intake during ICV infusion of urocortin plus angiotensin II with that during angiotensin II alone. Data are presented as means $\pm$ SEM.

Analytic Procedures. The glucose concentration of plasma and Na concentrations of urine and saliva were measured with a Beckman Synchron CX5 Clinical System. The cortisol concentration of plasma was analyzed by RIA (32).

Results

Effect of ICV Infusion of Urocortin, CRF, or ACTH on Ingestive Behavior of Na-Repleted Sheep. Under baseline conditions, the sheep consumed 25–1,500 (515 $\pm$ 74) ml of 0.5 M NaCl daily. Daily Na intake was decreased ($P < 0.05$ or less) during infusion of urocortin (second and fourth day of infusion), CRF (third day of infusion), or ACTH (first and third day of infusion). Compared with baseline, intake of Na was increased on the last 3 days of the postinfusion period after urocortin infusion. Na intake was not altered during ICV infusion of aCSF (Fig. 1 *Bottom*).

Under baseline conditions, the sheep consumed 850–4,000 (1,977 $\pm$ 101) ml of water daily. Daily water intake was decreased ($P < 0.001$) during the last 3 days of urocortin infusion and the first 3 days of the posturocortin infusion period. Relative to baseline, during urocortin infusion, average daily water intake was decreased by 62 $\pm$ 10%. Daily intake of water was decreased ($P < 0.001$) on the first day of CRF infusion only. Water intake during the first day of CRF infusion was less ($P < 0.01$) than that during the first day of urocortin infusion. Water intake during the last 3 days of CRF infusion was greater ($P < 0.01$) than that during the last 3 days of urocortin infusion. Intake of water was not altered during ICV infusion of either aCSF or ACTH (Fig. 1 *Middle*).

Under baseline conditions, the sheep consumed 500–1,400 (867 $\pm$ 46) g of food during the daily 2-h access period. The ICV infusion of either urocortin or CRF decreased ($P < 0.001$) food intake during the entire infusion period. Relative to baseline, during urocortin and CRF infusion, average daily food intake was decreased by 66 $\pm$ 13 and 54 $\pm$ 7%, respectively. On days 3 and 4 of infusion, food intake was less ($P < 0.01$ or less) during infusion of urocortin than that during infusion of CRF. When the infusions were stopped, food intake gradually returned to baseline values. Food intake was not significantly different from that during the baseline period by the third (CRF) or fourth (urocortin) postinfusion day. Intake of food was decreased ($P < 0.05$) for the first 2 days of infusion of ACTH and was not altered during ICV infusion of aCSF (Fig. 1 *Top*).

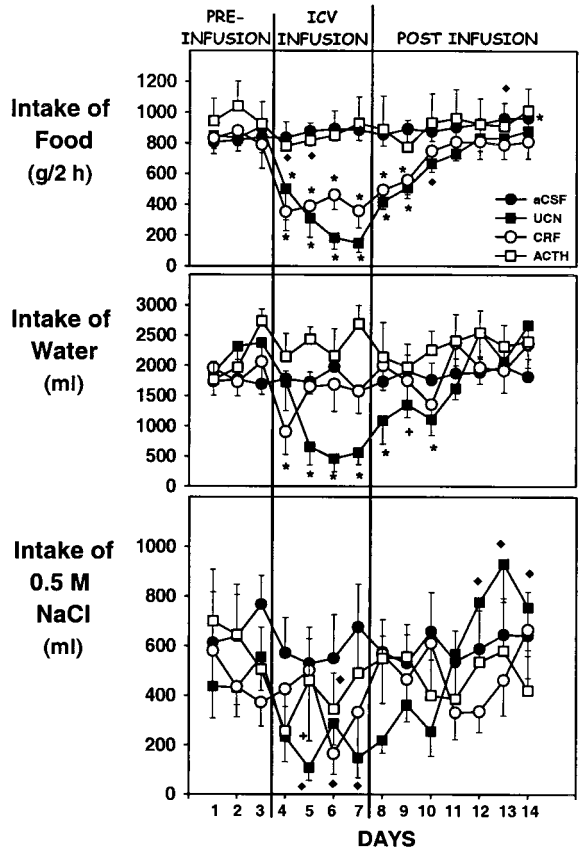

Fig. 1. Effect of 4-day ICV infusion of aCSF ($n = 7$), urocortin (UCN, 5 μg/h, $n = 5$), CRF (5 μg/h, $n = 5$), or ACTH (5 μg/h, $n = 5$) on intake of food, water, and 0.5 M NaCl of Na-repleted sheep. Statistical analysis was described in the text. *, $P < 0.001$; +, $P < 0.01$; ◆, $P < 0.05$ (vs. baseline).

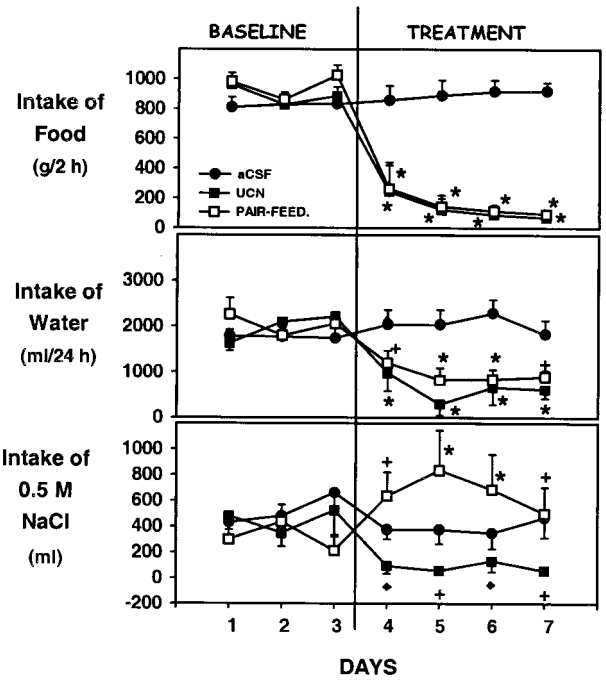

Fig. 2. Effect of 4-day ICV infusion of aCSF or urocortin (UCN, 5 μg/h) or pair-feeding (PAIR-FEED: food intake restricted to the amount of food consumed during ICV infusion of urocortin) on intake of food, water, and 0.5 M NaCl of Na-repleted sheep ($n = 5$). Statistical analysis was described in the text. *, $P < 0.001$; +, $P < 0.01$; ◆, $P < 0.05$ (vs. baseline).

Fig. 2 shows the effect of food restriction (*Top*) on water (*Middle*) and Na (*Bottom*) intakes of Na-repleted sheep (data from $n = 5$ sheep, three of which were previously included in Fig. 1). For comparison, intakes of these five animals during ICV infusion of aCSF and urocortin also are shown. In contrast to the decrease ($P < 0.05$ or less) in Na intake observed during ICV infusion of urocortin, Na intake was increased ($P < 0.01$ or less) during the period of pair-feeding. Intake of water was decreased ($P < 0.01$ or less) during pair-feeding and similar to that when the animals were infused with urocortin. Body weight was decreased ($P < 0.001$) during ICV infusion of urocortin (7.0 ± 0.8%) or pair-feeding (4.2 ± 0.3%). The decrease in body weight caused by urocortin was greater ($P < 0.01$) than that caused by pair-feeding. Body weight was not altered during ICV infusion of aCSF.

Effect of Intravenous Infusion of Urocortin, CRF, ACTH, or of Urocortin and ACTH Combined on Ingestive Behavior of Na-Repleted Sheep. Under baseline conditions, 25–1,650 (549 ± 81) ml of 0.5 M NaCl was consumed daily. Daily Na intake was increased ($P < 0.001$) during the last 2 days of infusion of ACTH. Na intake during infusion of ACTH was greater ($P < 0.01$ or less) than that during the infusion of aCSF during the last 3 days of the infusion period. Daily Na intake was decreased ($P < 0.05$) during infusion of urocortin (last 3 days of infusion), urocortin and ACTH combined (second day of infusion), or CRF (first day of infusion). Na intake recovered to baseline level by the last day of infusion of CRF or urocortin and ACTH combined. Na intake was not altered during i.v. infusion of normal saline (Fig. 3 *Bottom*).

Under baseline conditions, 1,025–3,075 (2,162 ± 115) ml of water was consumed daily. Daily water intake was decreased ($P < 0.05$ or less) for 3 (urocortin) or 4 (urocortin and ACTH) days of infusion and 1 or 2 days of the postinfusion period. Intake of water during the combined urocortin and ACTH infusion was similar to that observed during i.v. infusion of urocortin alone. Relative to baseline, during infusion of urocortin or urocortin and ACTH combined, average daily water intake was decreased by 40–50%. Intake of water was not altered during infusion of normal saline or CRF or ACTH (Fig. 3 *Middle*).

Under baseline conditions, 425–1,325 (960 ± 53) g of food were consumed during the 2-h access period. Relative to baseline, the i.v. infusion of urocortin or urocortin and ACTH combined decreased ($P < 0.01$ or less) food intake during the entire infusion period. Food intake gradually returned to baseline values during the postinfusion period. Intake of food during the combined urocortin and ACTH infusion was less ($P < 0.001$) than that during the urocortin alone on the first day of infusion. Average daily food intake during urocortin or urocortin and ACTH combined was decreased by 56 ± 13% and 77 ± 5%, respectively. Intake of food was not altered by infusion of normal saline, CRF, or ACTH (Fig. 3 *Top*).

Effect of ICV Infusion of Urocortin on Ingestive Behavior of Na-Depleted Sheep. Under baseline conditions, the sheep with a parotid fistula lost (losses in urine and saliva combined) 200–575 (386 ± 22) mmol of Na daily. The sheep consumed 450–1,025 ml of 0.6 M $NaHCO_3$ = 270–615 (403 ± 20) mmol of Na daily to correct this deficit. Daily Na intake was unaltered by either ICV infusion of aCSF, urocortin, or pair-feeding.

Under baseline conditions, 2,275–6,450 (4,275 ± 230) ml of water was consumed daily. Daily water intake was decreased ($P < 0.05$ or less) during the last 3 days of urocortin infusion and during 3 of the first 4 days (not shown) of the postinfusion period.

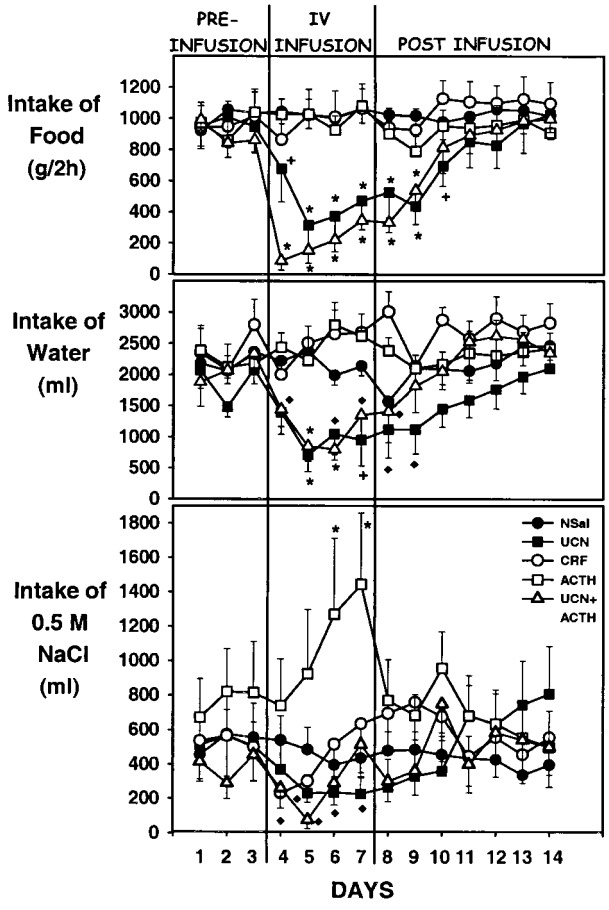

Fig. 3. Effect of 4-day i.v. infusion of normal saline (NSal), urocortin (UCN, 5 μg/h), CRF (5 μg/h), ACTH (5 μg/h), or urocortin (5 μg/h) and ACTH (5 μg/h) combined (UCN + ACTH) on intake of food, water, and 0.5 M NaCl of Na-repleted sheep ($n = 5$). Statistical analysis was described in the text. *, $P < 0.001$; +, $P < 0.01$; ◆, $P < 0.05$ (vs. baseline).

Intake of water was not altered during ICV infusion of aCSF. During the period of pair-feeding, intake of water was similar to that when the animals were infused with urocortin, i.e., decreased ($P < 0.01$ or less) on the last 3 days. The average daily water intake was decreased ($P < 0.001$) by 28 ± 8% during infusion of urocortin and by 34 ± 7% during the period of food restriction. Intake of water during the concurrent infusion of urocortin and angiotensin II was similar to that during infusion of angiotensin II alone (1,375 ± 249 vs. 1,250 ± 224 ml/30 min).

Under baseline conditions, 600–1,275 (871 ± 34) g of food was consumed during the 3-h access period. The ICV infusion of urocortin decreased ($P < 0.001$) intake of food during the last 3 days of the infusion period. When the infusion was stopped, food intake gradually returned to baseline values and was similar to baseline by the fifth postinfusion day (not shown). The average daily food intake was decreased ($P < 0.01$) by 38 ± 10% during infusion of urocortin. Intake of food was not altered during ICV infusion of aCSF.

Under baseline conditions, the sheep weighed 30–45 kg. Body weight was decreased ($P < 0.05$ or less) during ICV infusion of urocortin (6.6 ± 2.2%) or pair-feeding (4.1 ± 1.9%). Body weight was not altered during ICV infusion of aCSF.

Plasma Glucose and Cortisol Concentrations. Relative to baseline values (3.3 ± 0.1, $n = 8$), plasma glucose (mmol/liter) concentration was increased ($P < 0.05$ or less) by ICV infusion of urocortin (4.2 ± 0.2) or CRF (4.2 ± 0.2) and by i.v. infusion of urocortin (4.2 ± 0.2), ACTH (5.4 ± 0.3), or urocortin and ACTH (5.8 ± 0.5) combined. Relative to baseline values, plasma cortisol (nmol/liter) concentration was increased ($P < 0.01$) by ICV infusion of urocortin and by i.v. infusion of urocortin, CRF, or ACTH (Fig. 4).

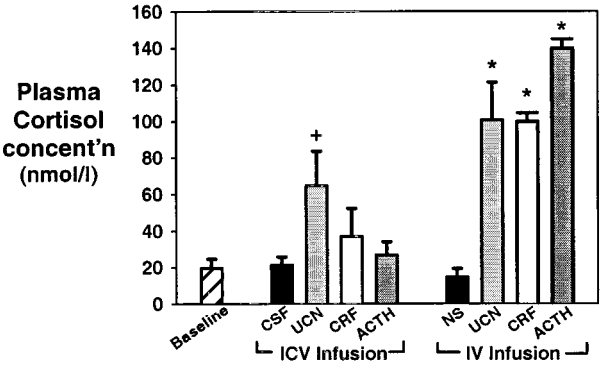

Fig. 4. Effect of the various infusions on plasma cortisol concentration of Na-repleted sheep ($n = 3$–10). Statistical analysis was described in the text. *, $P < 0.001$; +, $P < 0.01$ (vs. baseline). UCN, urocortin; NS, normal saline.

Discussion

This study reports evidence of an inhibitory influence of peptides associated with stress on need-free Na intake in Na-repleted sheep. This finding is intriguing given that stress (6–9) and systemic administration of ACTH (24) have been shown to cause clear-cut increases in Na intake. In contrast to these results in sheep, ICV, but not systemic, infusion of ovine CRF increased Na intake of rabbits (20) and mice (9). In rabbits (20) and rats (19, 33), ICV administration of CRF is associated with a sustained increase in ACTH, measured directly (19) or as indicated by increased plasma glucocorticoid hormone levels (20, 33). Although ICV administration of urocortin increased plasma cortisol levels in sheep, it failed to stimulate Na intake. Indeed, urocortin appears to have inhibited the expected steroid-induced Na appetite.

The inhibitory action of urocortin, administered ICV or i.v., was greater than that of CRF, at the same dose. During 4 days of ICV infusion, Na intake was decreased on 2–4 days (Figs. 1 and 2) with urocortin but only 1 day with CRF. The decrease in Na intake during ICV infusion of urocortin does not appear to be related to decreased food intake. Indeed, Na intake of Na-repleted sheep was increased by food restriction, as in food-deprived mice (34) and rats (unpublished observations). During i.v. infusion, both urocortin and CRF increased plasma cortisol concentration to levels approximating those observed during infusion of ACTH. Na intake was decreased on the first day of CRF infusion but returned to baseline level over the next 3 days, whereas it was decreased on the last 3 days of urocortin infusion, with no sign of recovery. Urocortin not only inhibited need-free Na intake but also the high Na intake induced by peripheral ACTH, further indicating a definite inhibitory action. Interestingly, Na intake during the last day of the combined urocortin and ACTH infusion was greater than that during the first day of infusion, suggesting that the inhibitory influence of urocortin was not insurmountable. This inhibition of Na intake cannot be attributed to the increase in blood pressure observed during i.v.-administered urocortin (35) because i.v.-administered ACTH causes a much greater increase in blood pressure (36) while still increasing Na intake. In contrast to its influence on the Na intake (need-free or steroid-induced) of Na-repleted sheep, ICV infusion of urocortin did not influence the Na intake of

Na-depleted sheep. It would appear that urocortin and CRF can influence some, but not all, of the mechanisms involved in Na appetite—possibly only those initiated by corticosteroids.

The mechanisms by which the CRF or urocortin inhibit Na intake are still to be determined. In rats, steroid-induced but not Na depletion-induced Na appetite is mediated in the medial amygdala (37) a brain area behind the blood-brain barrier. Although the brain nuclei that mediate Na appetite in sheep are not known, the observation that ICV infusion of urocortin interferes with need-free or steroid-induced Na intake but not that caused by Na depletion is consistent with an inhibitory action of the peptide in the amygdala. Regarding systemic infusion, it is unlikely that urocortin can cross the blood-brain barrier, thus brain areas lacking a blood-brain barrier or some peripheral action of urocortin has to be considered.

Urocortin or CRF infused into the CSF caused a marked reduction in food intake and body weight. Interestingly, intake of food did not recover during the 4-day ICV infusion of either urocortin or CRF. The decrease in food intake and body weight was similar in Na-repleted and Na-depleted sheep during chronic ICV infusion of urocortin. Body weight loss after ICV infusion of urocortin was greater (Na-repleted sheep) or tended to be greater (Na-depleted sheep) than that after food restriction (pair-feeding). These data suggest that although food intake appears to be the major explanation it may not be the only factor contributing to the decreased body weight. In contrast to urocortin and CRF, food intake and body weight were altered minimally by ICV infusion of ACTH, clearly showing that the influence of CRF and urocortin on food intake and body weight is independent of their ability to stimulate a brain adrenocorticotropinergic pathway (38, 39).

Work in rats has suggested that the decrease in food intake caused by ICV administration of CRF (11, 18, 19, 33) or urocortin (18, 40) appears to be mediated by CRF-R2 receptors (18). The greater decrease in food intake caused by urocortin relative to that caused by CRF is presumably because CRF-R2 receptors have a 6–40 times greater affinity for urocortin than for CRF (14). CRF-R2 receptors (22, 41, 42) are found in hypothalamic nuclei (e.g., ventromedial nucleus, paraventricular nucleus) important for the control of food intake and body weight (40, 43–46). Actions of CRF (and presumably urocortin) via neural pathways from the paraventricular nucleus to brain nuclei controlling the sympathetic nervous system (e.g., locus coeruleus; refs. 1, 23, and 47) also may be involved in decreasing food intake and body weight (44, 45, 48). CRF has been shown to influence thermogenesis (48) and gastrointestinal functioning, e.g., gastric emptying and acid secretion (49, 50). In the present study, increased activation of the sympathetic nervous system during ICV administration of CRF and urocortin is suggested by the increase in plasma glucose concentration (13). Decreased activity of the parasympathetic nervous system, via decreased insulin secretion, also may contribute (48). Similar to rats (51), a comparison of body weight loss in sheep during ICV infusion of urocortin and pair-feeding, suggests an influence of urocortin on metabolic processes other than food intake, such as thermogenesis (48). Together, these results are consistent with an inverse relationship between activity of the sympathetic nervous system and food intake and/or body weight in sheep.

Unlike ICV infusion, i.v. infusion of CRF did not alter food intake or body weight, evidence that the influence of CRF on body energy homeostasis is centrally mediated. These observations are consistent with those previously made in sheep (52, 53), even with systemic doses of CRF 100 times greater than that given ICV (53). The decrease in food intake caused by the peripheral administration of urocortin could result from a decrease in rumen emptying, analogous to the decrease in gastric emptying reported in rats (49, 50). This action may be mediated by an action of urocortin on CRF-R2 receptors located in the gastrointestinal tract. In rats, the CRF-R2 receptor exists in two forms that show different distribution in the body with the α form being confined to the brain and the β form being found in both brain and peripheral tissue (42). Further investigation will be required to determine the role of these different forms of the receptor in mediating the actions of centrally and peripherally administered urocortin in sheep.

The failure of systemic administration of ACTH to alter food intake or body weight shows that the influence of urocortin on food intake is independent of its ability to cause the secretion of ACTH and the subsequent secretion of the adrenocorticoid hormones. Because systemic administration of ACTH does not increase activity of the sympathetic nervous system (54), the increase in plasma glucose caused by the systemic administration of ACTH, urocortin, or urocortin and ACTH combined most likely is related to the increase in glucocorticoid hormone secretion (36).

Water intake, in the main, followed food intake. Decreased food intake caused by ICV or i.v. infusion of urocortin or food restriction was associated with decreased water intake. Unchanged or minimally decreased food intake like that during control or ACTH infusions was associated with unaltered water intake. Interestingly, ICV infusion of both urocortin and CRF decreased food intake, but a sustained decrease in water intake was observed only during the infusion of urocortin. Although it has been shown that urocortin can inhibit water intake in rats (40), the results of the present study suggest that the effect of urocortin on water intake in sheep is secondary to its effect on food intake. With identical food consumption, sheep demonstrated an equal reduction in water intake whether or not infused ICV with urocortin. Furthermore, urocortin did not interfere with water intake caused by ICV infusion of angiotensin II. ICV infusion of CRF resulted in a sustained decrease in food intake, but in contrast to urocortin, only a transient decrease in water intake. Given the direct relationship between food and water intake, CRF may have a stimulatory action on water intake when administered centrally but not systemically. The mechanism by which CRF may stimulate water intake is unknown at present but it is noteworthy that increased water intake has been observed in sheep during stress (55).

The experiments were designed to delineate a probable locus of action of the peptides (brain or peripheral). The fact that ICV, but not i.v., administration of CRF decreased food intake, shows that the action of CRF on food intake is central. Given that the actions of urocortin are mediated by CRF receptors (14) and that not only ICV but also i.v. administration of urocortin decreased food intake, urocortin appears to have both central and peripheral actions. A peripheral action is supported by the striking cardiovascular effects of systemic infusion of urocortin (35). On the other hand, it would appear that in regard to Na intake, both CRF and urocortin have both central and peripheral actions. The physiological implication of effect by both ICV and systemic administration of these peptides is presently unknown and a matter for further investigation.

In conclusion, the decrease in food intake caused by stress (10, 11) is mimicked by ICV administration of CRF or urocortin (present study; refs. 11, 18, and 19). A physiological role of CRF has been shown in experiments where a CRF antagonist prevents the reduction in food intake caused by stress (11) and also by leptin (56), a peptide involved in the normal control of fat stores (46). In direct contrast, the present experiments have demonstrated that whereas systemic infusion of ACTH mimics the increase in Na intake observed in several different stressful situations (6–9), CRF or urocortin administered centrally actually inhibit Na intake, indicating a direct action overriding any

effect of these peptides on ACTH release. The inhibitory actions of the peptides cannot be attributed to a general malaise or to a nonspecific inhibition of ingestive behavior. For example, ICV administration of urocortin did not interfere with Na appetite caused by Na depletion or water intake caused by ICV administration of angiotensin II. Indeed, no inhibitory influence of the peptides on water intake was observed, and if anything, CRF appeared to have a stimulatory effect. Thus hormones associated with stress have both excitatory and inhibitory influences on Na intake. Presumably, other physiological processes entrained by stress will be important in determining the outcome on Na appetite.

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