FULL PAPER *Ethology*

Effects of AVP V1a and CRH Receptor Antagonist on Psychological Stress Responses to Frustrating Condition in Sheep

Ken-ichi YAYOU[1)]*, Masato NAKAMURA[2)] and Shuichi ITO[3)]

[1)]Laboratory of Neurobiology, National Institute of Agrobiological Sciences, 2, Ikenodai, Tsukuba, Ibaraki, 305–8602, [2)]Research Team for Dairy Production Using Regional Feed Resources, National Agricultural Research Center for Hokkaido Region, 1, Hitsujigaoka, Toyohira-ku, Sapporo, Hokkaido, 062–8555 and [3)]Department of Animal Science, Tokai University, Kawayu, Minamiaso, Aso-gun, Kumamoto, 869–1404, Japan

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ABSTRACT. Arginine vasopressin (AVP) and corticotropin-releasing hormone (CRH) are released in the brain to regulate behavioral and physiological stress responses. To elucidate the respective roles of these peptides under certain stressors, we examined the effects of intracerebroventricular infusions of either AVP V1a receptor antagonist, [Pmp[1], Tyr (Me)[2]]- Arg[8]-Vasopressin (Pmp, Tyr–AVP) or CRH receptor antagonist, alpha-helical CRF 9–41 (αhCRF) on stress responses induced by frustrating condition in sheep. Four ovariectomized Corriedale ewes were assigned to the experiment. In a "frustrating" condition (FC), food was withheld for 60 minutes from only the experimental ewe while this ewe was in the presence of the other ewes that were given food. As "non-frustrating" control condition (C), food was withheld for 60 minutes from all ewes, thereby controlling for the nonspecific effects of lack of food. FC induced a significant rise in the plasma cortisol concentration ($p < 0.05$) and increased the pawing number and rectal temperature compared with that in C ($p < 0.1$). The effects of either Pmp, Tyr–AVP or αhCRF on these stress responses were analyzed. The rise in cortisol restored nearly to the control level by infusion of Pmp, Tyr–AVP or αhCRF. The pawing number restored nearly to the control level by αhCRF. The hyperthermia restored nearly to the control level by Pmp, Tyr–AVP. These data suggest that both endogenous CRH and AVP might be concerned with inducing physiological and behavioral stress responses to frustrating condition in sheep.

KEY WORDS: arginine vasopressin V1a receptor antagonist, frustration, hyperthermia, hypothalamo-pituitary-adrenal axis, ovine corticotropin-releasing hormone antagonist.

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Neuropeptides arginine vasopressin (AVP) and corticotropin-releasing hormone (CRH) are released in the hypothalamus, amygdala, and lateral septum during stress to regulate behavioral and physiological stress responses in rats and mice [9, 19]. We previously reported the central effects of intracerebroventricular infusions of AVP and CRH in sheep [38, 40]. Centrally infused CRH had equal potential to release cortisol into the systemic circulation as AVP [38, 40], although many studies confirm that adrenocorticotropic hormone (ACTH) release is under the predominant control of AVP in sheep [10, 11, 31]. Centrally infused AVP also induced several types of stress-related behaviors, such as oral stereotyped behaviors [40], whereas CRH induced only stereotypical bleating [38]. Neither CRH nor AVP had an effect on autonomic nervous function, such as heart rate (HR) and rectal temperature (RT) [38, 40]. Although we had showed that intracerebroventricular infusions of CRH and AVP could partly mimic the adrenocortical and behavioral stress responses, there was no context or environmental challenge such as anxiety-eliciting or stress-related stimuli. To confirm the roles of CRH and AVP in regulating endocrine, behavioral, and autonomic stress responses in sheep, the inhibiting effects of intracerebroventricular infusion of antagonists for these peptides on stress responses should be examined.

Many studies in rodents have used several types of specific receptor antagonists for AVP or CRH and have confirmed the role of these peptides in regulating stress responses under stress-related contexts [13, 15, 17, 18, 20, 22, 23, 32, 33]. In the present study, we designed a psychological stress context using delayed feeding paradigm: food was withheld from only the experimental animal while this animal was in the presence of its peers that were given food, thereby "frustrating" the hungry experimental animal especially in social feeders such as sheep. This context induced physiological and behavioral stress responses and we examined the effects of centrally infused antagonists for AVP and CRH on these stress responses in sheep.

MATERIALS AND METHODS

Experimental procedures and care of the animals were carried out according to the guide for the care and use of agricultural animals in agricultural research and teaching [6].

Animals and surgery: Four mature Corriedale ewes (56 ± 0.8 kg) were used in the experiment. They were ovariectomized at least two months before the experiment. After ovariectomy, the animals were individually reared in a stanchion cage in an experimental room (20 ± 2 °C, lights on for 24 hrs). The animals were trained to be fed alfalfa hay cubes from 0900 to 1100hr daily at a maximum amount of

* CORRESPONDENCE TO: YAYOU, K., Laboratory of Neurobiology, National Institute of Agrobiological Sciences, 2, Ikenodai, Tsukuba, Ibaraki, 305–8602, Japan.
e-mail: ken318@affrc.go.jp

1.5 kg. Water was available *ad libitum*. The animals were given access to mineral blocks, except during the feeding period.

At least twenty days before the experiment, a guide-cannula consisting of an 18-gauge stainless steel outer tube fitted with an inner 22-gauge stainless steel tube was stereotaxically implanted into the third cerebral ventricle of each ewe under isoflurane anesthesia according to the procedure described in our previous report [38].

During the pre-experimental period, the ewes were continually tamed and allowed to adapt to their environment to minimize the impact of the stressor of handling associated with intracerebroventricular infusions and blood sampling. On the day before the experiment, each animal was fitted with an indwelling jugular catheter (Terufusion IVH catheter kit, Terumo, Tokyo, Japan) to collect blood samples.

Treatment solutions: The infusion vehicle was artificial cerebrospinal fluid (aCSF: NaCl, 125 mM; KCl, 2.5 mM; NaH_2PO_4, 0.5 mM; Na_2HPO_4, 1.2 mM; $CaCl_2$, 1.2 mM; $MgCl_2$, 1.0 mM; $NaHCO_3$, 27 mM) [14]. The pH of the medium was adjusted to 7.4 with 4–6 m*l* of 0.5 N HCl for 1000 m*l* aCSF. Synthetic arginine vasopressin V1a receptor antagonist, [Pmp^1, Tyr $(Me)^2$]-Arg^8-Vasopressin (Pmp, Tyr-AVP; Peptide Institute, Inc., Osaka, Japan), was dissolved in the aCSF solution at a concentration of 20 nmol/m*l*, and synthetic nonspecific CRH receptor antagonist, alpha-helical CRF 9–41 (αhCRF; Sigma Chemical Co., St Louis, MO), was dissolved in the aCSF solution at a concentration of 40 nmol/m*l*. The solutions were divided into 1-m*l* aliquots, which were stored at –20 °C before use. We determined previously that a 30-minutes pre-infusion of Pmp, Tyr-AVP 45 minutes before the AVP infusion (10 nmol/0.5 m*l* aCSF for 30 minutes) significantly inhibited the plasma cortisol and behavioral responses to the AVP infusion and that a 30-minutes pre-infusion of αhCRF 45 minutes before the CRH infusion (2 nmol/0.5 m*l* aCSF for 30 minutes) significantly inhibited the plasma cortisol and behavioral responses to CRH infusion. Infusion of neither Pmp, Tyr-AVP alone nor αhCRF alone changed plasma cortisol concentration, HR, RT, and behavior of sheep. The effective dosages of AVP and CRH for plasma cortisol concentration and behavioral responses used in the preliminary tests were determined according to our former reports [38, 40].

Experimental procedure: Experiments were performed between 0830h and 1100h (Fig. 1). At 0630h, telemetry devices for HR and RT recording (Polygraph 360 system, NEC Sanei, Tokyo, Japan, or Multi telemeter system WEB-5000, Nihon Kohden, Tokyo, Japan) were attached to the experimental ewe. At 0730h, the indwelling 22-gauge stylette was removed from the outer tube and a 22-gauge inner tube flushed with aCSF was lowered to the third ventricle. After confirming the efflux of cerebrospinal fluid, a polyethylene tube 1.3 m in length and 4/3 mm in diameter was connected to the inner tube. The tube was filled with treatment solution at 0800h. Experiments started at 0830h, and twenty five minutes after the start of the experiment (0855h), either AVP antagonist (FC_Pmp, Tyr-AVP), CRH antagonist (FC_αhCRF), or aCSF (FC) was infused into the third ventricle over 35 minutes at a speed of 0.5 m*l*/30 minutes. Considering the dead volume of the inner tube to be 80 μl, the infusion vehicle reached the third ventricle in 5 minutes, and the total amount of AVP antagonist infused was 10 nmol for a 20-nmol/m*l* infusion. The total amount of CRH antagonist infused was 20 nmol for a 40-nmol/m*l* infusion. The 0 min point of the experiment was set 30 minutes after the start of the experiment (0900h) when the infusion fluid reached the third ventricle.

Three of four ewes were fed normal feed as usual at 0 min. Only the experimental animal was not fed until 60 min, and then all the ewes were fed until 180 min. As a control treatment, the experimental animal was infused with aCSF from -5 min over 35 minutes as described above, none of the sheep including experimental animal was fed until 60 min, and then all ewe were fed for 2 hrs (C). Serial blood

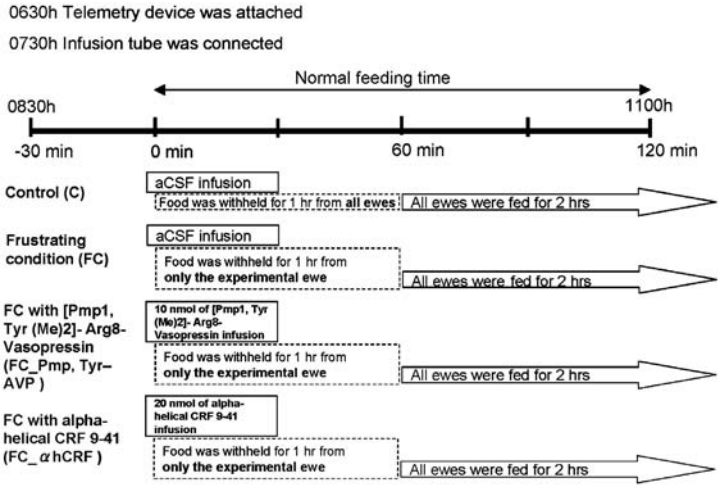

Fig. 1. Timing of experimental procedure. Four ewes were randomly assigned to each of four treatments.

Table 1. List of behaviors analyzed

Measure	Definition
Pawing (No.)	Scooping leg movemen on the cage floor. One sequence of several pawing was counted as one.
Sham Chewing (No.)	Chewing actions performed without the presence of food in the oral cavity. One sequence of several chewing was counted as one.
Teeth Grinding (No.)	Grinding upper and lower molar teeth without the presence of food in the oral cavity. One sequence of several grinding was counted as one.
Stamping Foot (No.)	Striking of the sole of a forefoot on the ground. One sequence of several striking was counted as one.
Abnormal Licking (No.)	Frequency of licking or gnawing objects in the surroundings. One sequence of several licking or gnawing was counted as one.
Bending Front Leg (No.)	Lowering of the body by bending front legs.
Rubbing (No.)	Any repeated or rhythmical rubbing action against objects in the surroundings. One sequence of several rubbing was counted as one.
Head Shaking (No.)	Rapid rotatory oscillation of the head. One sequence of several oscillation was counted as one.
Bleating (No.)	All types of vocalization.
Self-grooming (No.)	Licking body or scratching head with a foot. One sequence of several licking or scratching was counted as one.
Defecation (No.)	Elimination of feces from the body.
Urination (No.)	Discharge of urine from the body.
Water Access (No.)	Consumption of water.
Mineral Block Licking (No.)	Frequency of licking or gnawing mineral block. One sequence of several licking or gnawing was counted as one.

samples for cortisol measurement were collected via indwelling jugular catheters at –30, 0, 30, 60, and 120 min. HR and RT were continuously recorded via a telemetry system in an ECG processor (SBP-8, Softron, Tokyo, Japan). A time-lapse video recorder was used to record the behavior of the animal continuously. To minimize the carryover effect by each treatment, each ewe was randomly assigned to each of the four treatments, and the intervals between successive treatments were at least two days, after considering the clearance rate of the administered antagonists from the cerebrospinal fluid and the recovery of the animals.

Data analysis: Blood samples were collected into pre-chilled tubes containing heparin sodium and were stored on ice until the end of the experiment. After the experiment, the tubes were centrifuged, and plasma samples were stored at –20 °C until assayed. Plasma cortisol concentrations were obtained from an automatic magnetic particle enzyme immunoassay (Kainos Lab. Inc., Tokyo, Japan). The sensitivity of the assay was 1 *n*g/m*l*, and the intra-assay coefficient of variation (CV) was 3.3 % at 10 *n*g/m*l*. The inter-assay CV was 4.8 % at 10 *n*g/m*l*.

Mean HR was obtained at –30, 0, 10, 20, 30, 40, 50, 60, 90, and 120 min, and mean RT was obtained at –30, 0, 30, 60, and 120 min using the ECG processor. Raw data for HR and RT were converted to percentage changes from the pre-infusion value (mean of –30 and 0 min).

Continuous behavior sampling was performed from 0 to 60 min using a videotaped recording. Total number of pawing, sham-chewing, teeth-grinding, foot stamping, abnormal licking, bending of front legs, rubbing, head shaking, bleating, self-grooming, defecation, urination, water access, and licking of a mineral block were analyzed (Table 1). Pawing was defined as a scooping leg movement generally on the ground that is displayed in play situations, as a warning signal, when searching for food in snow-covered terrain, when in pain, or when frustrated [16].

Statistical analysis: The effects of time and treatment on plasma cortisol concentrations and the percentage changes in HR and RT were analyzed using repeated statements of the SAS GLM procedure with animals and treatment as the sole source of variation in the whole plot and time as the source of variation in the subplot. Differences in variation with time depend on whether time-treatments interactions are significant. Moreover, after transposing the data set with respect to each animal, the variations with time within each treatment were analyzed as a randomized blocks design using the SAS GLM procedure with animals and time as the main effects in the model. If there was a significant main effect of time, the statistical differences from the pre-infusion value (–30 and 0 min) within each treatment were analyzed by using the contrast statement of the SAS GLM procedure followed by calculations of Scheffe F-values. In addition, the areas under the cortisol curves (AUC) were calculated from 0 to 60 min during each treatment. The AUCs were analyzed as a randomized blocks design using the SAS GLM procedure with animals as the block and treatments as the main effect in the model. If there was a significant main effect of treatment, the statistical differences among the treatments were analyzed using the contrast statement of the SAS GLM procedure followed by calculations of Scheffe F-values. For HR and RT, the maximum rate of change from the pre-infusion value (mean of –30 and 0 min) during frustrating condition (0-60 min) were statistically assessed using Friedman's test followed by Nemenyi multiple comparison if there was a significant effect of treatments.

Behavioral data were statistically assessed using Friedman's test followed by Nemenyi multiple comparison if there was a significant effect of treatments.

RESULTS

There were no significant time-by-treatment interactions for cortisol. Significant main effects of time were seen with

the FC ($p < 0.05$) and the FC_αhCRF ($p < 0.01$) treatments but not with the FC_Pmp, Tyr-AVP treatment. Compared with the concentrations in the pre-infusion period (–30, 0 min), cortisol concentrations rose significantly at 60 min with the FC ($p < 0.01$) and FC_αhCRF ($p < 0.01$) treatments (Fig. 2A). There was a significant main effect of treatment ($p < 0.01$) on the AUC during the FC period (from 0 to 60 min). The AUC response to FC was significantly higher than the response to C ($p < 0.05$), and the AUC responses to FC_αhCRF and FC_Pmp, Tyr-AVP tended to be lower than that in response to FC ($p < 0.1$) (Fig. 2B).

There was no significant time-by-treatment interaction for percentage change in HR. Significant main effects of time were seen with the C ($p < 0.01$), FC ($p < 0.01$), FC_Pmp, Tyr-AVP ($p < 0.01$), and FC_αhCRF ($p < 0.01$) treatments. Compared with values in the pre-infusion period (–30, 0 min), the percentage change in HR was significantly higher in feeding period: at 90 ($p < 0.01$) and 120 min ($p < 0.05$) with the C treatment, at 120 min ($p < 0.05$) with the FC treatment, at 90 ($p < 0.01$) and 120 min ($p < 0.01$) with the FC_Pmp, Tyr-AVP treatment, and at 90 ($p < 0.01$) and 120 min ($p < 0.01$) with the FC_ αhCRF treatment (Fig. 3A). There was not a significant difference among the four treatments in the maximum rate of change in HR from the pre-infusion value (mean of –30 and 0 min) during frustrating condition (0–60 min) (Friedman's test, $\chi^2 = 0.90$, $p > 0.1$) (Fig. 3B).

There was no significant time-by-treatment interaction for percentage change in RT. Significant main effects of time were seen with the FC ($p < 0.01$), FC_Pmp, Tyr-AVP ($p < 0.01$), and FC_αhCRF ($p < 0.01$) treatments. Compared with values in the pre-infusion period (–30, 0 min), the percentage change in RT was significantly higher at 60 ($p < 0.01$), 90 ($p < 0.01$), and 120 min ($p < 0.01$) with the FC treatment; at 90 ($p < 0.01$) and 120 min ($p < 0.01$) with the FC_Pmp, Tyr-AVP treatment; and at 60 ($p < 0.05$), 90 ($p < 0.01$) and 120 min ($p < 0.01$) with the FC_ αhCRF treatment (Fig. 4A). There was a significant difference among the four treatments in the maximum rate of change in RT from the pre-infusion value during frustrating condition (Friedman's test, $\chi^2 = 8.10$, $p < 0.05$). The maximum rate of change in RT from the pre-infusion value during frustrating condition tended to be higher in FC and FC_αhCRF treatment than in C treatment (Nemenyi multiple comparisons: $p < 0.1$) (Fig. 4B).

The behaviors exhibited during the 60-min FC period are summarized in Table 2. There was a significant difference among the four treatments in the total number of pawing (Friedman's test, $\chi^2 = 10.85$, $p < 0.01$) and a tendency for a difference among the four treatments in the total number of sham chewings (Friedman's test, $\chi^2 = 6.69$, $p<0.1$) and in the total number of water accesses (Friedman's test, $\chi^2 = 6.41$, $p<0.1$). FC and FC_Pmp, Tyr-AVP treatment induced pawing more than did the C treatment (Nemenyi multiple comparisons: $p<0.1$ and $p<0.05$, respectively).

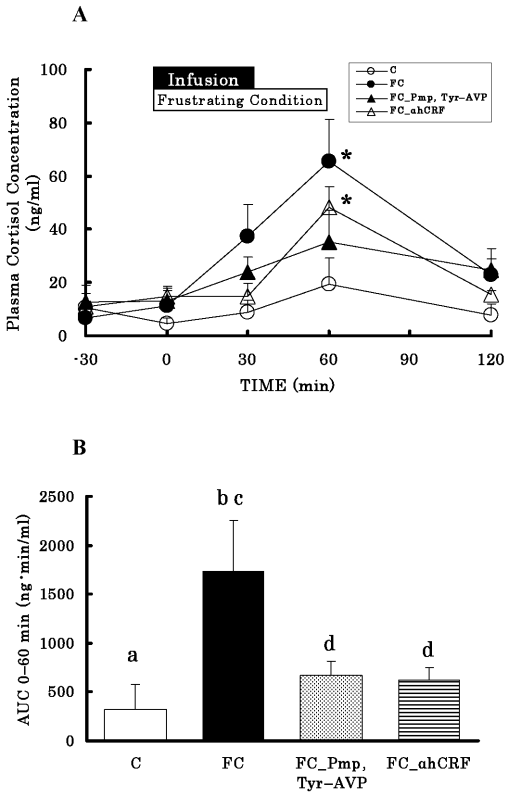

Fig. 2 A, Plasma profiles of cortisol (mean + SEM, n = 4) during the control (C), frustrating condition (FC), FC with arginine vasopressin V1a receptor antagonist, [Pmp[1], Tyr (Me)[2]]- Arg[8]-Vasopressin (FC_Pmp, Tyr-AVP), and FC with nonspecific CRH receptor antagonist, alpha-helical CRF 9–41 (FC_αhCRF). Asterisks indicate a significant difference from pre-treatment values (–30 and 0 min) ($p < 0.05$). The white bar indicates the period of FC, and the black bar indicates the period of infusion. The ewes started feeding at 60 min. B, Plasma profiles of cortisol analyzed as the mean area under the curve (AUC) of the plasma cortisol concentration from 0 to 60 min after the onset of treatment (mean + SD, n = 4) in C, FC, FC_Pmp, Tyr-AVP, and FC_αhCRF. Different superscript letters indicate statistical differences ($p < 0.05$: between a and b, $p < 0.1$: between c and d).

DISCUSSION

In the present study, frustrating condition (FC) in which food was withheld for 60 minutes from only the experimental animal in the presence of other ewes that were given food induced a significant rise in cortisol and hyperthermia at the end of the FC period (60 min), and increased the frequency of pawing during the FC period compared with the C treatment in which food was withheld from all the ewes controlling for the nonspecific effects of lack of food in the absence of seeing peers eating. The increase in cortisol which appears to be specific to FC and resolved after eating tended to be inhibited by infusion of Pmp, Tyr-AVP or αhCRF.

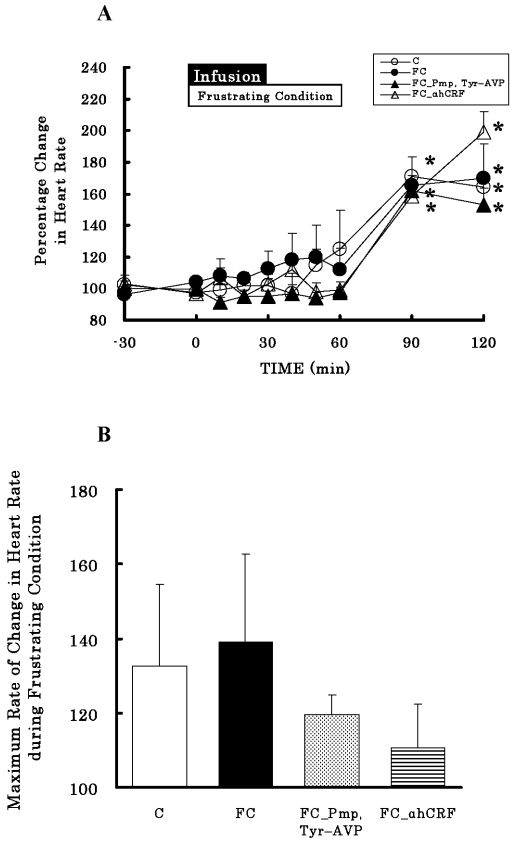

Fig. 3. A, Profiles of percentage change in heart rate from pre-treatment value (means of –30 and 0 min) (mean + SEM, n = 4) during the control (C), frustrating condition (FC), FC with arginine vasopressin V1a receptor antagonist, [Pmp[1], Tyr (Me)[2]]- Arg[8]-Vasopressin (FC_Pmp, Tyr-AVP), and FC with nonspecific CRH receptor antagonist, alpha-helical CRF 9–41 (FC_αhCRF). Asterisks indicate a significant difference from pre-treatment values (–30 and 0 min) ($p < 0.05$). The white bar indicates the period of FC, and the black bar indicates the period of infusion. The ewes started feeding at 60 min. B, The maximum rate of change in heart rate from the pre-infusion value (mean of –30 and 0 min) during frustrating condition (0–60 min) (mean + SEM, n=4) in C, FC, FC_Pmp, Tyr-AVP, and FC_αhCRF. There was not a significant difference among the four treatments (Friedman's test, $\chi^2 = 0.90$, $p > 0.1$).

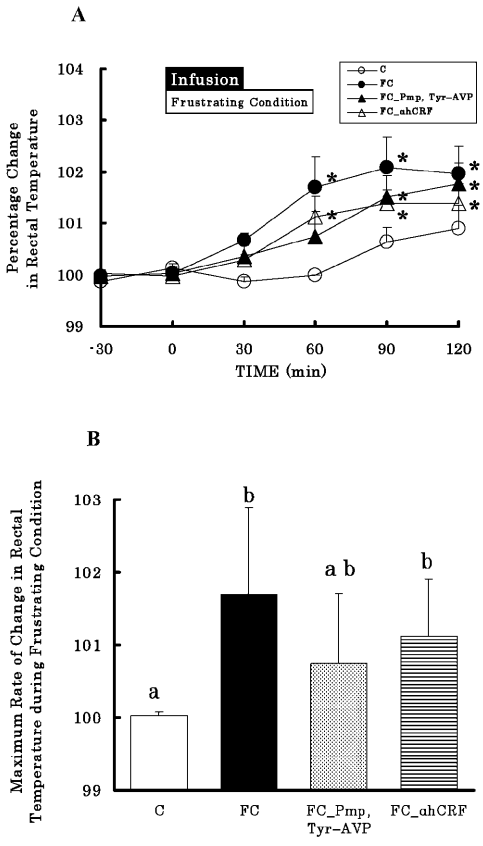

Fig. 4. A, Profiles of percentage change in rectal temperature from pre-treatment value (means of –30 and 0 min) (mean + SEM, n = 4) during the control (C), frustrating condition (FC), FC with arginine vasopressin V1a receptor antagonist, [Pmp[1], Tyr (Me)[2]]- Arg[8]-Vasopressin (FC_Pmp, Tyr-AVP), and FC with nonspecific CRH receptor antagonist, alpha-helical CRF 9–41 (FC_αhCRF). Asterisks indicate a significant difference from pre-treatment values (–30 and 0 min) ($p < 0.05$). The white bar indicates the period of FC, and the black bar indicates the period of infusion. The ewes started feeding at 60 min. B, The maximum rate of change in rectal temperature from the pre-infusion value (mean of –30 and 0 min) during frustrating condition (0–60 min) (mean + SEM, n=4) in C, FC, FC_Pmp, Tyr-AVP, and FC_αhCRF. Different superscript letters indicate statistical differences (Nemenyi multiple comparisons, $p < 0.1$: between a and b).

The hyperthermia which also appears to be specific to FC but did not resolved after eating restored nearly to the control level by Pmp, Tyr-AVP infusion. The conflict behavior, pawing, also restored nearly to the control level by αhCRF infusion. These data suggest that both endogenous CRH and AVP in the central nervous system are concerned with activating hypothalamo-pituitary-adrenal axis, that endogenous AVP might have certain role to induce hyperthermia, and that endogenous CRH might be concerned with inducing conflict behavior, under psychological stress, "frustration", in sheep.

The rise in cortisol, hyperthermia, and the increase in pawing with FC treatment compared with C treatment indicate that FC is a stressor for sheep. These responses are in part similar to those reported for food thwarted goats [5] and pigs required to wait for operant food reward [8, 24]. Food thwarted goats responded with an overall increase in activity such as higher incidences of pawing, head movements, and mouthing behaviors; chewing and biting. Plasma norepinephrine but not cortisol concentration increased in food

Table 2. Occurrence of behaviors during 60-minutes frustrating condition period

		Control	Frustrating Condition (FC)	FC_ Pmp, Tyr-AVP	FC_ αhCRF	χ^2	p
Pawing (No.)	Mean	6.5	42.3†	56.8*	12.8	10.85	6.580E-05
	SD	10.4	38.8	43.2	11.2		
	Number of animals exhibiting the behavior	(3/4)	(4/4)	(4/4)	(3/4)		
Sham Chewing (No.)	Mean	3.3	19.0	41.5	4.5	6.69	0.053
	SD	2.5	12.8	35.4	4.7		
	Number of animals exhibiting the behavior	(3/4)	(3/4)	(4/4)	(3/4)		
Teeth Grinding (No.)	Mean	5.3	11.8	3.8	15.0	1.88	0.657
	SD	5.6	22.8	4.1	26.2		
	Number of animals exhibiting the behavior	(3/4)	(2/4)	(3/4)	(2/4)		
Stamping Foot (No.)	Mean	58.0	100.3	78.8	134.8	3.00	0.432
	SD	47.0	83.9	55.6	129.9		
	Number of animals exhibiting the behavior	(3/4)	(4/4)	(4/4)	(4/4)		
Abnormal Licking (No.)	Mean	3.8	2.3	5.0	3.3	3.32	0.381
	SD	3.9	1.5	2.8	3.3		
	Number of animals exhibiting the behavior	(3/4)	(4/4)	(4/4)	(3/4)		
Bending Front Leg (No.)	Mean	3.3	3.0	4.3	3.0	4.80	0.185
	SD	3.3	2.9	3.0	3.2		
	Number of animals exhibiting the behavior	(3/4)	(3/4)	(4/4)	(3/4)		
Rubbing (No.)	Mean	3.5	1.8	3.5	1.5	1.76	0.681
	SD	4.4	1.0	3.7	1.7		
	Number of animals exhibiting the behavior	(3/4)	(4/4)	(3/4)	(3/4)		
Head Shaking (No.)	Mean	1.3	1.0	0.8	1.0	0.93	0.858
	SD	0.5	0.8	1.0	0.8		
	Number of animals e xhibiting the behavior	(4/4)	(3/4)	(2/4)	(3/4)		
Bleating (No.)	Mean	4.3	14.5	8.5	8.5	2.68	0.494
	SD	6.7	25.1	5.2	6.0		
	Number of animals exhibiting the behavior	(2/4)	(3/4)	(4/4)	(4/4)		
Self-grooming (No.)	Mean	0.8	0.0	0.5	1.3	3.63	0.333
	SD	1.0	0.0	1.0	1.9		
	Number of animals exhibiting the behavior	(2/4)	(0/4)	(1/4)	(2/4)		
Defecation (No.)	Mean	2.0	2.3	2.0	1.5	1.13	0.818
	SD	0.8	1.5	1.2	0.6		
	Number of animals exhibiting the behavior	(4/4)	(4/4)	(4/4)	(4/4)		
Urination (No.)	Mean	1.8	1.3	1.5	1.3	1.13	0.818
	SD	1.0	1.3	1.0	1.0		
	Number of animals exhibiting the behavior	(4/4)	(3/4)	(3/4)	(4/4)		
Water Access (No.)	Mean	4.5	6.8	8.3†	7.3	6.41	0.066
	SD	3.5	2.6	2.5	3.6		
	Number of animals exhibiting the behavior	(4/4)	(4/4)	(4/4)	(4/4)		
Mineral Block Licking (No.)	Mean	1.5	5.8	5.8	3.3	3.81	0.306
	SD	1.9	5.6	4.1	3.2		
	Number of animals exhibiting the behavior	(3/4)	(3/4)	(4/4)	(4/4)		

p: P-values obtained by Friedman's test.
*: Significantly different from Control (Nemenyi multiple comparison: $p < 0.05$)
†: Tended to differ from Control (Nemenyi multiple comparison: $p < 0.1$).

thwarted goats [5]. Frustrated pigs vocalized frequently, became extremely active, and exhibited increased plasma cortisol levels [8]. Frustrated pigs did not exhibit hyperthermia [24]. Species differences exist in frustration-induced HPA-axis activity; non-reward frustration induced increases in plasma glucocorticoid concentrations in pigs [8], rats [28], chickens [2], and squirrel monkeys [21], but not in goats [5]. Although the responses to food frustration paradigm differ depending on species and experimental protocol, the frustrating context in which only the experimental animal could not access feed in the presence of other ewes that were given food might induce such physiological and behavioral stress responses in the present study, as we carefully controlled the nonspecific effects of lack of food, hunger. It was partly because sheep are social animals. As they tend to graze at the same time in the free-range condition [1], the lack of simultaneity affected the mood of FC sheep. A social facilitation in which the behavior is increased in rate or frequency by the presence of another animal carrying out that behavior in social animals [12, 37] also affected the mood.

That the increase in cortisol by FC tended to decline to the same level as control by infusions of Pmp, Tyr-AVP or αhCRF suggests that both AVP and CRH have certain role in the psychological-stress-induced increase in cortisol. Although many reports have shown that AVP or CRH receptor antagonist block stress-induced behavioral and physiological responses [18, 22, 23, 32, 33], relatively few reports have clearly shown their inhibiting effect on the stress-induced increase in ACTH and glucocorticoid [13, 27]. AVP and CRH concentrations in the portal vein were elevated after several stressors in sheep, and different stressors evoked different release patterns of both peptides into the portal vein [3, 4]. Moreover, intracerebroventricular infusion of AVP and CRH increased plasma cortisol concentrations equally in equimolar concentrations in sheep [38, 40]. Although these studies indicate that AVP and CRH could be major secretagogues to stimulate ACTH secretion in sheep, other factors such as catecholamines also play a role [25, 35, 36]. Moreover, the relative significance of AVP and CRH on the pituitary-adrenocortical system was reported to be stress-specific [3, 26, 29]. Thus, at least in the psychological stress context applied in the present study, both AVP and CRH have important roles in stimulating glucocorticoid secretion.

In studies involving rats, stress-induced hypertension, tachycardia, and hyperthermia were inhibited by CRH antagonist [22, 23]. In these studies, a mild stressor, cage-switch stress, was used to induce a mild stress response that included increases in HR, blood pressure, and body temperature indicative of sympathetic nervous system activation. In the present study, no change in HR but an increase in RT was observed during the FC period. During feeding time after the FC period, significant increases in HR and the maintenance of high RT compared with pre-infusion period was observed at 90 and 120 min that might be caused by feeding activity. The maximum rate of change in RT from the pre-infusion value during the FC period was partly inhibited by Pmp, Tyr-AVP treatment nearly to the control level, whereas no effect was observed with αhCRF treatment. Although AVP infusion into the third cerebral ventricle did not cause hyperthermia in our former report [40] and even hypothermic actions of exogenous AVP during pyrogen-induced fever was reported in sheep [7, 30], the present data suggest that in the psychological stress context applied here, AVP might have certain role in inducing the hyperthermic response to the stressor.

The behavioral change induced by FC was an increase in pawing. Food-thwarted goats also showed an increase in pawing [5]. The increase in pawing was inhibited to almost the control level by αhCRF infusion, whereas Pmp, Tyr-AVP infusion had no measurable effect on the behavior in the present study. CRH antagonist inhibited the duration of shock-induced freezing in rats [20, 33], anxiety behavior induced by a social stressor in primates [13], and locomotor activation induced by mild cage-switch stress in rats [22]. AVP V1b receptor antagonist was active in suppressing separation-induced pup vocalization and suppressing depression in a forced swim test in rats [15]. In our previous report in sheep and cattle [38–40], intracerebroventricular infusion of CRH induced stereotypical bleating and AVP induced oral stereotyped behaviors. Types of stress-induced behaviors inhibited by antagonists for these peptides and types of behaviors induced by the synthetic peptides introduced exogenously were context-dependent. The relative significance of AVP and CRH in regulating stress-induced behavioral responses may differ according to the type and intensity of stressors. In the present study, CRH might have certain role in inducing conflict behavior, pawing, to a psychological stressor, frustration.

In this study, intracerebroventricular infusion of AVP antagonist inhibited the increase in plasma cortisol concentration and hyperthermia, and intracerebroventricular infusion of CRH antagonist inhibited the increase in plasma cortisol concentration and pawing, induced by FC, a frustrating stress context. Although further study is necessary with a large number of animals to confirm the general applicability of these findings, these results suggest that at least in the psychological, frustrative context used in this experiment, both endogenous CRH and AVP in the central nervous system have important role to stimulate cortisol secretion, that endogenous AVP might have certain role to induce hyperthermia, and that endogenous CRH might have certain role to induce conflict behavior in sheep.

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