The Effects of Water Deprivation on Cerebrospinal Fluid Constituents During Feeding in Sheep

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ABSTRACT : The internal humoral factors in the central regulation of dry feed intake during water deprivation in sheep were investigated by measurement of cerebrospinal fluid (CSF) constituents. Five animals were fed dried alfalfa chaff for 2 hours once a day. Sheep in the water deprivation treatment were deprived of water for 28 hours, while the sheep in the control treatment were given free access to water. During the first hour of the 2 hour feeding period, a rapid reduction in blood volume occured in both treatments (water deprivation and free access to water). The CSF concentrations of Na, Cl and osmolality during the second hour of the 2 hour feeding period in both treatments were greater ($p<0.01$) than those during the first hour. The drinking behaviors in sheep were concentrated during the second hour of the 2 hour feeding period in periods of free access to water. Water intake during feeding in periods of free access to water was 1110 ml/2 h. The levels of increase in CSF osmolality with feeding during water deprivation were greater ($p<0.01$) than during periods of free access to water. The changes in CSF osmolality with feeding during water deprivation produced more vigorous thirst sensations in the brain compared to during periods of free access to water. The eating rates for the first hour of the allotted 2 hour feeding period were the same under both treatments. However, the eating rates for the second hour during water deprivation periods decreased significantly ($p<0.05$) compared to those during periods of free access to water. The decreased eating rates for the second hour during water deprivation may be due to the vigorous thirst sensations produced in the brain. The results suggest that the increase in CSF osmolality with feeding during water deprivation acts as a thirst and satiety factor in brain mechanisms controlling feeding to decrease dry feed intake in water-deprived sheep. (*Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 4 : 467-473*)

Key Words : CSF Osmolality, Brain, Thirst, Dry Feed Intake, Sheep

INTRODUCTION

Feed intake decreases during water restriction or water deprivation in animals (Langhans et al., 1995). It is thought that there may be a relationship between the mechanisms controlling water and feed intake in animals.

In sheep fed on alfalfa hay cubes, Otani et al. (1983) reported that the hematocrit value increased soon after feeding. Mathai et al. (1997) also reported that the plasma protein concentration in sheep fed on alfalfa dry chaff increased by 15% within 30 mins of feeding. Sato (1975) reported that circulating plasma volume estimated with Evans blue dye dilution method, decreased 10% during feeding in sheep fed alfalfa hay cubes. Despite the fact that the sheep in this experiment have free access to water during the 2 hour feeding period (in which they were fed dry feed) they may become hypovolemic during the first hour.

The intraruminal infusion of hyperosmotic NaCl or polyethylene glycol-400 (PEG) in sheep decreased feed intake, whereas intraruminal infusion of excessive water increased feed intake (Baile et al., 1969; Ternouth and Beattie, 1971; Kato et al., 1979). The water intake of sheep after the completion of intraruminal hyperosmotic solution infusion increased markedly. On the other hand, the water intake of sheep after the completion of excessive intraruminal water infusion decreased compared to that during non infusion. These results show that the thirst levels produced by hyperosmotic solution infusion were greater than those during water infusion in sheep.

The sensations of hunger, satiety and thirst are produced in the brain as a result of the integration of neuronal and humoral information (Nijima, 1969; Schmit, 1973; Fitzsimons, 1979; Oomura, 1980; Ono et al., 1981). Neuronal information is transported via the autonomic nerve (especially the vagus nerve) from the peripheral organs chemoreceptors and the mechanoreceptors in the internal visceral organs. A broad range of internal humoral information is transported via the blood and cerebrospinal fluid. However, to date the internal humoral factors regulating feed intake in ruminants have not yet been found.

The aim of the present research was to clarify the internal humoral factors in brain mechanisms controlling feed intake during water deprivation. We

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investigated changes in CSF constituents during feeding in water-deprived sheep.

MATERIALS AND METHODS

Animals

Five crossbred Merino ewes, 34-45 kg body weight, were used. The sheep were ovariectomized and had both carotid arteries exteriorized in a 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 surgical and experimental procedures were approved by the Institutes Animal Experimentation Ethics Committee, and adhered to the Australian code of practice for the care and use of animals for scientific purposes.

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

We examined the effect of water deprivation on plasma and CSF constituents during feeding in sheep adapted to a 2 hour, once a day feeding period. The sheep were offered a 1.5 kg daily ration of dried alfalfa chaff (Na+ 90-100 mmol/kg, K+ 250-400 mmol/kg) once a day (11:00 to 13:00) (Ruckebush and Malbert, 1986; Spina et al., 1996). All feed intake data are expressed on a dry matter basis. The temperature of the room in which this experiment was carried out was maintained at 20℃.

Cerebrospinal fluid and blood sampling

For taking cerebrospinal fluid (CSF), an obturator was removed from one of the guide tubes, and a LV (lateral ventricle) probe (20-guage needle attached to a metal Luer-Lock cap) of the appropriate length was inserted through the guide tube into the lateral brain ventricle. The probe was connected via a polyethylene cannula to a 10 ml syringe, and outflow of CSF was collected in the cannula.

The blood samples were taken via cannula from a carotid artery.

Experimental design

Two treatments were performed: 1) a control treatment (free access to water), and 2) a water deprivation treatment. Each treatment was performed on five sheep. Animals had free access to water and NaCl solution *ad libitum* in the control treatment. In the water deprivation treatment, the water pedal was locked for 28 hours from 11:00 on Monday to 15:00 on Tuesday, and from 11:00 on Thursday to 15:00 on Friday. The control experiments were conducted on Tuesday and Friday. The water deprivation experiments were conducted on Tuesday and Friday in the following week. CSF samples (0.5 ml) and blood samples (10 ml) were taken at 11:00, 11:30, 12:00, 13:00 and 15:00 on each day. Feed intake was measured at 30 min intervals for 2 hours from 11:00 to 13:00. Water and sodium intake were also measured daily.

Chemical analysis

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

Alfalfa chaff was ground using a Willey mill (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: digestibility (%)=(dry matter intake-fecal output)/dry matter intake × 100%.

Statistical analysis

As the confounding effect of previous treatments was not found in the results of the experiments conducted on Friday, a statistical analysis was performed using pooled data from Tuesday and Friday (ten observations from 5 sheep). Data are presented as means ± S.E. from the ten observations of 5 sheep. A two-way ANOVA and subsequent Dunnetts test (repeated measurement) were performed to compare the

Table 1. Chemical composition and nutritive values of alfalfa chaff

	Alfalfa chaff
Dry matter(%)	89.0 ± 0.17
Chemical composition (% of DM)	
Organic matter	92.8 ± 0.05
Crude protein	12.9 ± 0.21
Crude fat	3.5 ± 0.11
Crude fiber	24.5 ± 0.19
Nitrogen-free extracts	52.0 ± 0.29
NDF[1)]	45.6 ± 0.21
ADF[2)]	26.2 ± 0.23
Nutritive values(% of DM)	
DCP[3)]	9.2 ± 0.13
TDN[4)]	61.6 ± 0.01

[1)] NDF: Neutral detergent fiber, [2)] ADF: Acid detergent fiber, [3)] DCP: Digestible crude protein, [4)] TDN: Total digestible nutrients, Values are means ± SE from five determinations.

differences in data between treatments (free access to water and water deprivation) (Gill, 1978; SAS, 1990). Further data analysis using the same methods was carried out to compare changes prior to, during and after feeding within each treatment.

RESULTS

The results of feed intake are shown in figure 1. The eating rates for the first hour of the allotted 2 hour feeding period were the same in both treatments (water deprivation and free access to water). However, the eating rates for the second hour during water deprivation periods decreased significantly compared to those during periods of free access to water. The drinking behaviors in sheep were concentrated during the second hour of the 2 hour feeding period in periods of free access to water. Water intake during feeding in periods of free access to water was 1110±93 ml/2 h and the total daily water intake was 2275 ±140 ml/day (table 2). The plasma total protein concentration after 30 mins of the commencement of feeding during water deprivation increased significantly compared to that during periods of free access to water (figure 2). The plasma osmolality prior to, during and after feeding in water deprivation periods increased significantly compared to that during periods of free access to water. However, the plasma concentrations of Na, K and Cl during water deprivation were not different from those during periods of free access to water (figure 3).

The CSF osmolality prior to, during and after feeding in water deprivation periods increased significantly compared to that during periods of free access to water (figure 5). On the other hand, the

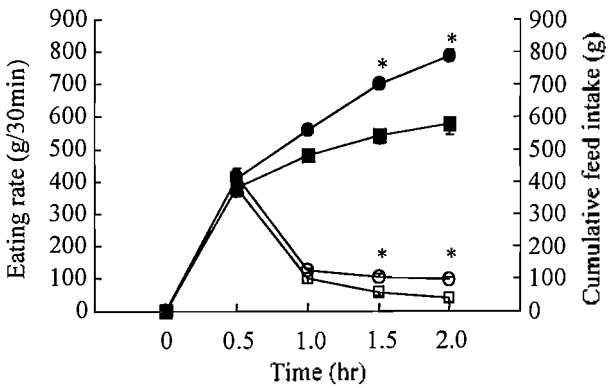

Figure 1. Cumulative feed intake and eating rate during the 2 hr feeding period in periods of water deprivation (□, ■) and periods of free access to water (○, ●). Each point represents the mean±S.E. of 5 sheep. Significant differences from the value during periods of free access to water are indicated by * $p<0.05$.

Table 2. Water and salt intake in Na replete sheep

Water intake	
ml/2 h	1110.0 ± 93.0
ml/day	2275.5 ± 139.8
0.5 M NaCl intake	
ml/2 h	57.5 ± 12.9
ml/day	628.0 ± 110.2

Values are means±S. E. from ten observations of 5 sheep.

CSF concentrations of Na, K and Cl during water deprivation were not different from those during periods of free access to water (figure 4). The levels

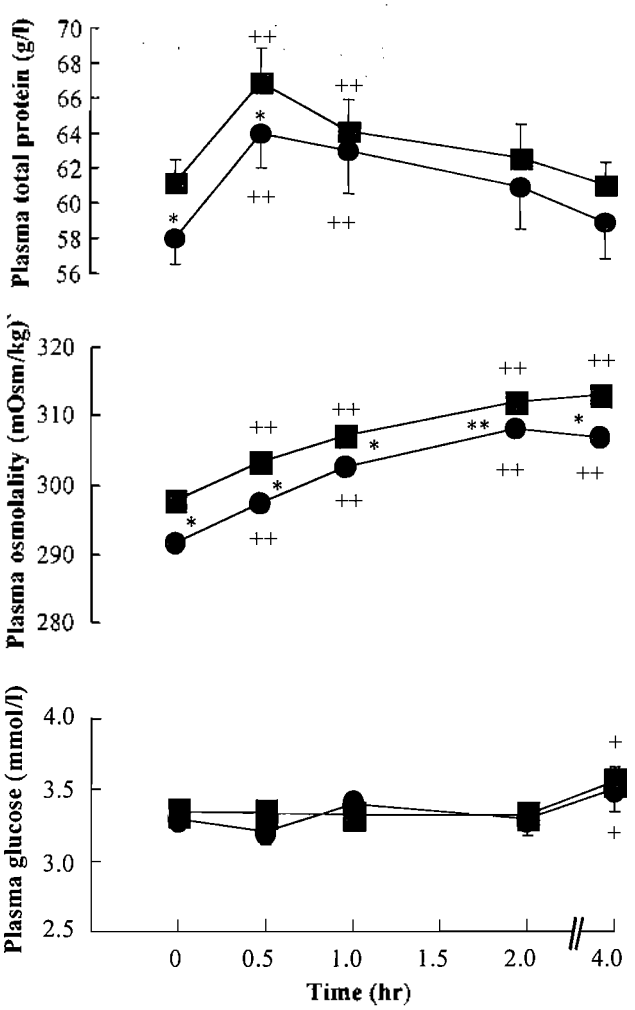

Figure 2. Plasma total protein concentration, plasma osmolality and plasma glucose concentration during the 2 hr feeding period in periods of water deprivation (■) and periods of free access to water (●). Each poin represents the mean±S.E. of 5 sheep. Significant differences from the value during periods of free access to water are indicated by * $p<0.05$, ** $p<0.01$. Significant differences from prefeeding values are shown by + $p<0.05$, ++ $p<0.01$.

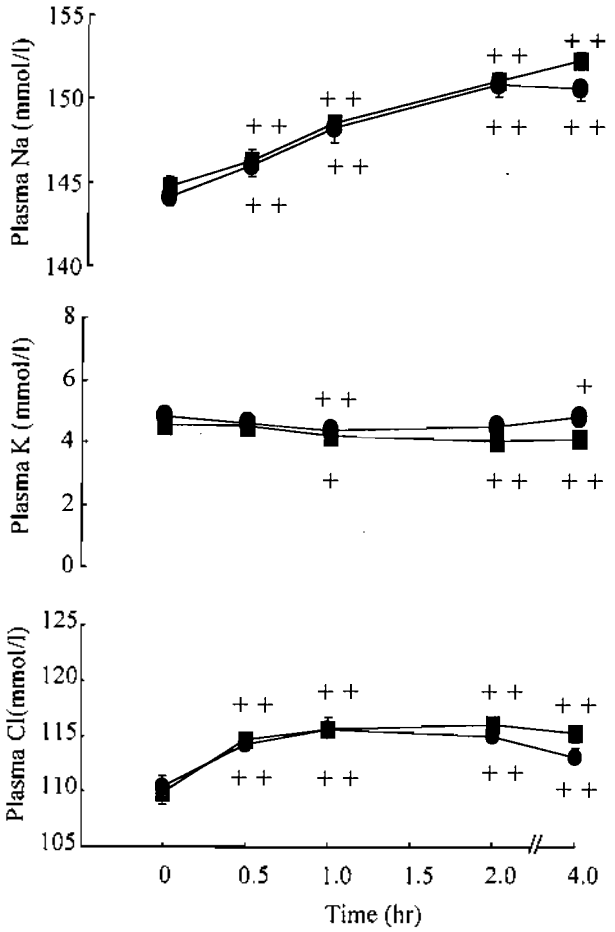

Figure 3. Plasma concentrations of Na, K and Cl during the 2 h feeding period in perods of water deprivation (■) and periods of free access to water (●). Each point represents the mean±S.E. of 5 sheep. Significant differences prefeeding values are shown by + $p<0.05$, ++ $p<0.01$.

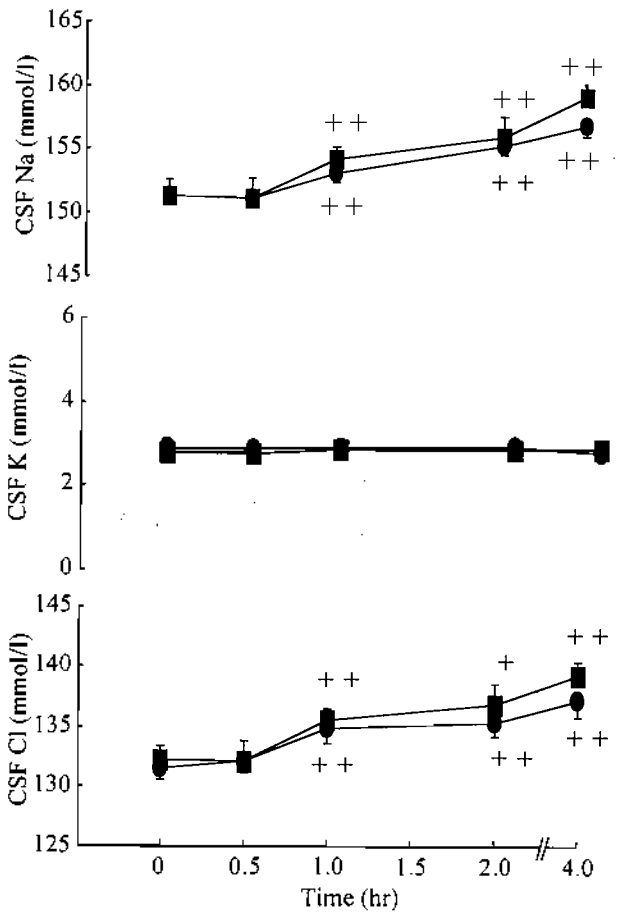

Figure 4. CSF concentrations of Na, K and Cl during the 2 hr feeding period in periods of water deprivation (■) and periods of free access to water. Each point represents the mean±S.E. of 5 sheep. Significant differences from prefeeding values are indicated by + $p<0.05$, ++ $p<0.01$.

of increase in CSF osmolality during water deprivation were larger than the increases in plasma osmolality.

DISCUSSION

In this experiment, sheep were offered dry alfalfa chaff for 2 hours once a day, and their feeding drive was strong. While eating continued for the entire duration of the feeding period, the highest eating rates were observed in the first 30 mins of the 2 hour feeding period. The concentrations of plasma total protein, plasma osmolality and CSF osmolality prior to feeding were significantly higher during water deprivation than during periods of free access to water (figures 2 and 5). The levels of increase in plasma total protein concentration during water deprivation in sheep were smaller than those in cats (Schultze et al., 1972). Although sheep during water deprivation experienced greater thirst than during periods of free access to water, their feed intake for the first hour of the allotted 2 hour feeding period was the same under both treatments (figure 1). The similarity in eating rates between both treatments for the first hour of the allotted 2 hour feeding period were due to a 2 hour once a day feeding system. These results indicate that animals in both treatments experienced the same degree of hunger before feeding and exhibited a similar appetite during the first phase of feeding.

It was reported that the feed intake of alfalfa pellets was regulated by changes in ruminal fluid osmolality (Baile et al., 1969; Kato et al., 1979; Grovum, 1995). The same sized dose of hyperosmotic NaCl, polyethylene glycol-400 (PEG), sodium acetate or sodium propionate produced the same increases in rumen fluid osmolality when intraruminally infused. These increases in rumen fluid osmolality resulted in the same sized decrease in feed intake (Grovum, 1995). On the other hand, when the rumen fluid

osmolality was decreased by the intraruminal infusion of an excessive amount of warm water (39.8℃), feed intake increased markedly (Kato et al., 1979). It has been thought that the changes in ruminal fluid osmolality were sensed by the osmoreceptors in the rumen wall and these signals were then transported into the central nervous system (Leek and Harding, 1975). However, the effect of internal humoral factors on the intake of grass has not been investigated under these experimental conditions. During the first hour of feeding, a rapid reduction in blood volume probably occured in both treatments, as indicated by an initial increase in plasma protein concentration (figure 2). It is likely that hypovolaemia was caused by fluid moving from the circulating blood into the saliva and gut soon after dry feed had been ingested. Within the second hour, plasma protein concentration returned to pre-feeding levels, but plasma osmolality, Na and Cl concentration continued to increase (figures 2 and 3). The increase of plasma Na and Cl concentrations appeared to depend on the continuous Na, Cl absorption from the rumen (Stacy and Warner, 1966; Warner and Stacy, 1972). These increases of rumen fluid and plasma osmolality, and hypovolaemia with feeding in both treatments resulted in increased CSF osmolality (figure 5).

It was observed in this experiment that drinking behaviors in sheep were concentrated in the second hour of feeding during periods of free access to water. The water intake during feeding in periods of free access to water was 1110 ml/2 h (table 2). The CSF concentrations of Na, Cl and osmolality during the second hour of feeding in both treatments were greater than those during the first hour of feeding (tables 4 and 5). It was reported that osmoreceptors exist in the organum vasculosum lamina terminalis (OVLT) and Na sensors in the subfornical organ (SFO) (Weisinger et al., 1985). Assuming that Na sensors and osmo-recepters in the brain are involved in thirst, the increases in CSF Na concentration and CSF osmolality may produce thirst sensations in the brain (Weisinger et al., 1985; McKinley et al., 1994). The levels of increase in CSF osmolality with feeding were greater during water deprivation than during periods of free access to water (figure 5). These feeding related changes in CSF osmolality during water deprivation produced greater thirst sensations in the brain than during periods of free access to water.

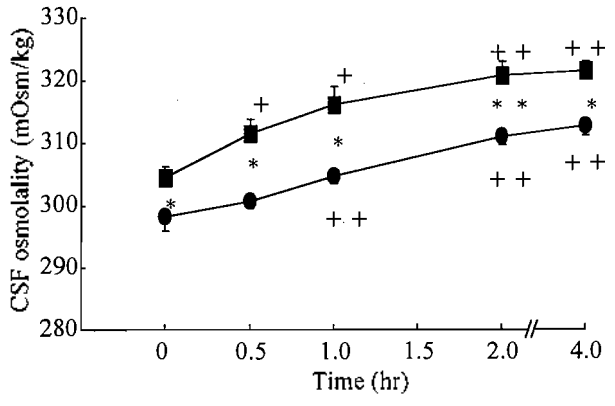

Figure 5. CSF osmolalities during the 2 hr feeding period in periods of water deprivation (■) and periods of free access to water (●). Each point represents the mean±S.E. of 5 sheep. Significant differences from the value during periods of free access to water are indicated by * $p<0.05$, * $p<0.01$. Significant differences from prefeeding values are shown by + $p<0.05$, ++ $p<0.01$.

When rats were deprived of water or when ANGII produced during water deprivation was injected into the lateral ventricle, thirst sensations were produced in the brain (Nazarali et al., 1987; Weisinger et al., 1997). Under these conditions, increased formation of c-fos was observed in the hypothalamic praventricular nucleus (PVN), supraoptic nucleus (SO) as well as in the circumventricular organs including OVLT, SFO and median preoptic nucleus (MnPO) (Morien et al., 1999). The c-fos expression indicates increased activity of neurons, in a range of neural systems (Hunt et al., 1987; Dragunow et al., 1989). While the circumventricular organs are outside the blood-brain barrier and thus are exposed to changes in circulating constituents, the PVN and SO are inside the blood-brain barrier. These nuclei are related to the central control of feed and water intake, and are activated during water deprivation (Arnauld et al., 1975; Morley et al., 1987; Nazarali et al., 1987; Vaughan et al., 1995).

Lesioning of the SFO abolishes the dipsogenic response to intravenous administration of angiotensinII (ANGII), however, central administration of the peptide elicits drinking even in the presence of such a lesion (Phillips, 1978). In this experiment, CSF osmolality prior to, during and after feeding in water deprivation periods increased significantly compared to that during periods of free access to water (figure 5). Infusion of ANGII into the lateral ventricle of sheep produced thirst sensations which resulted in the decrease of feed intake (Sunagawa et al., 2000). The neuronal activity of ventromedial hypothalamic neurons decreased by intraventricular administration of hyper osmotic NaCl solution in freely behaving rats (Ono et al., 1987). From these results, it is thought that the marked increases in CSF osmolality during the second hour of feeding in water deprivation periods acts as a thirst and satiety factor in brain mechanisms controlling water and feed intake. The similarity in CSF Na and Cl concentrations in both treatments may be due to the fact that the loss of Na and Cl from the

circulating blood into saliva and the gut, and the absorption of Na and Cl from the rumen into the circulating blood was the same.

The results suggest that the maked increase in CSF osmolality with feeding during water deprivation acts as a thirst and satiety factor in brain mechanisms controlling feeding to decrease dry feed intake in water-deprived sheep.

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