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Changes of Blood and Cerebrospinal Fluid Constituents after Feeding in Sheep

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Summary

Changes in blood and cerebrospinal fluid (CSF) constituents were observed in sheep that were fed alfalfa haycubes for 2 hours. During feeding, plasma IRI concentration increased but plasma FFA concentration decreased. The depression of plasma HCO_3^- concentration was accompanied by the depression of blood pH during feeding. Mg and Ca concentrations in plasma increased soon after feeding and gradually decreased thereafter. These changes were similar to the changes of haematocrit value. There were, however, no changes of these constituents in CSF during feeding. On the contrary, osmolality and Na concentration in CSF increased gradually during feeding periods as the result of similar changes of osmolality and Na concentration in plasma. It was therefore suggested that Na, which was rising at the end of feeding periods, might act as a signal to reduce food intake.

Many studies have been made on various kinds of animals to determine the factors in blood which play a part in the food intake regulatory system as humoral signals. Although glucose in blood is regarded as the dominant feeding control factor in monogastric mammals, no significant constitutent has been confirmed yet in ruminants. It has been reported that the hypothalamus functions as the control center for food intake in ruminants, just like in the case of monogastric mammals (1). Recent research on the factors which stimulate the feeding center has been carried out mainly by means of the technique of cerebroventricular injection (2, 3).

The so called blood-brain barrier (BBB), located in between the blood and brain tissue, restricts the transport of many blood substances to the brain by means of a special permeability mechanism. In order to clarify the factors in blood that can act as control signals of the feeding center, it is necessary to know the changes of constituents not only in the blood side but also in the brain side of

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BBB during feeding. In the present experiment, we adopted cerebrospinal fluid (CSF) in order to investigate these movements across BBB. We observed the concentration changes in blood and CSF constitutents in sheep after feeding. The comparative measurement of blood and CSF constitutents can elucidate the possible blood factors which regulate the food intake in ruminants.

Materials and Methods

Cross-bred sheep weighing 28–58 kg were used. All animals had their left common carotid artery chronically placed in a loop of skin. At least 2 days before the experiments began, polyethylene catheters were inserted about 8 cm into the lumbar subarachnoid space through the lumbo-sacral site to permit repeated CSF collection. Alfalfa haycubes were given for 2 hours daily, at 11:00–13:00 in fed experiments or at 14:00–16:00 in control experiments. In fed experiments, food intakes were recorded every 15 minutes for 2 hours. Water was given *ad libitum*.

Blood samples were collected through the polyethylene catheter inserted into the carotid artery and CSF smaples were collected through the polyethylene catheter inserted into the lumbar subarachnoid space. Samples were taken every 30 minutes from 10:30 to 14:00. Immediately after the collection of all samples, pH and Pco_2 in blood and CSF and haematocrit value were measured. In order to analyze other constituents, blood samples were centrifuged, and separated plasma and CSF were stored at –20°C until analysis. Physiological responses were observed from 10:30 to 14:00, with intervals of 15 minutes only from 11:00 to 13:00, and of 30 minutes for other experimental periods.

Pco_2 and pH in blood and CSF were measured by the blood gas analyzer (Radiometer, BMS3–MK2) and, from these values, plasma and CSF HCO_3^- concentrations were determined with the Siggard-Andersen Alignment nomogram. Glucose, free fatty acids (FFA), and immunoreactive insulin (IRI) in plasma and CSF were assayed by the glucose oxidase method (4), Itaya & Ui method (5) and Mougan & Lazarow method (6) respectively. Plasma and CSF osmolality were measured by the osmometer (Advanced Osmometer, model 3L). Na and K concentrations in plasma and CSF were measured by the flame emission spectrophotometer, and Mg and Ca concentrations were measured by the atomic absorption spectrophotometer (Nippon Jarrell-Ash, AA–825).

All results are expressed as mean±SE of mean. The Student's t test was used to compare the initial value (the mean of –30 min value and 0 min value) with the value after feeding.

Results

1. *Food Intake, Physiological Response and Haematocrit value*

Maximal food intake, 339±12 g/15 min, was recorded soon after the start of feeding. It decreased gradually until the deprivation of ration. Total food

intake during 2 hours was 1013±62 g (Table 1.). Respiratory rate and heart rate increased suddenly after the animals began to eat and these high levels continued during feeding periods. Rectal temperature increased to a peak at 45 minutes after the start of feeding. The haematocrit value also increased to about

TABLE 1. *Food Intake* (g) in Sheep for Fed Experiments*

Intervals, *min*								Total
0-15	15-30	30-45	45-60	60-75	75-90	90-105	105-120	
339±12	206±21	160±23	88±12	69±8	65±8	47±6	39±7	1013±62

* Values are expressed as mean±S.E., n=14 observations on 6 sheep.

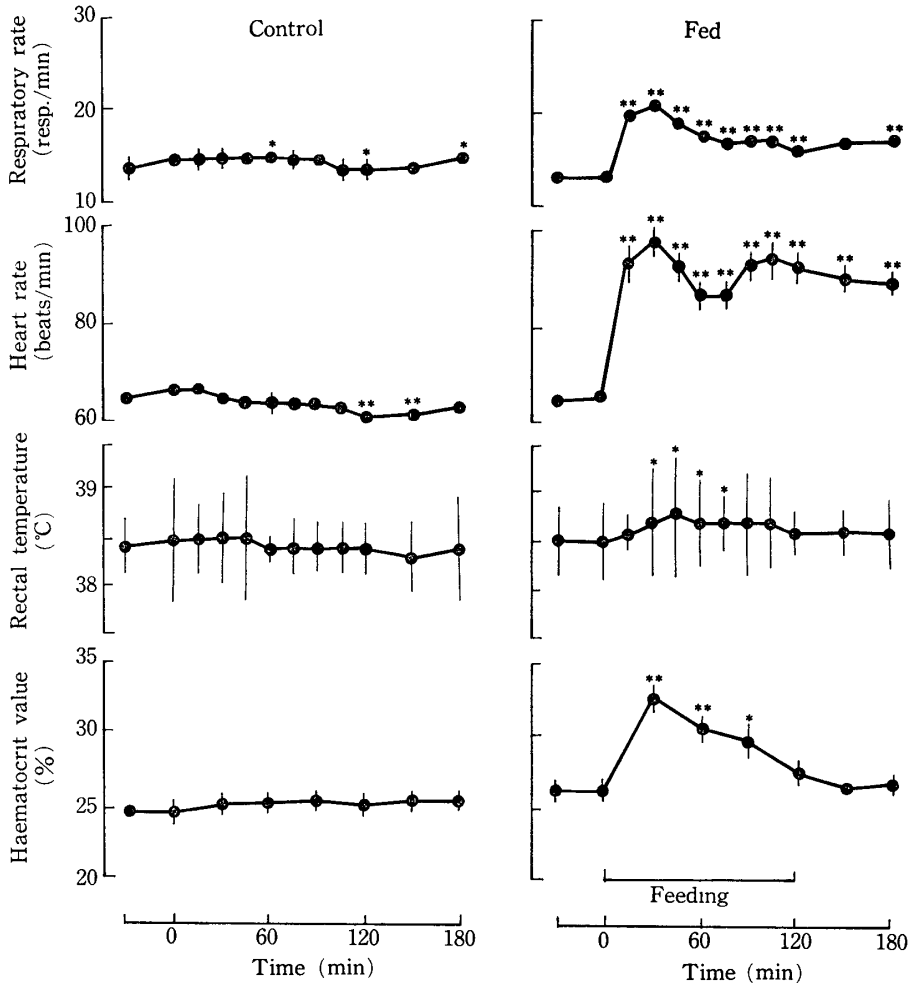

FIG. 1. Changes of physiological responses and haematocrit value in 4 sheep. *Left*: control experiments (n=12 for respiratory rate, heart rate and rectal temperature, n=4 for haematocrit value); *right*: fed experiments (n=13 for respiratory rate and heart rate, n=11 for rectal temperature, n=4 for haematocrit value). Ration was given from 11:00 (0 min) to 13:00 (120 min) in fed experiments. Values are expressed as mean±SE. Values significantly different from the mean of −30 min value and 0 min value: *P<0.05, **P<0.01.

6 per cent of the initial value soon after the start of feeding, but it gradually decreased and returned to the initial level at the end of feeding periods (Fig. 1).

2. *Substances Related to Glucose Metabolism*

Plasma and CSF glucose concentrations tended to decrease during the experimental periods both in fed and control experiments, but these decreases were not statistically significant. Plasma IRI concentration gradually increased up to 22.8±4.3 μU/ml. On the other hand, plasma FFA concentration that was at a high level before feeding (191.9±45.3 μEq/l) decreased remarkably to about a quarter of the pre-feeding level. Nevertheless, there were not any changes of these constiutents in CSF during feeding (Fig. 2).

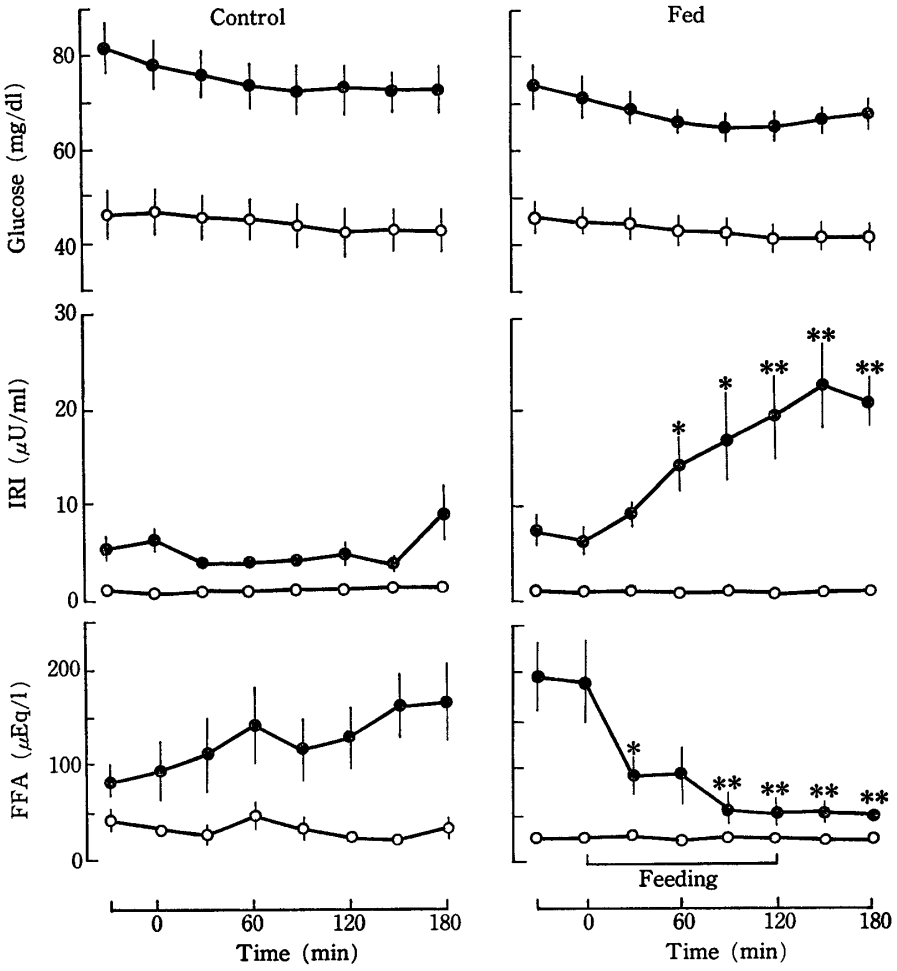

FIG. 2. Changes of glucose, FFA and IRI concentration in plasma (●) and CSF (○) in 6 sheep. *Left*: control experiments (n=6); *right*: fed experiments (n=6). Ration was given from 11:00 (0 min) to 13:00 (120 min) in fed experiments. Values are expressed as mean±SE. Values significantly different from the mean of -30 min value and 0 min value: *P<0.05, **P<0.01.

3. *Acid-base Status*

Blood pH and plasma HCO_3^- concentrations decreased soon after the start of feeding and maintained their levels during feeding. All 30–180 min values in blood pH and 90 min values in plasma HCO_3^- showed a statistically significant decrease compared with the initial values. Blood Pco_2, however, did not change during feeding. In CSF, we could not observe any changes of pH, Pco_2 and HCO_3^- concentration during feeding (Fig. 3).

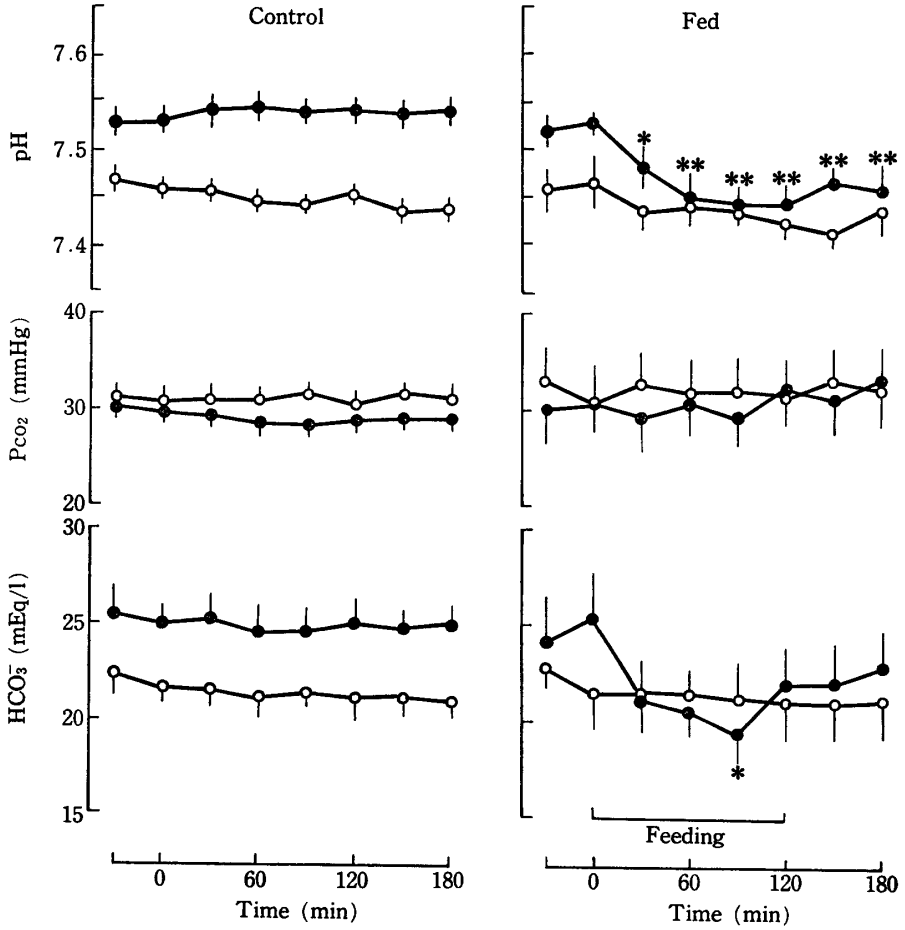

FIG. 3. Changes of pH and Pco_2 in blood (●) and CSF (○) and of HCO_3^- concentration in plasma (●) and CSF (○) in 6 sheep. *Left*: control experiments (n=6); *right*: fed experiments (n=6). Ration was given from 11:00 (0 min) to 13:00 (120 min) in fed experiments. Values are expressed as mean±SE. Values significantly different from the mean of −30 min value and 0 min value; *$P<0.05$, **$P<0.01$.

4. *Osmolality and Minerals*

Osmolality and Na concentration both in plasma and CSF increased in a similar pattern after the supply of ration and these increases continued after the deprivation of ration. Compared with the initial levels and 180 min values, osmolality and Na concentration showed about 20 mOsm/kg and 10 mEq/l increases

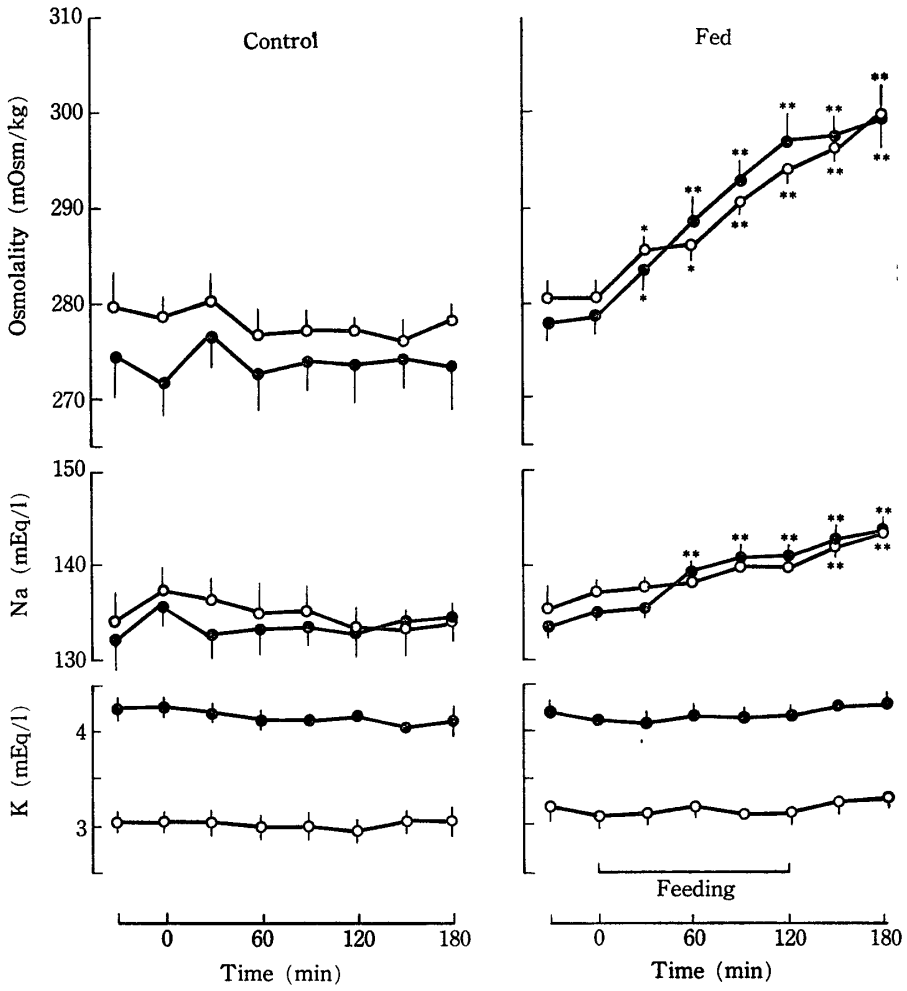

FIG. 4. Changes of osmolality, Na and K concentration in plasma (●) and CSF (○) in 6 sheep. *Left*: control experiments (n=6); *right*: fed experiments (n=8). Ration was given from 11:00 (0 min) to 13:00 (120 min) in fed experiments. Values are expressed as mean±SE. Values significantly different from the mean of -30 min value and 0 min value: *P<0.05, **P<0.01.

respectively both in plasma and CSF. K concentration in plasma was higher than in CSF, but we could not find any changes during feeding in these two fluids (Fig. 4). Mg level was similar in these two fluids but CSF Ca level was half of the plasma level. Plasma Ca and Mg concentrations showed a similar pattern of increase soon after the start of feeding and then decreased gradually. But we could not observe these changes of Mg and Ca concentrations in CSF. In control experiments, plasma Mg concentration increased significantly after the deprivation of ration (Fig. 5).

Discussion

Although it has already been pointed out that plasma insulin concentration in ruminants increases after feeding just like in monogastric mammals, its secretory

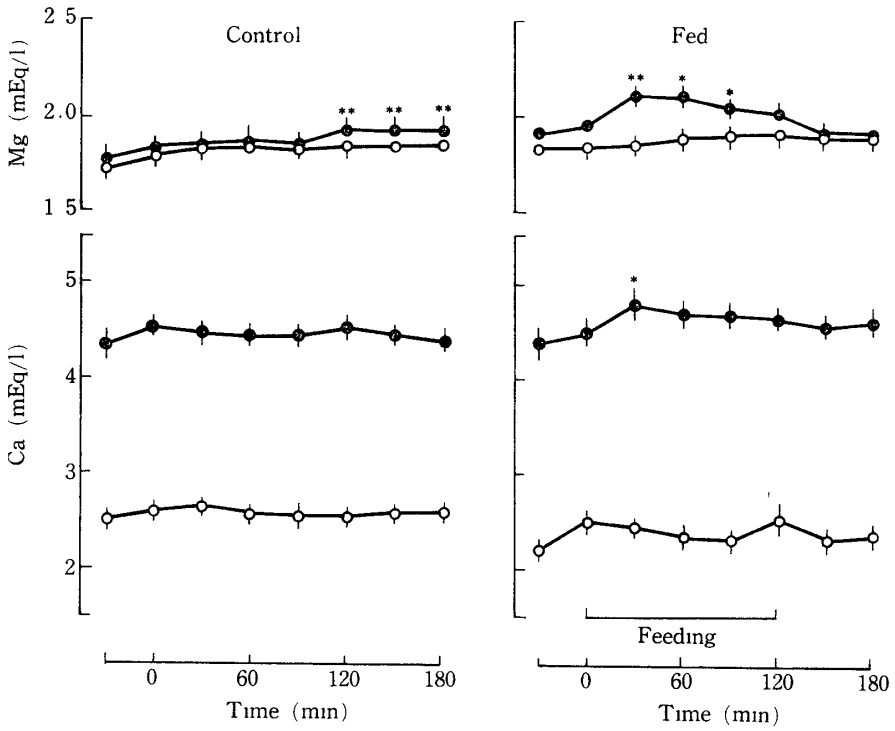

FIG. 5. Changes of Mg and Ca concentration in plasma (●) and CSF (○) in 6 sheep. *Left*: control experiments (n=6); *right*: fed experiments (n=8). Ration was given from 11:00 (0 min) to 13:00 (120 min) in fed experiments. Values are expressed as mean±SE. Values significantly different from the mean of −30 min value and 0 min value: *P<0.05, **P<0.01.

mechanism has not been fully clarified. The generally accepted view (7) that the increase of VFA concentration in blood after feeding stimulates insulin secretion has been denied by some researchers (8, 9). Recently, it was suggested that some gastrointestinal hormones may be included in this mechanism (10). In our study, the increase of plasma IRI concentration after feeding was obvious but plasma glucose concentration and CSF IRI concentration did not change significantly. It has been reported that intravenous infusion of glucose raises the endogenous plasma insulin concentration and leads to a slight increase of CSF insulin concentration (11). In our experiment, however, the increase of 15 μU/ml or so of IRI concentration in plasma did not affect IRI concentration in CSF. FFA concentration in CSF was at a very low level and was kept constant, although the plasma FFA level changed before and after feeding. FFA concentration in plasma is easily altered by the stress of the change of energy balance, while that in CSF is kept constant.

Sasaki *et al.* suggested that the fall in blood pH during feeding in ruminants was associated with the large loss of $NaHCO_3$ from blood via saliva into the rumen (12). In our study, we observed a similar change in acid-base status during feeding. It is, however, known that the passing rate of HCO_3^- between blood and CSF is too slow to change CSF HCO_3^- concentration (13). In fact, HCO_3^- con-

centration in CSF did not change in our experiment, so we could not observe the fall of CSF pH in spite of the fall of blood pH.

The temporary increase of plasma Mg and Ca soon after the beginning of eating appeared to be associated with the increase in haematocrit value as a result of the decrease of extracellular fluid due to the acceleration of saliva secretion. It was therefore considered that blood was condensed and plasma Mg and Ca concentrations might have increased relatively. Subsequently, these concentrations appeared to decrease to the initial levels because of recovery of extracellular fluid due to reabsorption of water from the digestive tract or to the increase of Ca and Mg excretion in urine (14). These temporary increases of Mg and Ca in plasma were, however, slight, and it was also found that these minerals were extremely stable in CSF (15, 16).

The increase of plasma Na concentration appeared to depend on Na absorption from the digestive tract (17) and on the decrease of Na excretion in urine soon after the start of feeding (14). The increase of plasma osmolality, being the sum total of all osmotic constituents, was supposed to be due mainly to the increase of Na concentration which is the largest osmotic constituent. In CSF also, the increase of Na concentration may be considered as the main cause of the increase of osmolality, because this changing pattern of Na concentration and osmlality was similar and because there were no changes in K, Mg, Ca or other osmotic constiutents concentrations. Because the transport of Na from plasma to CSF was obstructed by BBB, the increase of plasma Na concentration was thought not to influence the CSF Na concentration during a short time in normal conditions (18). However, it has been reported that the rate of transport of Na through BBB is enhanced by the presence of vasopressin (19). As vasopressin was secreted during feeding in sheep (14, 20), it was supposed that this hormone might contribute to the increase of CSF Na concentration. In addition, because water transport occurs quickly in BBB and because water moves from CSF to blood by means of osmotic gradient when plasma osmolality increases (18), it was thought that CSF Na concentration increases relatively.

It has been hypothesized that Na is able to act on the feeding center as a signal to restrain food intake because Na injected into CSF decreases spontaneous feeding in cats (21), rats (22) and sheep (3). The result of our experiment that CSF Na concentration as a putative food intake regulatory factor increased at the end of feeding periods appeared to emphasize that hypothesis. To make another reference to this hypothesis, it is known that Na injected into the third ventricle makes animals increase their water intake (23), and the fact that drinking behavior in sheep is concentrated in the latter period of feeding may be connected with the high Na concentration in CSF observed at the end of feeding periods in our experiment. On the other hand, although CSF osmolality increased similarly to Na concentration in our study, some studies whereby hyper solutions were injected into

the cerebro-ventricle showed that the effect on feeding may be either suppressive or stimulative (24, 25). It is therefore difficult to make a definite conclusion on the possible role of osmolality in the food intake regulatory system and we must wait for the results of further research.

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