INTRODUCTION

Originally isolated from the hypothalamus, somatostatin (somatotropin release-inhibiting factor, SRIF) is a 14- or 28-amino acid peptide and was initially characterized as an inhibitor of growth hormone release from the pituitary (Brazeau et al., 1973). SRIF is present in assayable quantities in human CSF (Black, 1982; Atack et al., 1988). The SRIF concentration in the brain regions of rats was highest in the hypothalamus (Ho et al., 1989). The SRIF receptors were distributed in the arcuate nucleus, medial preoptic area, suprachiasmatic nucleus, paraventricular nucleus, ventromedial nucleus and the dorsomedial nucleus in the hypothalamus of rats (Beaudet et al., 1995).

Sunagawa et al. (2001a) gave a continuous ICV infusion of somatostatin 1-28 at a rate of 5 µg/0.2 ml/h for 27 h from day 1 to day 2. Goats (n = 5) were fed roughly crushed alfalfa hay cubes for 2 h twice daily and water was given ad libitum. Feed intake was measured during ICV infusion of artificial cerebrospinal fluid (ACSF) and SRIF. The feed intake during SRIF infusion increased significantly compared to that during ACSF infusion. In comparison to the ACSF treatment, plasma osmolality during the SRIF treatment significantly decreased during the first half of the 2 h feeding period. The factor causing the decrease in plasma osmolality during the ICV infusion of SRIF was a decrease in plasma Na, K, Cl, and Mg concentrations. In comparison to the ACSF infusion treatment, parotid saliva secretion volumes during the 2 h feeding period in the SRIF infusion treatment were significantly larger. While there was no significant difference in cumulative water intake (thirst levels) between the SRIF and the ACSF treatments upon conclusion of the 2 h feeding period, based on the plasma osmolality results it is thought that thirst level increases brought about by alfalfa hay cube feeding in the first half of the feeding period were reduced. It is thought that the somatostatin-induced increases in feed intake during the 2 h feeding period in the present experiment were caused by decreases in plasma osmolality brought about by the somatostatin infusion. As a result, it is suggested that the significant decrease in feed intake during the initial stages of feeding in large-type goats given roughly crushed alfalfa hay cubes, was due to the actions of thirst-controlling peptides. (Key Words: Dry Forage Intake, Saliva Secretion, Thirst Controlling Peptides, Brain Somatostatin, Large-type Goats)

ABSTRACT : An intracerebroventricular (ICV) infusion of somatostatin 1-28 (SRIF) was used as a thirst-controlling peptide antagonist to investigate whether or not thirst-controlling peptides are involved in the significant decrease in feed intake during the initial stages of feeding large-type goats on dry forage. A continuous ICV infusion of SRIF was conducted at a small dose of 4 µg/0.2 ml/h for 27 h from day 1 to day 2. Goats (n = 5) were fed roughly crushed alfalfa hay cubes for 2 h twice daily and water was given ad libitum. Feed intake was measured during ICV infusion of artificial cerebrospinal fluid (ACSF) and SRIF. The feed intake during SRIF infusion increased significantly compared to that during ACSF infusion. In comparison to the ACSF treatment, plasma osmolality during the SRIF treatment significantly decreased during the first half of the 2 h feeding period. The factor causing the decrease in plasma osmolality during the ICV infusion of SRIF was a decrease in plasma Na, K, Cl, and Mg concentrations. In comparison to the ACSF infusion treatment, parotid saliva secretion volumes during the 2 h feeding period in the SRIF infusion treatment were significantly larger. While there was no significant difference in cumulative water intake (thirst levels) between the SRIF and the ACSF treatments upon conclusion of the 2 h feeding period, based on the plasma osmolality results it is thought that thirst level increases brought about by alfalfa hay cube feeding in the first half of the feeding period were reduced. It is thought that the somatostatin-induced increases in feed intake during the 2 h feeding period in the present experiment were caused by decreases in plasma osmolality brought about by the somatostatin infusion. As a result, it is suggested that the significant decrease in feed intake during the initial stages of feeding in large-type goats given roughly crushed alfalfa hay cubes, was due to the actions of thirst-controlling peptides. (Key Words: Dry Forage Intake, Saliva Secretion, Thirst Controlling Peptides, Brain Somatostatin, Large-type Goats)
were prevented by SRIF. Wang et al. (1987a; 1987b) reported that ICV infusion of SRIF inhibited vasopressin secretion during haemorrhage in sheep.

In this study, it was hypothesized that the significant decrease in feed intake in large-type goats fed dry forage, was caused by thirst controlling peptides.

**MATERIALS AND METHODS**

**Animals**

Five male large-type goats (1 Japanese Saanen goat, 6 yr, weighing 72.5 kg, 4 crossbred Japanese Saanen/Nubian male goats, 4 to 6 yr, weighing 82.8±3.7 kg) were used in this experiment. Sunagawa et al., Faculty of Agriculture, University of the Ryukyus have, through heterosis, produced a large-type goat for meat production purposes. The hybrid goat grows rapidly achieving a body weight of over 60 kg in its first year. As illustrated in Sunagawa et al. (2005), it is thought that the physiological state of the goats used in this experiment did not change after parotid gland fistulation because the collected saliva was infused into the rumen every day. Prior to the morning feeding, the collected saliva (3 to 5 kg) was infused into the rumen via the extension tube using a bathtub pump (Minipandy, KP-30F, Kosin, Tokyo). The physiological state of the goats used in this experiment did not change after parotid gland fistulation because the collected saliva was infused into the rumen every day (Sungawa et al., 2005). During the morning 2 h feeding period (10:30 to 12:30 h), the animals were fed 2 to 3 kg of roughly crushed (3×3×1 cm) alfalfa hay cubes. At 16:00 h each day, the animals were fed again with 0.8 kg of alfalfa hay cubes, 200 g of commercial ground concentrate and 20 g of sodium bicarbonate (Table 1). A more than adequate supply of water (8 to 10 L) was also provided twice a day during the feeding periods.

**Intracerebroventricular infusion procedure**

For intracerebroventricular (ICV) infusion, an obturator was removed from one of the guide tubes, and a LV (lateral ventricle) probe (20-gauge needle attached to a metal Luer-Lock cap) of the appropriate length was inserted through the guide tube into the lateral brain ventricle. In order to collect parotid saliva, the aperture of one of the parotid ducts was surgically prepared, more than 6 months prior to the experiment, to exteriorize it via the cheek of the animal. Either an Atom Disposable Multiple Purpose Tube (o.d. 2.75 mm, 8 fr, Atom, Tokyo) approximately 10 cm in length or, depending on the animal, a fluid infusion tube (o.d. 4.00 mm, Terumo, Tokyo) was inserted into the parotid duct and fixed to the cheek. To enable the return of saliva collected from the parotid fistula, an extension tube (o.d. 4.50 mm, X3-50, Top, Tokyo) was inserted into the dorsal sac of the rumen. The other end of the tube was fixed to the skin. Parotid saliva flowing from the parotid fistula was collected in a plastic bucket. The goats were maintained in individual metabolism cages (length 2 m×width 1 m× height 2 m) that allowed for the separate collection of urine, feces and saliva. The laboratory room was maintained under thermoneutral conditions (24.9±0.1°C, 76.5±1.3% relative humidity).

The animals were fed twice a day at 10:30 h and again at 16:00 h for 2 h each time. Prior to the morning feeding, the collected saliva (3 to 5 kg) was infused into the rumen via the extension tube using a bathtub pump (Minipandy, KP-30F, Kosin, Tokyo). The physiological state of the goats used in this experiment did not change after parotid gland fistulation because the collected saliva was infused into the rumen every day (Sungawa et al., 2005). During the morning 2 h feeding period (10:30 to 12:30 h), the animals were fed 2 to 3 kg of roughly crushed (3×3×1 cm) alfalfa hay cubes. At 16:00 h each day, the animals were fed again with 0.8 kg of alfalfa hay cubes, 200 g of commercial ground concentrate and 20 g of sodium bicarbonate (Table 1). A more than adequate supply of water (8 to 10 L) was also provided twice a day during the feeding periods.

**Table 1. Chemical composition of alfalfa hay cubes and ground concentrate feed**

<table>
<thead>
<tr>
<th></th>
<th>Alfalfa hay cubes</th>
<th>Ground concentrate feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (%)</td>
<td>84.3</td>
<td>86.9</td>
</tr>
<tr>
<td>Chemical composition (% of DM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude protein</td>
<td>18.7</td>
<td>13.4</td>
</tr>
<tr>
<td>Crude fat</td>
<td>2.4</td>
<td>3.6</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>29.7</td>
<td>3.7</td>
</tr>
<tr>
<td>Nitrogen-free extracts (NFE)</td>
<td>39.7</td>
<td>71.0</td>
</tr>
<tr>
<td>NDF</td>
<td>45.9</td>
<td>14.6</td>
</tr>
<tr>
<td>ADF</td>
<td>36.6</td>
<td>5.4</td>
</tr>
<tr>
<td>Na</td>
<td>0.10</td>
<td>0.25</td>
</tr>
<tr>
<td>K</td>
<td>2.39</td>
<td>0.71</td>
</tr>
<tr>
<td>Cl</td>
<td>0.47</td>
<td>0.31</td>
</tr>
<tr>
<td>Ca</td>
<td>1.40</td>
<td>0.78</td>
</tr>
<tr>
<td>Mg</td>
<td>0.29</td>
<td>0.25</td>
</tr>
<tr>
<td>P</td>
<td>0.23</td>
<td>0.48</td>
</tr>
</tbody>
</table>

DM: dry matter, NDF: neutral detergent fiber, ADF: acid detergent fiber.

**Table 2. Mineral and glucose concentrations in plasma and cerebrospinal fluid (CSF)**

<table>
<thead>
<tr>
<th></th>
<th>CSF</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na (mmol/L)</td>
<td>151.9±1.01</td>
<td>146.5±0.73**</td>
</tr>
<tr>
<td>K (mmol/L)</td>
<td>3.3±0.55</td>
<td>4.8±0.12**</td>
</tr>
<tr>
<td>Cl (mmol/L)</td>
<td>110.9±0.39</td>
<td>111.0±0.71</td>
</tr>
<tr>
<td>P (mg/dl)</td>
<td>1.4±0.16</td>
<td>5.1±1.02**</td>
</tr>
<tr>
<td>Mg (mg/dl)</td>
<td>2.7±0.09</td>
<td>2.7±0.07</td>
</tr>
<tr>
<td>Ca (mg/dl)</td>
<td>6.3±0.22</td>
<td>9.5±0.10**</td>
</tr>
<tr>
<td>Glucose(mg/dl)</td>
<td>50.6±1.94</td>
<td>62.4±0.94**</td>
</tr>
</tbody>
</table>

Significant differences between CSF and plasma are indicated by **p<0.01.

Glucose(mg/dl) 50.6±1.94
K (mmol/L) 6.3±0.22
Mg (mmol/L) 2.7±0.09
Ca (mg/dl) 6.3±0.22
Glucose(mg/dl) 50.6±1.94

Alfalfa hay cubes
Concentrate feed
DM: dry matter, NDF: neutral detergent fiber, ADF: acid detergent fiber.
The alfalfa hay cubes (2.0 to 3.0 kg) were placed in a feed box attached to a 6 kg measuring scale. The weight of the remaining feed was measured every 10 min for the 2 h duration of feeding. Water intake was measured for 30 min following the completion of the 2 h feeding period. Fluid intake is regulated by thirst mechanisms (Guyton and Hall, 1996; Prasetiyono et al., 2000) and therefore the level of thirst (water appetite) in this experiment was evaluated quantitatively by water intake. Blood samples (5 ml) were collected through the polyethylene cannula into heparinized tubes at 09.30, 10.30, 10.45, 11.00, 11.15, 11.30, 12.00, 12.30, 13.00 and 13.15 h. Blood plasma was obtained by centrifugation (16,260×g, 10 min, 4°C).

Saliva secretion rates of parotid gland fistulated goats were measured every 10 minutes from prior to the commencement of feeding until the completion of the feeding period. The parotid saliva was collected in a measuring cylinder from a tube attached to the unilateral parotid gland fistula. Each sample of the collected saliva was transferred to a test tube and stored on ice until it could be analyzed.

All surgical and experimental procedures were approved by the Animal Experimental Ethics Committee of the University of the Ryukyus and were in compliance with the Japanese code of practice for the care and use of animals for scientific purposes.

**Chemical analysis**

The chemical composition of the alfalfa hay cubes and ground concentrate feed is indicated in Table 1. Alfalfa hay cubes were ground with a Wiley mill (Type 40-525P, Ikemoto Rika Kougyou, Tokyo, Japan). The chemical components of the feeds were quantified using the procedures described by the Japanese Feed Association (Kato, 1988).

Blood samples were placed in a hematocrit centrifuge (HC-12A, Tomy Seiko, Tokyo, 5 min, 16,260×g) to separate plasma and red blood cells. A hematocrit reader was used to determine hematocrit. Plasma total protein concentration and osmolality were measured using a refractometer (Atago, Tokyo) and an osmometer (OM-6010, Kyoto Daichi Kagaku, Kyoto), respectively. The plasma concentrations of Na, K and Cl were measured using Spotchem EL (SE-1520, Arklay, Kyoto). The plasma and cerebrospinal fluid concentrations of P, Mg, Ca and glucose were measured using Spotchem EZ (SP-4430, Arklay, Kyoto). Saliva osmolality was measured using the same method as that for plasma osmolality.

**Statistical analysis**

The experiments were conducted according to a Latin square design. A two-way analysis (animal, treatment) of variance was performed. After this, a t test was used to
compare treatments. For statistical analysis, GLM procedures (SAS, 1990) were adopted. Data are presented as the means±SE of five goats.

RESULTS

Feed intake

Figure 1 shows the effects of ICV infusion of SRIF on cumulative feed intake. The cumulative feed intake of both treatments upon the conclusion of the 2 h feeding period was ACSF: 972±61 g/2 h and SRIF: 1,404±44 g/2 h. Compared with the ACSF treatment, cumulative feed intake in the SRIF treatment was 44% larger (p<0.05).

Following the conclusion of the 2 h feeding period, the animals were given access to drinking water for 30 min. Water intake in this experiment was indicative of thirst levels. Thirst level in the SRIF infusion (4,920±1,020 g/30 min) was not significantly different from that in the ACSF infusion (4,700±690 g/30 min).

Humoral parameters

Figure 2 shows the effects of ICV infusion of SRIF on plasma osmolality, plasma total protein concentration and hematocrit in the blood sampled at 60 min before feeding.
and 0, 15, 30, 45, 60, 90, 120, 150, 165 min after feeding had commenced. Plasma osmolality slowly increased in both infusion treatments over the course of the 2 h feeding period. Compared with the ACSF infusion treatment, plasma osmolality of the SRIF infusion treatment decreased significantly (p<0.05) in the first half of the feeding period. However, in the latter half of the feeding period, there was no significant difference. Plasma total protein concentrations and hematocrit in the SRIF infusion treatment did not significantly differ from those in the ACSF infusion treatment during the 2 h feeding period.

**Plasma mineral concentrations**

Figure 3 shows the effects of ICV infusion of SRIF on plasma concentrations of Na, K and Cl. Plasma concentrations of Na, K and Cl before and during the initial stages of feeding in the SRIF infusion treatment were lower than those in the ACSF infusion treatment. Plasma Mg concentrations in the SRIF infusion treatment were lower than those in the ACSF infusion treatment over the 2 h feeding period, while plasma Ca concentrations in the SRIF infusion treatment were not different from those in the ACSF infusion treatment.

**Parotid saliva secretion volumes**

The cumulative saliva volumes secreted from unilateral parotid gland during the 2 h feeding period are shown in Figure 5. The cumulative saliva secretion volumes in the SRIF infusion treatment (548±64.8 ml/60 min) were significantly (p<0.01) higher than those in the ACSF infusion treatment (375±9.3 ml/60 min) during the 2 h feeding period.

**Mineral and glucose concentrations in plasma and cerebrospinal fluid**

Table 2 shows mineral and glucose concentrations in plasma and cerebrospinal fluid before feeding. Na concentration in CSF was higher (p<0.01) than that in plasma. However, K, P, Ca and glucose concentrations in CSF were lower (p<0.01) than those in plasma. Cl and Mg concentrations in CSF were not significantly different from those in plasma.

**DISCUSSION**

In countries such as Japan where ruminants are raised in...
ruminants, most farmers feed their stock a diet of dry forage twice a day. Dry forage has very low water content, is bulky, and has low energy levels. Because of these factors, ruminants raised on dry forage eat large volumes in order to satisfy their nutritional requirements for maintenance and production levels. During the initial stages of dry forage feeding in large-type goats, copious amounts of saliva are secreted (Sunagawa et al., 2003). Ruminant saliva is mainly composed of water and NaHCO₃. Therefore, during the initial stages of dry forage feeding, circulating plasma volumes and plasma pH decrease in ruminants (Blair-West and Brook, 1969; Sásaki et al., 1975). In the present experiment, a decrease in plasma volume estimated by increases in hematocrit and plasma total protein concentrations was apparent within 15 min of the commencement of feeding (Figure 2).

When the circulating plasma volume decreases during dry forage feeding, dehydration or hemorrhage, Angiotensin II (ANG II) is produced in the blood through the activation of the renin-angiotensin system, and vasopressin (AVP) is released (Mann et al., 1980; Fisher and Brown, 1984; Cameron et al., 1986; Mathai et al., 1997). Blair-West and Brook (1969) found that sheep fed lucerne chaff once a day showed a marked reduction in plasma volume within 15 min of the commencement of feeding and an increased plasma renin concentration.

Intraperitoneally injected vasopressin decreased feed intake in sheep (Meyer et al., 1989) and intravenous injection of ANG II suppressed parotid saliva secretion (McKinley et al., 1979). ICV infused ANG II also decreased intake of alfalfa chaff in sheep (Sunagawa et al., 2001a).

Feifel and Vaccarino (1990) reported that ICV infusion of 0.4-40 pmol of somatostatin in rats increased feed intake while a 3 nmol infusion decreased it. Danguir (1988) reported that feed intake in rats was increased by prolonged ICV infusion of the SRIF analogue SMS 201-995 at a small dose of 5 µg/50 µl/day over 3 consecutive days. Mid-range doses (0.31 nmol) of SRIF or SMS 201-995 produced no effect on feed intake (Shibasaki et al., 1988; 1998). Sunagawa et al. (2001a) showed that a continuous ICV infusion of somatostatin 1-28 at a rate of 5 µg/0.2 ml/h to sheep fed once a day on alfalfa chaff that had been trained to use a pedal system in order to access drinking water and a 0.5 M NaCl solution. They reported that feed intake markedly increased after infusion. The mechanism behind this response however, is unclear.

In the present experiment, eating rates were recorded every 10 minutes and blood samples were collected periodically. Cumulative feed intake for the 2 h feeding period in the SRIF treatment was significantly (p<0.05) larger than that in the ACSF treatment (Figure 1). Cumulative parotid saliva secretion volumes for the 2 h feeding period in the SRIF treatment were also larger (p<0.05) than that in the ACSF treatment (Figure 5). Plasma osmolality both prior to and during feeding in the SRIF treatment of the present experiment tended to be significantly lower than the ACSF treatment (Figure 2). This was due to a reduction in plasma Na, K, Cl, and Mg concentrations brought about by an ICV infusion of somatostatin (Figures 3 and 4). Thirst is a subjective perception that provides the urge for humans and animals to drink fluids (McKinley and Johnson, 2004). The desire to drink, that is, is completely satisfied only when plasma osmolality or blood volume returns to normal. Prasetyono et al. (2000) reported that thirst levels were measured quantitatively using water intake. The sensation of thirst is produced in the brain as a result of the integration of neuronal and humoral information (Fitzsimons, 1979). Neuronal information is transported via the autonomic nerves (especially the vagus) from chemoreceptors in the internal visceral organs. A broad range of internal humoral information is transported via the blood and cerebrospinal fluid. Increased extracellular fluid osmolality, decreases in extracellular fluid volume, the production of angiotensin II and dryness of the mouth stimulate the sensation of thirst (Guyton and Hall, 1996). While there was no significant difference in water intake (thirst levels) between the SRIF and ACSF treatments upon conclusion of the 2 h feeding period, based on osmolality results it is thought that hay feeding-induced thirst level increases in the first half of the feeding period were reduced. It is suggested that the reason behind the somatostatin ICV infusion-induced increases in feed intake and saliva secretion were a reduction in plasma osmolality brought about by somatostatin infusion.

Because of the copious amounts of saliva secreted by large-type goats during the initial stages of dry forage feeding, circulating plasma volumes decrease (Sunagawa et al., 2003). Blair-West and Brook (1969) reported that circulating plasma volumes decreased, plasma renin concentrations increased and urine excretion decreased during the initial stages of feeding in sheep fed on dry alfalfa chaff. In the ACSF treatment of the present experiment, despite a continuous ICV infusion of ACSF, a decrease in plasma volume estimated by increases in hematocrit and plasma total protein concentrations was apparent within 15 min of the commencement of feeding (Figure 2). Because of this, it is thought that the renin-angiotensin system was activated in goats fed on roughly crushed alfalfa hay cubes. Sunagawa et al. (2001b) reported that in sheep fed once a day on dry alfalfa chaff, an ICV infusion of ANG II induced thirst sensations and reduced feed intake. The intravenous injection of ANG II suppressed parotid saliva secretion (McKinley et al., 1979). Weisinger et al. (1991) reported however, that the increases in Na appetite and aldosterone secretion caused by ICV infusions of ANG II were prevented by SRIF. Wang et al. (1987a;
1987b) reported that ICV infusion of SRIF inhibited vasopressin secretion during haemorrhage in sheep. These reports suggest that ICV infused SRIF inhibits the release of vasopressin and ANG II production usually brought about by dry forage consumption. Wang et al. (1987a; 1987b) reported that ICV infusion of SRIF inhibited haemorrhage-induced ACTH and vasopressin release. From these reports and the results of the present experiment, it is surmised that the significant decrease in feed intake during the initial stages of feeding in large-type goats fed on roughly crushed alfalfa hay cubes was due to the action of peptides controlling thirst sensation.

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