The Role of Brain Somatostatin in the Central Regulation of Feed, Water and Salt Intake in Sheep

Katsunori Sunagawa*, Richard S.Weisinger, Michael J. McKinley, Brett S. Purcell, Craig Thomson and Peta L. Burns

Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne
Parkville, Victoria 3052, Australia

ABSTRACT : The physiological role of brain somatostatin in the central regulation of feed intake in sheep was investigated through a continuous intracerebroventricular (ICV) infusion of somatostastin 1-28 (SRIF) at a small dose of 5 μg/0.2 ml/hr for 98.5 hours from day 1 to day 5. Sheep (n=5) were fed for 2 hours once a day, and water and 0.5 M NaCl solution were given *ad libitum* . Feed, water and salt intake were measured during ICV infusion of artificial cerebrospinal fluid (CSF) and SRIF. The feed intake during SRIF infusion on days 2 to 5 increased significantly compared to that during CSF infusion. Water intake, when compared to that during CSF infusion, only increased significantly on day 4. NaCl intake during SRIF infusion was not different from that during CSF infusion. Mean arterial blood pressure (MAP) and heart rate during SRIF infusion were not different from those during CSF infusion. The plasma concentrations of Na, K, Cl, osmolality and total protein during SRIF infusion were also not different from those values during CSF infusion.There are two possible mechanisms, that is, the suppression of brain SRIF on feed suppressing hormones and the direct actions on brain mechanisms controlling feed intake, explaining how SRIF works in the brain to bring about increases in feed intake in sheep fed on hay. The results indicate that brain SRIF increases feed intake in sheep fed on hay. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 7 : 929-934)*

Key Words : Somatostatin, Brain, Dry Forage Intake, Sheep

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). Subsequent investigation in rats revealed that the weak, growth hormone releasing factor (GRF)-induced GH response observed during trough periods of the pulsatile GH secretion rhythm is due to antagonization by endogenous circulating SRIF (Plotsky and Vale, 1985., Tannenbaum and Ling, 1985). Thus, it is thought that SRIF works in close concert with GRF in the hypothalamus to produce the pulsatile rhythm of GH release from the pituitary (Plotsky and Vale, 1985; Tannenbaum and Ling, 1985).

The effects of brain SRIF on the feeding behavior in rats have been investigated. Vijayan and McCann (1977) found that ICV injections of SRIF (3 nmol) decreased feed intake. No effect on feed intake following ICV injections of SRIF (0.31 nmole) was reported by either Lotter et al. (1981) or by Levine and Morey (1982). Feifel and Vaccarino (1990) reported that low pmol doses (0.4-40) ICV administered during the light photoperiod increased feed intake whereas 3 nmol decreased it. None of the doses tested during the dark photoperiod significantly altered feed intake in rats. As indicated by the above reports, the effect of ICV SRIF injection on feed intake is equivocal.

While saliva secretion increased markedly after the start of feeding in sheep fed on alfalfa hay cubes, it drcreased to its lowest level after 30 mins of the feeding period had elapsed (Sato, 1975). Prasetiyono et al. (2000) reported that the rate of eating decreased sharply after the first 30 min of feeding in goats fed on alfalfa hay cubes. They indicated that the reason for the decreased eating rate was hypovolemia (decrease in plasma volume) that occurred after dry feed had been ingested. The hypovolemia was caused by fluid moving from the circulation into the saliva (Sunagawa et al., 2001). When the circulating plasma volume decreases during 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., 1997; Fisher and Brown, 1984; Cameron et al., 1986; Mathai et al., 1997). Wang et al. (1987) reported that ICV infusion of SRIF inhibited vasopressin secretion during haemorrhage in sheep. The intravenous injected vasopressin decreased feed intake in sheep (Meyer et al., 1989). The ICV infused ANG II also decreased intake of alfalfa chaff in sheep (Sunagawa et al., 2001a). From these reports, ICV infused SRIF may inhibit vasopressin release and ANG II production usally brought about by dry feed consumption. This may result in

* Address reprint request to Katsunori Sunagawa, Dept. of Animal Physiology, Faculty of Agriculture, University of the Ryukyus, 1 Senbaru, Nishihara-cho, Okinawa 903-0213, Japan. Tel: +81-98-895-8798, Fax: +81-98-895-8734, E-mail: b986094@agr.u-ryukyu.ac.jp.

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an increase in feed intake in sheep. However, there are no reports as to the effect of brain SRIF on feed intake in ruminants.

Although the secretion pattern of SRIF into the CSF has never been investigated, 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). From these reports, a direct action of SRIF on brain mechanisms controlling feed intake can also be expected in ruminants.

The aim of the present research was to investigate the role of brain SRIF in the central regulation of feed intake in sheep. We gave a continuous ICV infusion of the peptide at a small dose of 5 μg/0.2 ml/hr for 98.5 hours from day 1 to day 5.

MATERIALS AND METHODS

Animals

Five crossbred Merino ewes, 35-48 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 Institute's Animal Experimentation Ethics Commitee, 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 faeces. In addition, the cages contained two pedals. The animals were trained to press the left pedal to receive 25 ml of 0.5 M NaCl (=12.5 mmol Na) and the right pedal to receive 50 ml of water. All deliveries were consumed. The number of deliveries were counted and recorded continuously by computer.

We examined the effects of ICV SRIF infusion in sheep adapted to a 2 hour, once a day feeding scheme. The sheep were offered a 1.5 kg daily ration of dried alfalfa chaff (Na^+ 90-100 mmol, K^+ 250-400 mmol) once a day (12:00 to 14:00) before and during the experiment (RucKebusch and Malbert, 1986; Spina et al., 1996).

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. The probe was connected via a polyethylene cannula to a 10 ml syringe held in an infusion pump (Perfusor, Braun, Germany). Infusion experiment was of 98.5 hours in duration (0.2 ml/h), during which a continuous ICV infusion of the peptide was given at a dose of 5 μg/0.2 ml/h. The infusate used, Somatostatin 1-28 (3149 MW, Auspep, Australia), was dissolved in artificial cerebrospinal fluid (CSF: 151 mM Na^+, 157.5 mM Cl^-, 2.8 mM K^+, 1.1 mM Ca^{2+}, 0.9 mM $Mg^{2+}$and 0.5 mM HPO_4^-). All animals received a control infusion of artificial CSF (0.2 ml/h). The dose of ICV SRIF infusion in the present experiment was equal to the ICV infused Corticotropin releasing factor (CRF) dose which had earlier resulted in decreased feed intake in sheep (Sunagawa et al., 2000a).

Blood pressure measurements and blood sampling

Mean arterial blood pressure was measured from the carotid artery via a heparin-saline filled 18 gauge needle and polyethylene tube connected up to a COBE disposable transducer model 345-931-009. The pressure recording system was a JRACK (Australia) rack type RK8 pressure amplifier with a GRAPHTEC Thermal Arraycorder model WR7700. Blood samples were taken via this cannula from a carotid artery.

Experimental design

Firstly, CSF was continuously infused into the lateral ventricle of five sheep for 98.5 hr. Secondly, the same five sheep were given a continuous ICV infusion of SRIF at 5 μg/hr for 98.5 hr. All infusions were at a rate of 0.2 ml/hr. The infusion started at 11:30 on day 1 and ended at 14:00 on day 5. Blood pressure was measured at 10:00 a.m. each day and a 10-ml blood sample was taken after that. Feed, water and sodium chloride intake were also measured daily. All feed intake data were expressed on a dry matter basis. In the text, baseline is prior to infusion. Sheep were weighed once a week. Typically, there was a minimum of 9 days between CSF infusion experiment and SRIF infusion experiment in each sheep.

Chemical analysis

Plasma Na^+, K^+, Cl^-, osmolality 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 by 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:

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
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. DCP and TDN were calculated using the digestibilities of crude protein, crude fat, crude fiber and NEF (71.0±2.05, 45.0±2.75, 49.0±0.70, 71.0±0.455 %).
* Values are means±S.E. from five determinations.

digestibility (%)=(dry matter intake-feacal output)/dry matter intake×100 %.

Statistical analysis

A one-way classification and subsequent Duncan's Multiple Range Tests were used to compare the data during ICV CSF infusion and ICV SRIF infusion. For statistical analysis, GLM procedures (SAS, 1990) were adopted. Data are presented as the means±S.E. of five sheep.

RESULTS

The results of feed, water and salt intake are shown in table 2. Feed intake during SRIF infusion on days 2 to 5 increased significantly ($p<0.01$) compared to that during CSF infusion. Water intake, when compared to that during CSF infusion, only increased significantly ($p<0.05$) on day 4. NaCl intake during SRIF infusion was not different from that during CSF infusion.

In this experiment, blood samples were taken, and mean carotid arterial blood pressure (MAP) and heart rate were measured prior to feeding. The plasma concentrations of Na, K, Cl, osmolality and total protein are shown in figures 1 and 2. These concentration values during SRIF infusion (143.5±0.2, 4.3±0.04, 111.1±0.3 mmol/l, 290.5±0.3 mOsm/l, 58.5±0.5 g/l) were the same as those values during CSF infusion (144.1±0.1, 4.4±0.01, 111.3±0.3 mmol/l, 292.1±0.5 mOsm/l, 61.0±0.4 g/l). MAP and heart rate are shown in figure 1. MAP and heart rate during SRIF infusion (63±0.7 mmHg, 67±0.8 beats/min) were not different from those during CSF infusion (69±0.5 mmHg, 64±1.0 beats/min). That is, ICV infusion of SRIF had no effect on the sympathetic nervous system and the plasma electrolyte concentrations.The body weight of the sheep following the conclusion of ICV SRIF infusion (41.5±2.6 kg) was relatively unchanged compared to pre-infusion body weight (39.8±3.2 kg).

DISCUSSION

To clarify the biological activities of the peptides in small animals, most studies have employed intravenous (IV) or ICV bolus injections of the peptide at a dose larger than the endogenous release. However, this method has not been successful in clarifying the biological activity of endogenous peptides in large animals due to the large size of the lateral ventricle and the rapid flow rate of cerebrospinal fluid (Okita et al., 1998). In order to elucidate the effect of endogenous brain SRIF on brain mechanisms controlling feed, water and salt intake in sheep, we gave a continuous ICV infusion of the peptide at a small dose for long periods. The influence of ICV SRIF infusion on MAP and heart rate was not observed in this experiment (figure 1). All animals remained calm during ICV SRIF infusion. Therefore, these results indicate that the continuous ICV infusion of SRIF at a small dose for long periods had no stressful effects on sheep.

The important finding of this experiment was the fact that continuous ICV infusion of small doses of SRIF increased feed intake during the allotted 2 hour feeding period in sheep.

ICV injection of large doses of SRIF (3 nmol) decreased

Table 2. Effect of ICV infusion of CSF and SRIF on feed, water and salt intake in sheep fed on alfalfa chaff*

Parameter	Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
Feed intake	CSF	$733±60^{Aa}$	$684±70^{Aa}$	$697±64^{Aa}$	$718±38^{Aa}$	$675±36^{Aa}$
(g/2 h)	SRIF	$808±84^{Aa}$	$951±80^{Bbc}$	$894±50^{Bac}$	$978±47^{Bbc}$	$945±65^{Bbc}$
Water intake	CSF	$1,883±142^{Aa}$	$1,751±178^{Aa}$	$1,936±127^{Aa}$	$1,948±256^{Aa}$	$1,909±101^{Aa}$
(ml/day)	SRIF	$2,219±325^{Aab}$	$2,314±407^{Aab}$	$2,742±339^{Aa}$	$2,659±283^{Ba}$	$2,044±204^{Ab}$
NaCl intake	CSF	$586±68^{Aa}$	$636±69^{Aa}$	$631±102^{Aa}$	$681±112^{Aa}$	$501±116^{Aa}$
(ml/day)	SRIF	$537±39^{Aa}$	$541±38^{Aa}$	$657±113^{Aa}$	$436±85^{Aa}$	$338±44^{Aa}$

* Values are means±S.E. of 5 sheep.
$^{a,b,c\ A,B}$ Values having same letters in the same row and column are not significantly different ($p>0.05$). Large and small letters indicated infusion treatment and day effect, respectively.

feed intake in rats (Vijayan and McCann, 1977), while ICV injection of small doses of SRIF (0.4-40 pmol) during the light period increased feed intake (Feifel and Vaccarino, 1990). 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 concecutive days. Mid-range doses (0.31 nmol) of SRIF or SMS 201-995 produced no effect on feed intake (Shibasaki et al., 1988; Shibasaki et al., 1998). It would appear that there is some relationship between ICV injected doses and changes in feed intake. Although the type of animal and the administration methods of ICV SRIF in this experiment are different from those in the above mentioned reports, it is thought that brain SRIF increases feed intake in sheep fed on dry feed.

It is thought that there are two possible mechanisms explaining how SRIF works in the brain to bring about increases in feed intake in sheep. Firstly, it is thought that the feed intake suppression actions of vasopressin and angiotensin II may have been depressed through the ICV infusion of SRIF and thus feed intake was greater than that during ICV CSF infusion. The sheep, during ICV CSF infusion, continued eating for approximately 40 mins following the start of the 2 hour feeding period. After 40 mins had elapsed, the sheep began drinking (Sunagawa et al., 2001a). Due to this feeding system, plasma osmolality and CSF osmolality increased with feeding (Sunagawa et al., 2001b). The eating rate markedly decreased after approximately 40 mins of the feeding period had elapsed (Sunagawa et al., 2001a). In this experiment, during CSF infusion, eating rates for the first, second, third and fourth 30 min periods of the 2 hour feeding period were 365, 148, 130 and 77 g/30 min, respectively. During SRIF infusion, these rates were 538, 272, 182, 156 g/30 mins, respectively. In this experiment, blood samples were taken prior to feeding. Because the changes in plsama osmolality, Na, K, Cl and total protein concentration during feeding returned to pre-feeding levels within 12 hours, we were unable to

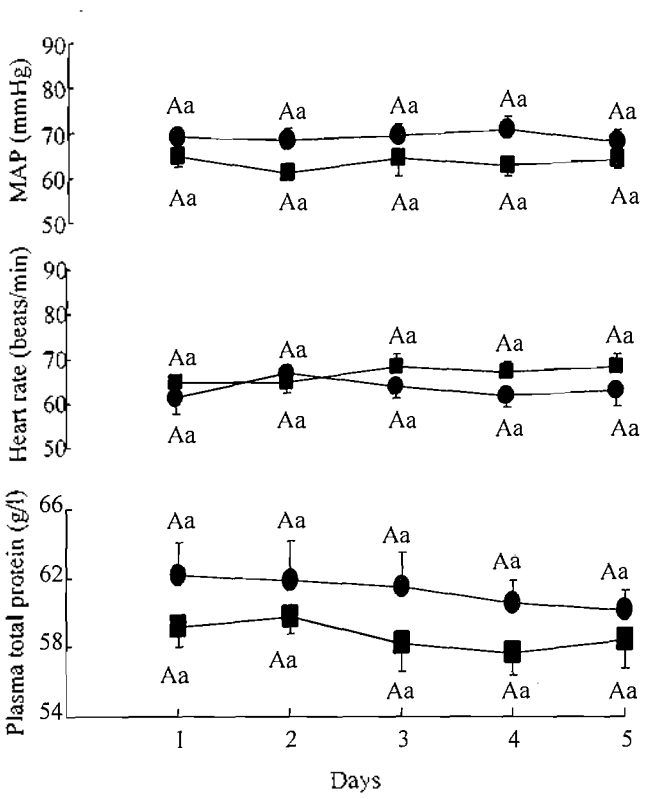

Figure 1. Effects of ICV infusion of CSF (0.2 ml/h,●) and SRIF (5 μg/0.2 ml/h,■) on MAP, heart rate and plasma total protein concentration. 1-5 represent the number of days of ICV CSF or SRIF infusion, respectively. Each point represents the means±S.E. of 5 sheep. The large letters are a comparison between CSF and SRIF within each day. The small letters are a comparison among days within each treatment. Means with different letters are significantly different ($p<0.05$).

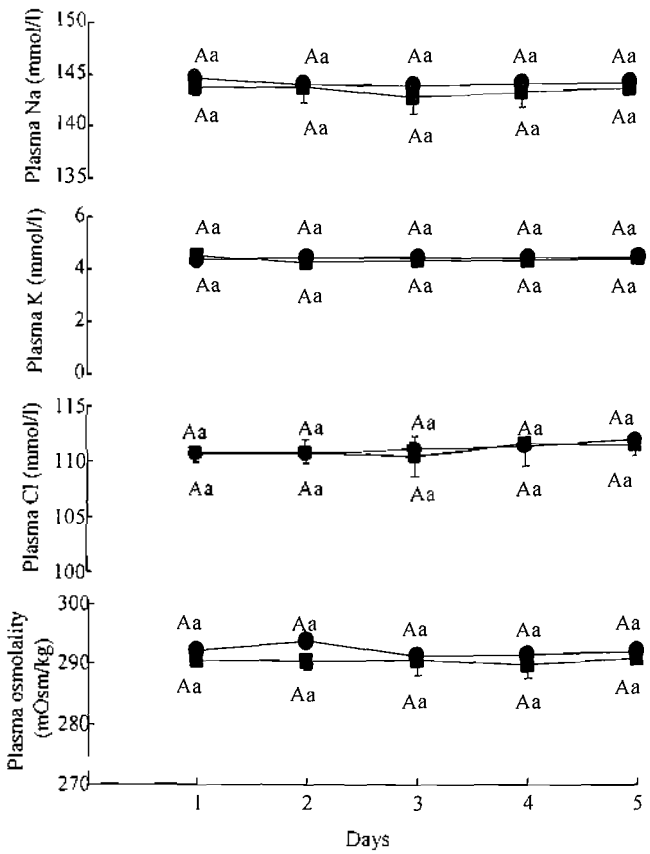

Figure 2. Effects of ICV infusion of CSF (0.2 ml/hr, ●) and SRIF (5 μg/0.2 ml/h, ■) on plasma concentrations of Na, K, Cl and osmolality. 1-5 represent the number of days of ICV CSF or SRIF infusion, respectively. Each point represents the means±S.E. of 5 sheep. The large letters are a comparison between CSF and SRIF within each day. The small letters are a comparison among days within each treatment. Means with different letters are significantly different ($p<0.05$).

observe the changes (McKinley et al., 1994). Sato (1975) reported that using the same feeding system circulating plasma volume decreased to its lowest level after 30 mins of the feeding period had elapsed, and then gradually recovered. Vasopressin is released and ANG II is produced in the blood when circulating plasma volume decreases and plasma osmolality increases in rats and sheep (Stacy and Brook, 1965; Blair-West and Brook, 1969; Mathai et al., 1997). Intraperitoneal injection of vasopressin decreased feed intake in goats (Meyer et al., 1989). ICV infusion of ANG II decreased feed intake in sheep (Sunagawa et al., 2001a). It has also been reported that ICV SRIF injection inhibits vasopressin and ACTH secretion during haemorrhage (Wang et al., 1987a; Wang et al., 1987b). From these reports, ICV infused SRIF in this experiment may have inhibited vasopressin release and ANG II production usually brought about by dry feed consumption, and thus feed intake increased. Secondly, it is possible that SRIF worked directly on feeding centers in the brain causing increases in feed intake. This possibility can not be excluded at the present time. However, the response to ICV SRIF injection on feed intake is not stoichiometric. Therefore, it is difficult to consider that brain SRIF acts directly on feeding centers to increase feed intake.

The results indicate that brain SRIF increases feed intake in sheep fed on hay. However, the way brain SRIF acts on brain mechanisms controlling feed intake remains unknown. Further study is required in this area. The effect of brain SRIF on Na appetite has not been reported. From the results, it is concluded that brain SRIF is not involved in the controlling mechanism for Na intake in sheep.

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