# The Role of Neuropeptide Y in the Central Regulation of Grass Intake in Sheep

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**ABSTRACT :** The physiological role of brain neuropeptide Y (NPY) in the central regulation of grass intake in sheep was investigated through a continuous intracerebroventricular (ICV) infusion of NPY at a dose of 5 $\mu$g/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 intake during ICV NPY infusion increased significantly compared to that during ICV artificial cerebrospinal fluid (CSF) infusion. Water and NaCl intake during ICV NPY infusion remained unchanged. Mean arterial blood pressure (MAP) and plasma osmolality during ICV NPY infusion were not significantly different from those during ICV CSF infusion. On the other hand, plasma glucose concentration during ICV NPY infusion increased significantly compared to that during ICV CSF infusion. The results suggest that brain NPY acts as a hunger factor in brain mechanisms controlling feeding to increase grass intake in sheep. (*Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 1 : 35-40*)

**Key Words :** NPY, Brain, Grass Intake, Sheep

## INTRODUCTION

The sensations of hunger and satiety are produced in the brain as a result of the integration of neuronal and humoral information (Nijima, 1969; Schmit, 1973; 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. A number of neurotransmitters are involved in the forming process of these sensations (Baile et al., 1974; Shimizu et al., 1987; Fujise et al., 1993). Despite the focus on the actions of neurotransmission and neuromodulation in neuropeptides, many of the physiological roles in brain mechanisms controlling feed intake are not known.

Neuropeptide Y (NPY) is a 36-amino acid neuropeptide that is synthesized in the hypothalamic arcuate nucleus (ARC) and released in the paraventricular nucleus (PVN) (Sawchenko and Polak, 1985; Morris, 1989). Food-deprived rats showed marked increases in NPY concentrations in the ARC, with some studies reporting several-fold increases within 48 hours of food withdrawal (Sahu et al., 1988; Beck et al., 1990). NPY levels also rise in the PVN, dorsomedial nucleus (DMH) and medial preoptic nucleus (MPO). Increases in regional NPY concentrations and NPY release within the PVN fall to normal levels after feeding (Sahu et al., 1988; Kalra et al., 1992). Lactating rats, which display striking hyperphagia, have increased NPY concentrations in the ARC, PVN, ventromedial nucleus hypothalamus (VMH) and DMH (Malabu et al., 1994). From these reports, it is thought that endogenous NPY is involved in the hypothalamic neural circuit that regulates energy balance.

A considerable amount of study has been done to establish the role of metabolic and physical factors in the control of forage intake in ruminants, and there is now adequate evidence that end-products of carbohydrate fermentation and distension of the rumen are involved in limiting feed intake (Campling and Balch, 1961; Montgomery et al., 1963; Ternouth, 1967; Bhattacharya and Warner, 1968; Baile and McLaughlin, 1970; Bergen, 1972; Anil et al., 1993). However, the physiological mechanisms regulating feed intake in ruminants are still largely unclear. In order to clarify these mechanisms, further research is necessary on the brain mechanisms controlling feed intake including how the brain processes peripheral information. ICV bolus injection of 10 $\mu$g of NPY in feed-satiated sheep increased feed intake of concentrate pellets, while an intravenous injection produced no effect (Miner, 1992). The response to the ICV bolus injection of NPY in sheep appeared inside 30 mins of injection, and was completed within 2-3 hours (Miner et al., 1989). There has been a number of studies in which ICV bolus injections of NPY have been used. Despite increases in feed intake, the injected doses were large and thus it is not known whether or not endogenous NPY is involved in the physiological mechanisms controlling feed intake in ruminants. Additionally, there are no reports concerning the effect

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of brain NPY on grass intake in ruminants.

The aim of the present research was to investigate the role of brain NPY in the central regulation of grass intake in sheep. We gave a continuous ICV infusion of the peptide at a very small dose of 5 $\mu$g /0.2 ml/hr for 98.5 hours from day 1 to day 5, and measured feed, water and salt intake.

## 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 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 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 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 ICV NPY infusion in sheep adapted to a 2 hour, once a day feeding period. The sheep were fed dried alfalfa chaff ($Na^+$ 90-100, $K^+$ 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 venricle. 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/hr). The infusate used, porcine Neuropeptide Y (4254 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.

### Blood pressure measurements and blood sampling

The mean arterial blood pressure was measured from the carotid artery via a heparin-saline filled 18 gauge needle and polyethylene tube hooked 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. The blood samples were taken via this cannula from a carotid artery.

### Experimental design

Firstly, feed, water and salt intake in the absence of ICV infusion were measured everyday for 5 days. Secondly, CSF was continuously infused into the lateral ventricle of five sheep for 98.5 hours. Thirdly, the same five sheep were given a continuous ICV infusion of Neuropeptide Y (NPY) at 5 $\mu$g/hr for 98.5 hours. 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. During the above infusions, blood pressure was measured at 10:00 on day 1, 2, 3, 4 and 5, and a 10 ml blood sample was subsequently taken. The feed, water and salt intake were also measured daily. Sheep were weighed once a week. Typically, there was a minimum of 9 days between infusions.

### Chemical analysis

The plasma glucose concentration was measured with a Beckman CX5 Clinical system. The plasma osmolality was measured with a Digimatic osmometer (Advanced Instrument, Denmark).

The alfalfa chaff was ground by Willey milling machine (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: the digestibility of feed (%)=(feed intake-feaces)/feed intake$\times$100.

**Table 1.** Chemical composition and nutritive values of alfalfa chaff

| | Alfalfa chaff |
|---|---|
| Dry matter (%) | 89.0±0.17 |
| Chemical cmposition (% 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, Values are means±SE from five determinations.

**Statistical analysis**

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

All feed intake data were expressed on a dry matter basis.

## RESULTS

The results of feed intake are shown in table 2. Feed intake during ICV CSF infusion was not significantly different from that during non-ICV infusion and was not influenced by the continuous 98.5 hour infusion. On the other hand, feed intake during ICV NPY infusion on days 3, 4 and 5 increased significantly compared to that during ICV CSF infusion. The results of water intake are shown in table 3. Water intake during ICV CSF infusion was not significantly different from that during non-ICV infusion. Water intake during ICV NPY infusion was also unchanged from that during ICV CSF infusion. The results of salt intake are shown in table 4. NaCl intake during ICV CSF infusion was not significantly different from that during non-ICV infusion. NaCl intake during NPY ICV infusion was also unchanged from that during ICV CSF infusion.

The results of MAP, plasma osmolality and plasma glucose concentration were shown in figure 1. MAP during ICV NPY infusion was the same as that during ICV CSF infusion. The plasma osmolality during ICV NPY infusion was also the same as that during ICV

**Table 2.** Effect of ICV infusion of CSF and NPY on feed intake (g/2 h) in sheep fed on alfalfa chaff

| | No infusion | CSF infusion | NPY infusion |
|---|---|---|---|
| Day 1 | 757±29[a] | 780±27[a] | 834±61[a] |
| Day 2 | 771±40[a] | 725±55[a] | 852±59[a] |
| Day 3 | 774±38[a] | 766±27[a] | 949±24[b] |
| Day 4 | 765±26[a] | 773±17[a] | 999±41[b] |
| Day 5 | 758±45[a] | 711±42[a] | 998±47[b] |

Values are means±SE of 5 sheep. Values not having same letters in the same row are significantly different ($p<0.01$).

**Table 3.** Effect of ICV infusion of CSF and NPY on water intake (ml/day) in sheep fed on alfalfa chaff

| | No infusion | CSF infusion | NPY infusion |
|---|---|---|---|
| Day 1 | 2255±245 | 1964±101 | 2149±460 |
| Day 2 | 2045±155 | 1832±165 | 2002±141 |
| Day 3 | 2025±210 | 2090±133 | 2471±207 |
| Day 4 | 1948± 73 | 2156±282 | 2214±184 |
| Day 5 | 2180±109 | 2005± 91 | 2258±237 |

Values are means±SE of 5 sheep.

**Table 4.** Effect of ICV infusion of CSF and NPY on NaCl intake (ml/day) in sheep fed on alfalfa chaff

| | No infusion | CSF infusion | NPY infusion |
|---|---|---|---|
| Day 1 | 680±121 | 665± 57 | 538± 71 |
| Day 2 | 731±120 | 581± 58 | 552± 93 |
| Day 3 | 501± 46 | 628±103 | 710±130 |
| Day 4 | 544± 62 | 806± 82 | 534± 61 |
| Day 5 | 543± 97 | 576± 71 | 657±100 |

Values are means±SE of 5 sheep.

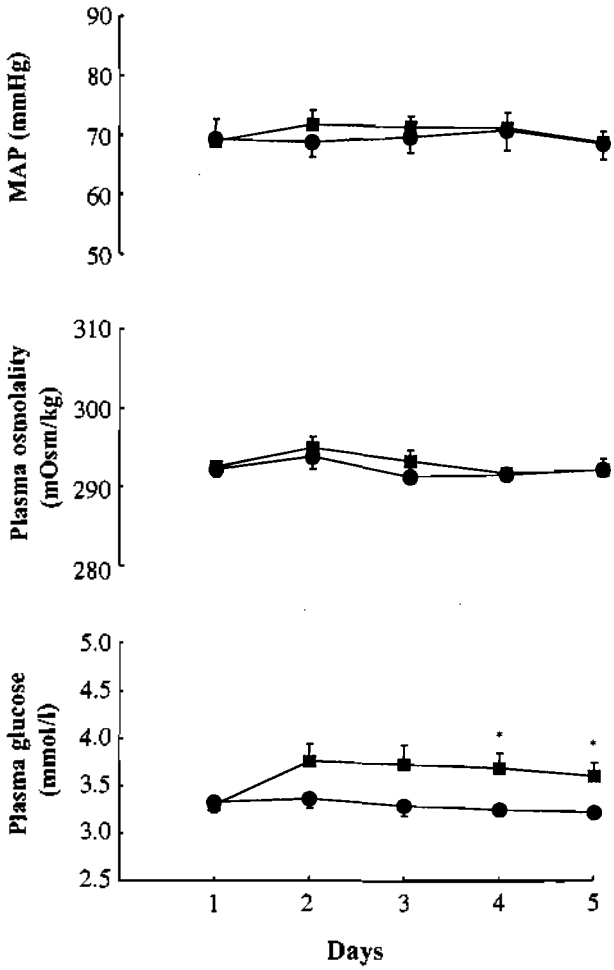


**Figure 1.** Effects of ICV infusion of CSF (0.2 ml/hr, ●) and NPY (5 μg/0.2 ml/hr, ■) on MAP, plasma osmolality and plasma glucose concentration. 1-5 represent the number of days of ICV CSF or NPY infusion, respectively. Each point represents the means ±SE of 5 sheep. Statistical analysis is descrived in the text : * $p<0.05$ (vs. CSF).

CSF infusion. On the other hand, plasma glucose concentration during ICV NPY infusion increased significantly compared to that during ICV CSF infusion.

The body weight of sheep after the completion of ICV NPY infusion (41.6±3.0 kg) was not significantly different from that prior to beginning the infusion (41.1±2.6 kg).

## DISCUSSION

To clarify the biological activities of the peptides in small animals, most studies have employed intravenous (IV) or ICV bolus injection of the peptide at a dose larger than the endogenous release. However, this method has not been successful in clarifying a 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 NPY 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 dose of ICV NPY 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., 2000). The influence of ICV NPY infusion on MAP was not observed in this experiment (figure 1). All animals remained calm during ICV NPY infusion. Therefore, these results indicate that the continuous ICV infusion of NPY at a small dose for long periods had no stressful effects on sheep.

It has been reported that conditions that induce major energy deficits, namely, food deprivation, lactation and diabetes increased NPY concentrations in the ARC and PVN of rats (Sahu et al., 1988; Beck et al., 1990; Brady et al., 1990; Kalra et al., 1992; Pelletier and Tong, 1992; Sahu et al., 1992; Malabu et al., 1994). ICV bolus injection of NPY specifically stimulated carbohydrate appetite in rats (Stanley et al., 1985). Three injections of ICV NPY per day for 10 days caused a dramatic increase in both daily feed intake and body weight in rats (Stanley et al., 1986). In this experiment, sheep were fed for 2 hours once a day, that is, sheep were deprived of feed for 22 hours of each day. During ICV CSF infusion on day 5, 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 ICV NPY infusion on day 5, eating rates for the first, second, third and fourth 30 min periods of the 2 hour feeding period were 463, 251, 186 and 130 g/30 min, respectively. These results indicate that ICV infused NPY increases the levels of hunger sensation in the brain of sheep. Therefore, endogenous brain NPY may act as a hunger factor in brain mechanisms controlling grass intake in sheep.

ICV bolus injection of NPY stimulated the intake of concentrate pellets in feed-satiated lambs (Miner et al., 1989). The response to the ICV bolus injection of NPY (10 $\mu$g) in sheep appeared inside 30 mins of injection, and was completed within 2-3 hours. ICV bolus injection of NPY in sheep did not, however, change total intake of feed or water for a 24-h period after injection (Miner et al., 1989). In this experiment, feed intake of alfalfa chaff during ICV NPY infusion on days 3, 4 and 5 increased significantly compared to that during ICV CSF infusion (table 2). Stanley et al. (1985) injected NPY into seven regions of the brain of rats and produced hyperphagia when they targeted the PVN or the lateral or ventromedial hypothalamic areas but not other brain regions. Sahu et al. (1997) reported that an early increase in NPY neuronal activity at the level of the ARC-PVN pathway was responsible for initiation of hyperphagia in experimental diabetes. From these reports, the results in this experiment suggest that brain NPY binds NPY receptors in feeding centers causing increased feed intake. The lack of change in feed intake during ICV NPY infusion on day 1 and day 2 in the present experiment was a result of the small dose employed.

The plasma osmolality during ICV NPY infusion was not different from that during ICV CSF infusion. On the other hand, plasma glucose concentration during ICV NPY infusion increased significantly compared to that during ICV CSF infusion (figure 1). Marks and Waite (1996) reported that ICV NPY injection acutely stimulated insulin and glucagon release, and reduced effects of insulin on glucose metabolism. The increased plasma glucose levels during ICV NPY infusion in this experiment might have been due to an increase in glucagon release. However, it is difficult to consider that the peripheral actions of these peptides increase feed intake. The reason for this is that the changes in plasma glucose concentration does not affect feed intake in ruminants (Baile and Mayer, 1969).

Generally, water intake follows feed intake. However, increased feed intake caused by ICV infusion of NPY on days 3 to 5 was not associated with increased water intake. This might have been due to a large variation of water intake among individuals. The effect of brain NPY on Na appetite has not been reported. NaCl intake during ICV NPY infusion remained unchanged in this experiment. Therefore, it is concluded that brain NPY is not involved in the controlling mechanisms for Na intake in sheep.

The present results suggest that brain NPY acts as a hunger factor in brain mechanisms controlling

feeding to increase grass intake in sheep.

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