EFFECT OF ABOMASAL INFUSION OF ALANINE AND ASPARTIC ACID ON GROWTH HORMONE SECRETION IN SHEEP

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Summary

Effects of amino acids infusion into the abomasum on plasma growth hormone (GH) concentration were investigated using three wethers of 54 kg of average body weight. Wethers were infused with either 3.25 mmol/kg BW/day of sodium chloride solution (control), 3 mmol/kg BW/day of alanine (Ala), or 3 mmol/kg BW/day of aspartic acid (Asp) continuously for five days through an abomasum catheter in a 3 × 3 Latin square desing. On the day of starting infusion (day 0) and day 4 blood samples were collected from a jugular vein every fifteen minutes for six hours after feeding, and their GH concentrations were measured. Blood samples were also collected immediately before starting infusion (day 0), and before feeding of day 1, day 2 and day 4, and their plasma free amino acid concentrations were measured. In the animals infused with Ala, plasma free Ala concentration was increased by Ala infusion and it continued for four days. Plasma GH concentration of these animals increased on day 0, but this phenomenon disappeared on day 4. In the animals infused with Asp, the increase in plasma Asp concentration was observed only on day 1. Plasma GH concentration of these animals was not affected by Asp infusion. These results suggest that continuous Ala infusion stimulates GH secretion for a short period, but the effect would not last long, and that continuous Asp infusion does not affect plasma GH concentration.
(Key Words: Alanine, Aspartic Acid, Abomasal Infusion, Growth Hormone, Sheep)

Introduction

It is reported that a GH administration promotes weight gain in growing calves (Wagner et al., 1988) and lambs (Johnsson, 1985), and increases milk production in milking cows (McGuire et al., 1992). The practical use of GH in animal production, however, is prohibited because there is a possibility that the administered GH remains in animal products. Kuhara et al. (1991) intravenously infused seventeen amino acids in sheep at a dose of 3 mmol/kg BW in thirty minutes, and found that some amino acids stimulate GH secretion. They also indicated that the effect of Asp is stronger than that of arginine (Arg), which had been recognized to stimulate GH secretion, and that the effect of Ala on GH secretion is compa rable to Arg. Therefore, there is a possibility that intake of a great amount of amino acids stimulates GH secretion in animals. By an experiment of continuous abomasal infusion of Arg, ornithine (Orn) and urea for seven days, Davenport et al. (1990) found that both Arg and Orn increase GH secretion in wether lambs. However, Kuhara et al. (1992) infused amino acids into the duodenum of sheep to find that the duodenal infusion of Asp, which was the most effective in increasing GH secretion by intravenous infusion (Kuhara et al., 1991), does not affect GH secretion. They suggested that the Asp infused into the duodenum might have been converted to other metabolites by the intestinal epithelium or might have been consumed by bacteria in the small intestine before being absorbed. However, they were unable to determine whether or not Asp was really absorbed, and why the Asp infusion did not increase GH secretion. It was because plasma amino acid concentrations were not assayed in their study. In the present study, sheep were continuously infused with Asp, which was the most effective of the seventeen amino acids used in the intravenous infusion study of Kuhara et al. (1991), and Ala, which was not used in the intraduodenal infusion study of Kuhara et al. (1992), into the abomasum to assay both plasma GH concentration and plasma amino acid concentrations.

Materials and Methods

Three Suffolk wethers (54 kg initial BW) were

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fitted with an abomasal cathether and fed a diet as in table 1 twice a day: 9 am and 5 pm. At each time they were given food at 1% of their BW. Water was given *ad lib.* The wethers were allotted to a control group, an Ala infusion group and an Asp infusion group in the 3 × 3 Latin square design. In the amino acid infusion groups, amino acid of 3 mmol/kg BW was dissolved in 1.5 liters of water, pH of the solution was set to 6.0 using NaOH, and NaCl was added to adjust the Na concentration to 3.25 mmol/kg BW. For the control group, NaCl solution of the same Na concentration as for the amino acid infusion groups was prepared. These solutions were continuously infused into the abomasum at the rate of 1.5 liters/day using a peristaltic pump. The infusion was started at 9 am of day 0 and continued for five days. For the measurement of plasma amino acid concentrations, blood samples were taken immediately before the start of infusion on day 0, and immediately before the morning feeding of days 1, 2 and 4. For the assay of plasma GH concentration, blood samples were taken every fifteen minutes for six hours, from 10 am to 4 pm, on day 0 and day 4. All the blood samples were collected through the intravenous catheters. Ten days of preliminary period was placed before each treatment.

TABLE 1. CONTENTS OF THE DIET USED IN THE STUDY ON THE DRY MATTER BASIS

Contents	Percentage
Timothy hay	65.0
Barley	34.5
$CaCO_3$	0.3
NaCl	0.2
DM	88.2
TDN	65.4
CP	9.9

The blood samples were heparinized and kept frozen at −20℃ before the analyses of free amino acids and GH. Plasma free amino acids were assayed by HPLC using o-Phthalaldehyde (Ishida et al., 1981) after plasma samples were deprotenized by trichloroacetic acid. Plasma GH was assayed by the double antibody method as described by Jaffe Behrman et al. (1978). NIDDK-oGH15 was used as antigen, standard and ^{125}I-labelled GH. Anti-rabbit IgG goat serum (I-RX17, Eiken co., Tokyo) was used as the second antibody. The assay revealed a minimal detectable concentration of 1.56 ng/ml. Interassay and intrassay coefficients of variation were 10.89% and 8.26%, respectively. Physiologic concentration of prolactin, thyroid stimulating hormone and adrenocorticotropic hormone did not affect this assay. All statistical analyses were conducted by the analysis of variance using GLM procedures of the SAS program.

Results and Discussion

Plasma free Asp and Ala concentrations increased by 72.5% and 53.7% respectively one day after starting the infusion of these amino acids (table 2). It shows that Asp and Ala infused into the abomasum were absorbed. However, plasma Asp concentration tended to decrease on day 2. On the other hand, plasma Ala concentration increased by 78.6% after four days of infusion. It suggests that the rate of metabolism of the infused Ala is slower than Asp. However, Wolff et al. (1972) report that the rate of Ala metabolism is faster than that of Asp in sheep fed hay. Therefore, when large amount of an amino acid comes into a body as in the present study, Asp metabolism may be accelerated while Ala metabolism is not affected so much.

Table 3 shows the areas (arbitrary units) and the number of peaks of plasma GH concentration. There was no effect of infusion detected in the Asp group. Kuhara et al. (1991) reported that plasma GH concentration increased when sheep were intravenously infused with 3 mmol/kg BW of Asp in 30 min. Subsequently, it was reported that four days of continuous duodenal infusion of Asp did not change plasma GH concentration (Kuhara et al., 1992). They indicated the possibility that the infused Asp was not absorbed, which was owing to catabolism by the intestinal epithelium or bacteria before absorption. Present study, however, shows that the infused Asp was absorbed since plasma Asp concentration increased by 72.5 % after one day of infusion. Hertelendy et al. (1970) report that the effect of intravenous Arg infusion on stimulation of GH secretion depends on the rate of infusion and that when the same amount of Arg is infused, faster infusion has stronger effect. If it is the case in Asp infusion

as well, it explains why the abomasal infusion of Asp in the present study did not stimulate GH secretion, while the intravenous infusion in the study of Kuhara et al. (1991) did, because plasma Asp concentration must have increased more rapidly when infused intravenously.

TABLE 2. CHANGE OF MEAN PLASMA FREE AMINO ACID (Asp, Ala) CONCENTRATIONS (μmol / l)

	Control	Asp group	Ala group	SE
Asp concentration				
Before infusion	9.10	13.12	13.24	2.63
Day 1	9.06	22.63*	12.37	4.57
Day 2	8.21	17.48	10.50	2.59
Day 4	11.45	16.12	12.51	4.22
Ala concentration				
Before infusion	127.90	133.97	132.25	13.22
Day 1	175.05	200.57	203.25	34.76
Day 2	128.53	196.19	208.14	26.98
Day 4	148.18	155.35	236.25*	45.81

* Significantly different ($p < 0.05$) from the control in the same row.

TABLE 3. CHANGE OF MEAN PLASMA GH CONCENTRATION BY ABOMASAL INFUSION

	Control	Asp group	Ala group	SE
Area (arbitrary units)				
Day 0	132.2	119.5	162.2	21.6
Day 4	147.4	132.8	151.6	23.8
Number of peaks				
Day 0	3.33	2.33	4.00	0.30
Day 4	2.33	2.33	2.33	0.31

In the Ala group, the area and the number of peaks increased in all the sheep on the day of starting infusion (22.7% and 20.1% of average increase, respectively) although the increase was not significant ($p > 0.05$). Since plasma Ala concentration increased by 53.7% after one day of infusion, it is considered that the infused Ala stimulated GH secretion. However, after four days of infusion, there was no difference in plasma GH concentration between the Ala group and the control group, although plasma Ala concentration in the Ala group was the highest at that time. The reason for that is not clear. But it may be possible that the effect of the stimulation of GH secretion by Ala infusion disappeared by some feedback mechanism through the long term infusion.

The mechanism of the stimulation of GH secretion by amino acid infusion has not been understood completely yet. Davenport et al. (1990) suggest that Arg and Orn infused into the abomasum are converted into glutamic acid, then it acts as a neurotransmitter in the central nervous system on either stimulation of growth hormone-releasing hormone or suppression of somatostatin secretion resulting in increase of GH secretion. In the present study, Ala can not be considered to have stimulated GH secretion through increase of plasma Arg and Orn concentrations since they did not change by Ala infusion. However, Ala is reported to have a strong effect of stimulating glucagon secretion (Kuhara et al., 1991). It is also reported that glucagon stimulates GH secretion (Martin, 1976). Therefore, it is possible that the Ala infusion into a digestive tract stimulates GH secretion via increase of plasma glucagon concen-

tration.

In a previous unpublished study, 7 mmol/kg BW/day of the amino acids were infused and they caused remarkable decrease of feed intake in both amino acid infusion groups. It showed that it was difficult to stimulate GH secretion without affecting feed intake. From the results of the present study, it was shown that temporal stimulation of GH secretion by Ala infusion into a digestive tract is possible, although the long term stimulation is difficult. It was also shown that Asp infusion does not stimulate GH secretion.

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