Effects of Intraruminal Isopropyl Alcohol Infusions on the Ruminating Behavior of Goats

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ABSTRACT : Isopropyl alcohol (IPA), produced from acetone by rumen bacterial action, was infused into the rumen of three female goats kept in a climatically controlled experimental room during feeding to investigate the mechanism and roles of IPA in ruminating behavior (number of boli and ruminating time). The ruminating behavior measured by the number of boli, ruminating time, number of remastications, and remasticating time increased ($p<0.05$) with intraruminal IPA infusion. The concentrations of IPA and acetone in the rumen and the plasma significantly increased ($p<0.05$) during intraruminal IPA infusion. These data suggest that rumination receptors sensitive to IPA and acetone may be in an area such as the rumen epithelium and the brain stem where they can respond to metabolite levels. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 8 : 1134-1138)***

Key Words : IPA, Acetone, Rumination, Intraruminal Infusion, Goats

INTRODUCTION

Many metabolite infusion experiments have been done to investigate the regulation mechanism of the masticating and ruminating behavior of ruminants from the chemostatic theory point of view where increases in the rumen and blood metabolites after feeding stimulate chemically sensitive receptors that may regulate the masticating and ruminating behavior by way of the nervous system.

Masticating and ruminating behavior of goats decrease after intravenous acetic, propionic and butyric acids infusion (Oshiro et al., 2000). Food intake of goats decreases with intraruminal acetic acid infusion (Baile and Mayer, 1968). Intraruminal volatile fatty acids (VFAs) infusion decrease food intake in sheep (Ulyatt, 1965) and cattle (Simkins et al., 1967). Increases in VFAs, especially butyric acid, increase blood acetone and ketone concentrations (Ulyatt, 1965; Simkins et al., 1967; Oshiro et al., 2000). Decreases in food intake during butyric acid infusion may be caused by increased ketones by way of the a chemostatic mechanism (Simkins et al., 1967).

Blood acetone concentrations in sheep increase 6 h after intraruminal acetic and butyric acids infusion, and food intake markedly decreases at the same time (Ulyatt, 1965). The masticating behavior of goats decreases and the ruminating behavior increases after intravenous acetone infusion, may be because of ruminating receptor sensitivity to acetone in the brain stem (Oshiro et al., 2000). However, acetone is not a metabolic end product. Acetone is further metabolized in cows (Luick et al., 1967; Black et al., 1972) and rats (Rudney, 1954).

Concentrations of acetone and isopropyl alcohol (IPA) in the blood and rumen increase with an increase in ketosis in cows that have intensified symptoms from subclinical to clinical, and IPA may form in the rumen from acetone by rumen bacterial action (Thin and Robertson, 1959). IPA can be produced by reducing acetone by bacterial action in vitro (Bruss and Lopez, 2000).

The ruminating behavior and masticating behavior of goats decrease with intravenous IPA infusions (Asato et al., 2001). We consider that IPA may have an important role in regulating the ruminating and masticating behavior of goats. The IPA metabolic pathway in the rumen and liver of ruminants is little understood. IPA absorbed by the rumen epithelium may circulate to the liver where IPA oxidizes to acetone (Thin et al., 1959; Bruss and Lopez, 2000). We believe no investigations have been done to clarify the effects of intraruminal IPA infusion on the ruminating and masticating behavior or its metabolite pathway in ruminants.

This study examined the relationship of IPA with the ruminating and masticating behavior and with the IPA metabolic pathway of goats.

MATERIALS AND METHODS

Animals and feeding

Three crossbred female goats (Saanen×Native Okinawan, 38.0±8.0 kg BW) were fed alfalfa hay cubes (12.6% moisture, 12.8% CP, 1.3% ether extract, 25.2% crude fiber, 9.6% crude ash, and 51.2% nitrogen-free extract) using an auto feeder by free choice (Oshiro and Katayama, 1987) and water at 1200 every day. Each goat surgically prepared with a jugular vein cannula and a rumen fistula was housed in a climatically controlled experimental room at 22.5±1.4°C and a relative humidity of 67.2±3.3%

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in individual metabolism cages. The test room (10.4 m^2) was continuously lit by fluorescent electric bulbs (4 bulbs×40 W, 55.8±4.0 Lux) during the trial. Goats were used that had become accustomed for four weeks to the experimental room and handling. Before the experiment, each goat was attached to chewing sensors to measure the jaw movements, and a polyethylene catheter (o.d. 0.7 mm, 63 mm length, TOP Co., Tokyo, Japan) for sampling was inserted into the jugular vein toward the heart.

Experimental design

We made three experiments: 1) a noninfusion experiment without intraruminal infusions; 2) using infused intraruminal Ringer's solution (147 mEq/L Na^+, 4 mEq/L K^+, 4.5 mEq/L Ca^{2+}, and 155 mEq/L Cl^-, Otuka Pharmaceutical Co., Ltd., Tokyo, Japan) once at 0000 (midnight), followed immediately by continuous infusion until 1200 (noon) of the next day (day 2); 3) using infused Ringer's solution containing IPA once at 0000 (midnight) at a concentration of 0.78 m mol/7.2 mL/kg, followed immediately by continuous infusion until 1200 (noon) at a concentration of 0.0038 m mol/0.01 mL/kg body weight/min. The pH of the solution was adjusted to 6.8 with 0.1 N NaOH solution. The infusions in all continuous infusion experiments were at a rate of 0.01 mL/kg body weight/min using a peristaltic pump (MP-3, Tokyo Rikakikai Co. Ltd., Japan).

Sampling and analysis

Ruminal fluid (7 mL) was sampled at -12, -8, -4, 0, 1, 3, 6, 9 and 12 h after the start of the metabolite infusion to measure ruminal pH and concentrations of VFA, acetone and IPA. Ruminal fluid pH was measured immediately after collection using a compact pH meter (B-211, HORIBA Ltd., Kyoto, Japan). A 5 mL aliquot from each sample of ruminal fluid was deproteinized with 1 mL of 25% metaphosphoric acid, and was centrifuged at 11,000 g at 4°C for 10 min. Then the supernatant was frozen at -20°C until it was analyzed for VFA, acetone and IPA concentrations using a gas chromatography (GC-6A, Shimazu Co., Kyoto, Japan).

Blood samples (8 mL) were placed in centrifuge tubes at -12, -8, -4, 0, 1, 3, 6, 9 and 12 h after the start of the metabolite infusion to measure the packed cell volume (PCV) and the concentrations of hemoglobin, glucose, non-esterealized fatty acids (NEFA), VFA, acetone and IPA. The PCV was measured immediately after collecting the blood using microhematocrit capillary tubes (o.d. 1.45-1.65 mm, 75 mm length, TERUMO Co., Tokyo, Japan) and the blood was centrifuged in the tubes at 11,000 g for 5 min. The concentration of hemoglobin was measured using the cyanmethemoglobin method (Cannan, 1965) with a commercial assay kit (Hemoglobin test wako, Wako Pure Chemical Industries Ltd., Osaka, Japan). Blood plasma was obtained by centrifugation at 3,000 g for 10 min within 24 h after collecting blood and was frozen at -20°C until analyzed. After thawing, the concentration of plasma glucose was analyzed using an assay kit (Glucose-B test wako, Wako Pure Chemical Industries Ltd., Osaka, Japan) based on the glucose oxidase method (Trinder, 1969). The NEFA were analyzed using the method of Duncombe (Duncombe, 1964) with an assay kit (NEFA test wako, Wako Pure Chemical Industries Ltd., Osaka, Japan). A 1 mL aliquot of each blood plasma sample was deproteinized with 1 mL of 20% trichloacetic acid to analyze for plasma VFA concentration using HPLC (LC-10S, Shimazu Co., Kyoto Japan), and concentrations of acetone and IPA using gas chromatography (GC-6A, Shimazu Co., Kyoto, Japan).

The goat's jaw movement was recorded continuously for 24 h from 1200 h of day 1 through to 1200 of day 2 using an autocounter system (LB-8801. TECMO Co. Ltd.) (Oshiro et al., 1987). The goats were allowed a period to adjust to the system before the experiment.

The results were reported as ruminations (number of boli; boli/12 h and ruminating time; min/12 h), mastications (number of mastications; chewing/ 12 h and masticating time; min/12 h) and resting time (min/12 h). The ruminations were further reported as number of remastications (chewing/12 h), remasticating time (min/ 12 h), intermittent time (min/12 h), bolus formation time (sec/bolus), number of remastications of each bolus (chewing/bolus), remasticating time (sec/bolus) and intermittent time between each bolus (sec/bolus) in each rumination and within each day.

Statistical analysis of all data were made between data collected before and after infusions using Tukey's method (Snedecor and Cochran, 1989).

RESULTS

Table 1 shows the results of the food intake, ruminating behavior, masticating behavior and ruminating behavior for each bolus in the noninfusion, inraruminal Ringer's solution and intraruminal IPA infusion experiments.

The ruminating behavior (number of boli, ruminating time, number of remastications, remasticating time) was constant at unvarying conditions during the noninfusion and Ringer's solution infusion experiments.

The number of boli, ruminating time, number of remastications, and remasticating time significantly increased after the intraruminal IPA infusion ($p<0.05$).

Food intake and masticating behavior (number of mastications, masticating time) was constant during the noninfusion and Ringer's solution infusion experiments. However, they tended to decrease during the intraruminal IPA infusion experiment.

Table 1. Food intake, ruminating behavior, masticating behavior and ruminating behavior per bolus in three experiments

Items		Controls		Treatment
		Non-infusion	Ringer infusion	IPA infusion
Food intake				
g/12 h	BI	658±128	625±204	688±118
	DI	645±184	613±212	500±178
Number of boli				
boli/12 h	BI	174±31	196±58	195±22
	DI	192±51	218±16	285±28*
Ruminating time				
min/12 h	BI	152±28	158±65	176±24
	DI	161±21	195±19	277±44*
Number of remastications				
chews/12 h	BI	16,558±9,427	13,698±1,094	15,974±755
	DI	17,016±7,381	18,782±5,752	25,213±3,418*
Remasticating time				
min/12 h	BI	136±28	138±52	155±21
	DI	141±15	166±23	247±37*
Number of mastications				
chews/12 h	BI	10,692±5,114	8,238±3,179	9,091±3,372
	DI	11,325±8,828	7,430±1,368	6,290±3,279
Masticating time				
min/12 h	BI	61±10	51±19	54±10
	DI	56±20	45±12	38±17
Intermittent time				
min/ 12 h	BI	16±6	30±2	21±4
	DI	18±7	25±3	30±7
Resting time				
min/12 h	BI	507±35	509±64	490±28
	DI	504±24	480±31	405±61
Bolus time formation time				
sec/bolus	BI	52.7±5.1	47.1±6.0	54.1±5.7
	DI	51.3±6.6	53.6±1.4	58.2±3.5
Remasticating time per bolus				
sec/bolus	BI	47.1±6.7	41.4±3.8	47.7±4.6
	DI	45.3±6.9	45.6±3.2	51.9±2.7
Intermittent time per bolus				
sec/bolus	BI	5.6±1.6	9.6±3.5	6.4±1.2
	DI	6.0±1.5	7.0±0.3	6.3±0.8
Number of remastications of each bolus				
chews/bolus	BI	94.9±52.5	73.8±21.5	82.3±5.3
	DI	96.6±62.4	87.5±32.9	88.3±3.8

BI: Before infusion, DI: During infusion.

* Means±S.D. are significantly different at $p<0.05$ compared with the values before infusion.

The bolus formation time, remasticating time per bolus, intermittent time between each bolus and number of remasticatons of each bolus were constant during the infusion periods in the three experiments.

Table 2 shows the PCV, glucose, NEFA, VFAs, acetone and IPA concentrations in the plasma in the three experiments. The PCV, glucose, NEFA and VFAs concentrations in the plasma did not significantly change in the three experiments, but the glucose concentration tended to increase after the intraruminal IPA infusion. However, the concentrations of acetone and IPA significantly increased after the IPA infusion experiment ($p<0.05$).

Table 3 shows the ruminal fluid pH, VFAs, acetone and IPA concentrations in the rumen in three experiments. Ruminal fluid pH and concentration of VFAs in the rumen were at the same level before and after the infusion in the three experiments, but the concentrations of acetone and IPA significantly increased after the IPA infusion experiment ($p<0.05$).

DISCUSSION

IPA metabolic pathway

The concentrations of acetone and IPA in the plasma increased after IPA infusion. We consider that the increases in plasma acetone in the blood circulation were due to activity of alcohol dehydrogenase (ADH) in the liver. After infusion, IPA absorbed from the rumen wall was circulated to the liver where the IPA was oxidized to acetone by alcohol dehydrogenase, and finally, passed into the blood circulation. Some studies have supported the hypothesis that IPA can be oxidized to acetone in rats (Nordmann et al., 1973; Lahman et al., 1980), and in cows (Thin et al., 1959). In the liver, IPA is oxidized to acetone at a constant rate but not all of it, so that the plasma IPA concentration increases because some IPA passes through the liver to the blood circulation.

The concentration of glucose that tended to increase during IPA infusion may be related to increases in blood acetone concentration (table 2). Acetone is metabolized to glucose in cows (Black et al., 1972), and glucose concentration increases after intravenous IPA infusion (Asato et al., 2001).

The concentration of acetone in the rumen increases during IPA infusion by increased acetone in the plasma entering the rumen through the rumen wall or in the saliva (Bruss and Lopez, 2000).

Effect of IPA on ruminating and masticating behavior

Masticating increases and ruminating decreases at the same time by feeding (Gordon and McAllister, 1970; Oshiro, 1985; Oshiro and Katayama, 1987; Oshiro and Koja, 1987). Ruminating behavior during feeding in continuous light either stays the same or decreases (Oshiro et al., 1988). Therefore, we consider that a decrease in the ruminating behavior after feeding (Oshiro, 1985) is related to an increase in VFAs in the rumen and plasma after feeding

Table 2. Concentration of plasma compounds in three experiments

Items	Controls		Treatment
	Non-infusion	Ringer infusion	IPA infusion
PCV, %			
BI	23.8±2.3	26.8±1.4	24.9±1.0
DI	23.4±1.6	26.2±1.4	24.4±1.7
Hemoglobin, g/dl			
BI	10.1±0.7	10.7±0.3	9.6±0.4
DI	9.9±0.5	9.9±0.5	10.0±0.5
Glucose, mg/dl			
BI	60.8±3.6	65.8±6.2	61.8±5.7
DI	63.2±1.3	68.2±8.7	70.4±9.7
NEFA, mEq/l			
BI	0.26±0.02	0.27±0.13	0.27±0.02
DI	0.27±0.02	0.24±0.10	0.31±0.03
Acetic acid, mmol/l			
BI	0.986±0.080	0.970±0.143	1.041±0.085
DI	1.054±0.201	1.040±0.332	0.934±0.208
Propionic acid, mmol/l			
BI	0.147±0.074	0.182±0.072	0.167±0.064
DI	0.129±0.023	0.168±0.082	0.171±0.052
Butyric acid, mmol/l			
BI	0.155±0.056	0.156±0.071	0.113±0.025
DI	0.186±0.033	0.123±0.067	0.154±0.067
Iso-valeric acid, mmol/l			
BI	0.158±0.039	0.182±0.029	0.164±0.073
DI	0.156±0.015	0.173±0.071	0.189±0.040
Acetone, mmol/l			
BI	0.615±0.179	0.613±0.225	0.647±0.191
DI	0.615±0.132	0.672±0.242	2.480±1.004*
IPA, mmol/l			
BI	0.039±0.005	0.026±0.003	0.067±0.031
DI	0.039±0.006	0.028±0.013	0.205±0.172*

BI: Before infusion, DI: During infusion.

* Means±SD are significantly different at $p<0.05$ compared with the values before infusion.

Table 3. Ruminal fluid pH, concentration of rumen compounds in three experiments

Items	Controls		Treatment
	Non-infusion	Ringer infusion	IPA infusion
pH			
BI	6.9±0.1	6.7±0.2	6.9±0.2
DI	6.8±0.1	6.7±0.4	6.8±0.1
Acetic acid, mmol/l			
BI	61.400±8.433	67.275±7.522	59.659±15.675
DI	66.698±4.558	73.542±7.235	61.921±10.957
Propionic acid, mmol/l			
BI	23.584±8.108	26.230±6.157	23.025±9.856
DI	23.535±4.069	26.507±4.637	24.946±8.768
Butyric acid, mmol/l			
BI	6.323±1.723	6.055±1.723	5.361±2.478
DI	6.557±1.268	6.345±1.258	4.988±2.538
Iso-valeric acid, mmol/l			
BI	0.568±0.276	0.387±0.099	0.531±0.179
DI	0.613±0.317	0.420±0.144	0.677±0.189
Acetone, mmol/l			
BI	0.273±0.118	0.359±0.040	0.384±0.089
DI	0.307±0.081	0.362±0.189	0.651±0.026*
IPA, mmol/l			
BI	0.077±0.012	0.087±0.010	0.100±0.050
DI	0.077±0.023	0.092±0.023	3.747±1.621*

BI: Before infusion, DI: During infusion.

* Means±SD are significantly different at $p<0.05$ compared with the values before infusion.

(Pothoven and Beitz, 1975). However, other metabolites, such as acetone produced from acetic and butyric acids, in addition to VFAs, are clearly also important in regulating food intake of cattle (Simkins et al., 1967), and the masticating and ruminating behavior of goats (Oshiro et al., 2000).

In this study, increased ruminating behavior during intraruminal IPA infusion was associated with an increased IPA and acetone in the rumen and plasma. However, intraruminal acetone infusion (Kawamura et al., 1999) and intravenous IPA infusion (Asato et al., 2001) decrease ruminating behavior. So, increased rumen acetone and plasma IPA may not be the cause of increased ruminating behavior. We speculate that increased ruminating behavior during the intraruminal IPA infusion was because of increased plasma acetone or rumen IPA.

Oshiro et al. (2000) supported the hypothesis that chemoreceptors in the brain stem may be sensitive to increased plasma acetone, and that increased plasma acetone may increase the ruminating behavior of goats after intravenous acetone infusion. In addition to receptors in the brain stem, afferent signals from chemoreceptors in the rumen epithelium are also important to control the ruminating and masticating behavior of ruminants (Forbes and Barrio, 1992). Therefore, increases in IPA concentration in the rumen may directly stimulate epithelial receptors in addition to acetone stimulating the brain stem.

The decreased masticating behavior by intraruminal IPA infusion during spontaneous meals suggests that ruminants may control their masticating behavior by not only VFAs but also other metabolites. A decreased food intake during butyric acid infusion may be caused by increased ketones produced by a chemostatic mechanism (Simkins et al., 1967). The masticating behavior of goats decreases during intravenous acetone (Oshiro et al., 2000) and IPA (Asato et al., 2001) infusions.

Montgomery and Baumgardt (1965) supported the hypothesis that ruminants adjust voluntary food intake in relation to the physiological demand for energy if the rumen

fill or the rumen load do not limit their consumption. The results of this study showed strong evidence that acetone and IPA decrease masticating behavior, although the food intake of goats does not decrease with acetic acid (Baile and Mayer, 1968) and VFA (Forbes, 1986) infusion into the jugular vein during spontaneous meals.

We conclude that increases in the ruminating behavior immediately after IPA infusion are due to increasing rumen IPA concentrations or plasma acetone concentrations themselves after IPA infusions.

CONCLUSION

Our data suggest that rumination receptors sensitive to the IPA and acetone in the rumen and blood may be in an area such as the rumen epithelium and brain stem (Anderson, 1951) where they can respond to metabolite levels.

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