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Methionine and lysine metabolism in the rumen and the possible effects of their metabolites on the nutrition and physiology of ruminants

Review Article

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Summary. This article briefly summarizes the metabolism of methionine and lysine in the rumen of ruminant animals and known and inferable effects of their metabolites on the nutrition and physiology of ruminant animals.

Keywords: Amino acids – Methionine and lysine metabolism – Rumen protozoa and bacteria – Methionine sulfoxide – Pipecolate – 2,6-Diaminopimelate – Nutrition in ruminants

Introduction

Feeding of rumen-protected methionine and lysine (chemically or physically protected from degradation by rumen microorganisms) has been known to be effective in enhancing the productivity of ruminant livestock (Nimrick et al., 1970; Richardson and Hatfield, 1978; Smith and Boling, 1984; Canale et al., 1990), though there have also been some negative data in some conditions (Chow et al., 1990; Broderick et al., 1991). These topics are included in one side of the category of amino acid nutrition for ruminants and seem important to improve the productivity of the animals. On the contrary, the present paper will concentrate on amino acid metabolism in the rumen from the alternative perspective, that is, the metabolites of the amino acids in unprotected forms in the rumen can be positively and negatively effective to the nutrition and physiology of host animals. This approach may lead us to insights into the utilization of protected, semi-protected and unprotected amino acids in ruminant animal production. Of the essential amino acids in animal nutrition, methionine and lysine are picked up in this paper and their metabolism in the rumen and by rumen micro-

organisms and the possible nutritional and physiological significance of their metabolites produced in the rumen will be discussed.

Metabolism of methionine

1. Metabolites of methionine in the rumen

Metabolism of methionine in the rumen has been investigated since 1955 (Lewis, 1955). He showed that ammonia and carbon dioxide productions from methionine was extremely lower than from cysteine using rumen bacterial suspension of sheep. Later Lewis and Emergy (1962a) found that methionine and valine were slowly degraded, arginine and threonine were rapidly degraded and the other essential amino acids (EAA) formed an intermediate group. These facts were confirmed by Chalupa (1976). He indicated that apparent in vitro degradation rates of methionine and valine by rumen microbes from cattle (average 300 kg) fed 4 kg/day of a 60% concentrate ration were 0.09 and 0.14 mM/h, respectively, whereas those of arginine and threonine were 0.88 and 0.50, respectively. In vivo EAA degradation rates were about 1.5 times greater than estimates derived from in vitro rate constants. Presence of other EAA in conjunction with methionine resulted in a decrease in methionine degradation rate in vitro (Skoch et al., 1975) and in vivo (Chalupa, 1976). Bacterial species contained in the rumen also affected methionine degradation rate (Scheifinger et al., 1976). By the way, rumen protozoa also tended to degrade arginine quickly (0.083 mM/h) (Onodera et al., 1983) and methionine very slowly (0.0036 mM/h) (Onodera and Ushijima, 1982), though the degradation rates of these EAA were less than one-tenth of those in bacteria (Chalupa, 1976). Glutamine and ornithine were degraded more quickly than arginine by rumen protozoa (Onodera et al., 1983).

Metabolic fate of methionine in the rumen and the fate of metabolites thereafter can be outlined as in Fig. 1. Methionine tended to be oxidized enzymatically by rumen microbes to give methionine sulfoxide (MSO) (Cook et al., 1965; Salsbury et al., 1971; Onodera and Ushijima, 1982; Onodera and Migita, 1984; Onodera and Takei, 1986). Oxidation rate of methionine in in vitro goat rumen microbial suspensions was very quick, converting approximately 10 to 20% of methionine into MSO within a few minutes, when 1 mM of methionine was added as a substrate (Takei and Onodera, unpublished data). This reaction seemed to be catalyzed by some enzyme, because boiled microbial suspensions did not produce MSO in our experiment. Salsbury et al. (1971) also pointed out that MSO could be produced by microbial action in the rumen fluid, though it could rise through nonbiological process (Shockman and Toennies, 1954). As to utilization of MSO, discussion will be made further in the next item.

The first product found in methionine catabolism by rumen microbes (mixed microbes and protozoa) was methanethiol (Zikakis and Salsbury, 1969; Salsbury et al. 1971; Bird, 1972; Merricks and Salsbury, 1974, 1976; Salsbury and Merricks, 1975). This compound can be produced by direct dethiomethylation by methionine γ-lyase (EC 4.4.1.11) (methioninase) in rumen protozoa (Merricks and Salsbury, 1974, 1976) like in *Escherichia coli* (Ohigashi et al., 1951), though

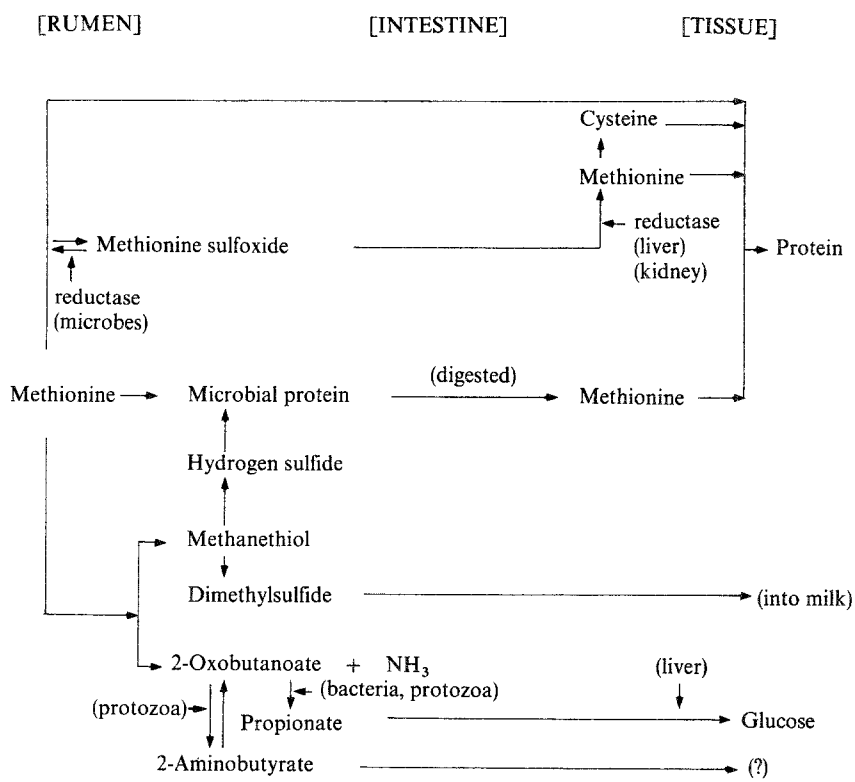

Fig. 1. Fate of methionine in the gastro-intestinal tract and tissues of ruminants

there has also been another suggestion that methanethiol might be formed by two-step reactions: oxidative deamination followed by dethiomethylation (Bird, 1972). Methanethiol can be incorporated directly into microbial protein (Zikakis and Salsbury, 1969) and converted to hydrogen sulfide (Bird, 1972), and hydrogen sulfide can be incorporated into microbial protein (Block et al., 1951; Bird and Moir, 1972). Methanethiol can also be converted to dimethyl sulfide (Salsbury et al., 1971; Salsbury and Merricks, 1975). This compound is thought to associate with milk flavor; at high concentration the flavor was described as malty or cowy (Reddy et al., 1967; Dunhum et al., 1968).

Alternative product in methionine metabolism by rumen microorganisms is thought to be 2-oxobutanoate (Onodera and Migita, 1984; Merricks and Salsbury, 1974, 1976). This compound can be converted to propionate by in vitro rumen protozoa (Onodera and Migita, 1984) and chiefly to propionate and to some extent to acetate and butyrate in in vivo rumen (Belasco, 1980). 2-Oxobutanoate can also be converted reversibly to 2-aminobutanoate by rumen protozoa (Onodera and Ushijima, 1982; Onodera and Migita, 1984). 2-Aminobutanoate was also found in the metabolic process of methionine by *E. coli* (Ohigashi et al., 1951). This compound has been shown to reduce growth of some strains of *Eubacterium ruminantium* (Stevenson, 1979) and seems to have important roles in rumen microbial ecosystem. Ruminal concentration of 2-aminobutanoate was higher in faunated goats than in defaunated (Itabashi and

Kandatsu, 1975). However, function of this compound in the host animal is not yet known.

2. *Methionine-S-oxide reductase activity in ruminant animals*

Interestingly Salsbury and Merricks (1972) suggested that degradation rate of methionine sulfoxide (methionine-S-oxide, MSO) was slower than that of methionine (60% of methionine) in in vitro rumen fluid. We (Takei and Onodera, unpublished) also examined this point and confirmed that it was true. As shown in Table 1, MSO degradation rates were slower than methionine degradation rates during 6 and 12 h incubation periods in mixed rumen microbial suspensions (50 and 65% for MSO vs 91 and 93% for methionine, respectively), when initial concentration of each substrate was 1 mM. In rumen bacterial suspensions, similar tendencies were also observed. Protozoa hardly decomposed MSO in these incubational conditions.

Table 1. Degradation rates (%) of methionine and methionine sulfoxide in in vitro rumen microbial suspensions

Incubations	Incubation time (min)					
	0	10	30	60	360	720
In case of methionine (1 mM)						
Without microbes	0	0	0	0	0	0
With protozoa	0	—	—	—	—	18.0
With bacteria	0	—	—	—	31.3	73.5
With mixed microbes	0	37.0	41.1	55.4	91.2	93.2
In case of methionine sulfoxide (1 mM)						
Without microbes	0	0	0	0	0	0
With protozoa	0	—	—	—	—	0
With bacteria	0	—	—	—	15.0	47.8
With mixed microbes	0	7.8	3.8	18.5	50.0	65.5

Cook et al. (1965) reported that in contrast with humans MSO was present in ruminant (goat) blood and was consistently higher in rumen vein blood than in jugular blood after methionine was added to the rumen (Fig. 2). They, of course, recognized an increase of MSO at a ruminal level of 0.32 mM 1 h after adding the amino acid mixture containing methionine to the rumen. From these results they concluded that MSO could be absorbed from the rumen, though they also demonstrated that in case of glycine a greater proportion of it was transferred through intestinal tissue than through any of the other tissues like rumen, reticulum, omasum and abomasum by means of an in vitro perfusion test. MSO has already been known to be reduced enzymatically to methionine in liver and kidney of rat (Aymard et al., 1979) and rabbit (Ganapathy and Leibach, 1982).

Based on these data, we came, at last, to have the hypothesis that ruminant animals might have a higher MSO reductase activity than other monogastric

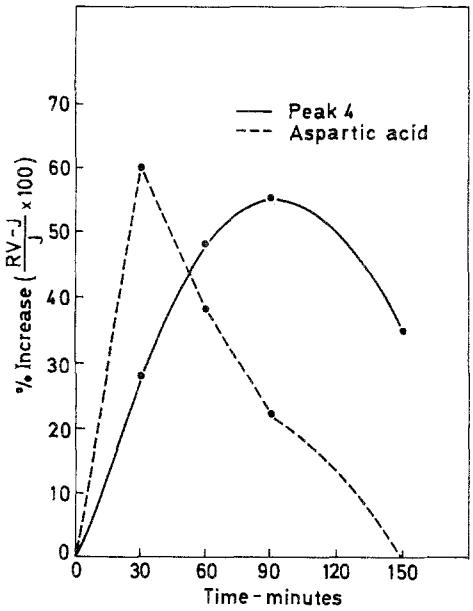

Fig. 2. Increase in aspartic acid and Peak 4 (MSO) levels in rumen vein vs. jugular blood (cited from Cook et al., 1965)

animals. Thus we compared the MSO reductase activities of livers and kidneys of cattle, swine and chickens. At first basic conditions for assaying MSO reductase activity were determined for both tissues of these animals after examining the characteristics of crude enzyme solutions prepared from homogenate of the tissues (Table 2) (data of cattle and swine are cited from the report of Nagamine et al., 1991). However, the conditions for chickens could not be determined and

Table 2. Assay conditions for MSO reductase activities in liver and kidney of cattle, swine, and chickens

Tissues	pH (optimum)	Temperature (physiological) (°C)	Reducing agent (1 mM)	Substrate concentration (mM)[a]
Cattle				
liver	6.0	39	NADH	0.80
kidney	6.7	39	NADPH	0.60
Swine				
liver	7.0	39	NADH	0.25
kidney	7.0	39	NADPH	1.00
Chikens				
liver and kidney	6.0[b] 7.0 8.0	42	NADH and NADPH	0.25[b]

[a] calculated from K*m*

[b] Optimum pH and K*m* could not be determined, because of very low activity

enzyme activity was assayed at three points of pH using NADH and NADPH as reducing agents, because very little activities were observed in the tissues of chickens. The first thing we should mention was that almost all of the enzyme activities were found in supernatant fluids of centrifuged homogenates in all tissues (Nagamine et al., 1989, 1991). The enzyme activities determined for cattle and swine were shown in Table 3 (from Nagamine et al., 1991). The activities of chickens were too low to be determined in the assay conditions tried. In expressing in per protein, the average enzyme activity of cattle liver was 2.51 times higher than that of swine liver, while broiler liver showed negligible activity as mentioned above, but not zero. The mean activity of cattle kidney was 2.78 times higher than swines. Broilar kidney also showed little activity. The enzyme activities expressed in per tissue had similar tendencies. These results supported our hypothesis that MSO reductase activity in ruminant animals might be higher than that in monogastric animals.

Table 3. Methionine-S-oxide reductase activities in livers and kidneys of cattle and swine (from Nagamine et al., 1991)

Tissues	Cattle (37 heads)	Swine (10 heads)	C/S ratio[a]
Liver:			
per protein[b]	2.18 ± 0.85	0.87 ± 0.67	2.51
per tissue[c]	220 ± 75	97 ± 77	2.27
Kidney:			
per protein[b]	8.80 ± 2.79	3.17 ± 0.98	2.78
per tissue[c]	586 ± 177	269 ± 62	2.18

[a] Ratio of the activity of cattle to that of swine
[b] Enzyme activity was expressed as nmol of methionine produced from MSO (substrate) per mg protein in the crude enzyme solution per h
[c] Enzyme activity was expressed as nmol of methionine produced from MSO (substrate) per g tissue per h

Incidentally, the MSO reductase of cattle liver has been purified and the molecular weight of the enzyme was shown to be about 20,000 (Nagamine et al., 1992). This is the first report concerning the purification of the enzyme from animal tissues.

In order to compare the possible abilities to reduce MSO to methionine between cattle and swine, the amount of methionine possibly produced by both liver and kidney was calculated using the values of MSO reductase activities shown in Table 3, provided that wet weights of livers of matured cattle and swine were 6,000 and 2,000 g, respectively, and those of kidneys of them were 650 and 225 g (single weight), respectively, and further that there was enough substrate in the blood. As a result, possible methionine producing-abilities from MSO were indicated as 7.46 and 1.12 g of methionine/head/day for cattle and swine, respectively. These values were equivalent to 26.5 and 12.4% of methionine requirements of cattle (28.2 g/600 kg/day: provisional requirement calculated

from mean values for steers and bulls, Buttery and Foulds, 1985) and swine (9 g/100 kg/day: NRC, 1979). Ruminant animals seem to be more efficient in utilizing MSO than swine.

Free methionine in the rumen is, thus, effectively utilized to appreciable extent in the nutrition of ruminant animals. Bird and Moir (1972) reasonably showed that the mean increase in wool growth rate above basal diet in sheep was 28 and 68% for the ruminal and abomasal infusions of methionine (2 g/day), respectively, and the mean increase in body weight gain above basal diet during a week was 4.6 and 7.3 fold, respectively.

Metabolism of lysine

1. Metabolites of lysine in the rumen

The first study concerning metabolic pathway of lysine in rumen microorganisms was reported by Dohner and Cardon (1954). They showed that 1 mole each of acetate and butyrate and 2 moles of ammonia were produced from lysine after incubation of 2 strains of *Escherichia coli* isolated from the bovine rumen. Conversely Lewis and Emery (1962a,b) showed that δ-aminovalerate (DAV) and cadaverine were produced from lysine using in vitro and in vivo rumen microbial ecosystem of cows. We also examined the metabolites of lysine (1 mM) by mixed rumen bacteria collected from goat rumen using L-[U-^{14}C]lysine (Onodera and Kandatsu, 1975). As a result, approximately 17% of total radioactivity was shown to be converted to volatile fatty acids (VFAs) and about 0.5% to CO_2 during 6 h incubation period. Of the VFAs, acetate and butyrate were main products similar to the result of Dohner and Cardon (1954) and their amounts calculated from radioactivities contained were 73.2 and 60.1 nmole/ml, respectively (Table 4). Thus the molar proportions of the radioactive acetate and butyrate produced from lysine did not seem equal in this mixed bacterial suspensions different from the results of *E. coli* by Dohner and Cardon (1954). Small amounts of radioactivity were also detected in propionate and C-6 acid fractions. In order to confirm the productions of DAV, cadaverine (Lewis and Emery, 1962a,b) and pipecolate (Onodera and Kandatsu, 1969, 1972: the only metabolite of lysine by rumen protozoa and will be described soon later in this paper) from lysine by mixed rumen bacteria, radioactive L-lysine was again incubated with enough amounts (5 mM) of these three compounds to trap them, if produced, in the mixed rumen bacterial suspensions mentioned above

Table 4. Amounts (mM) of VFAs produced from L-lysine (1 mM) by mixed rumen bacteria during 6 h incubation period (from Onodera and Kandatsu, 1975)

Experiment	Acetate	Propionate	Butyrate
I	0.0732	0.0025	0.0601
II[a]	0.0867	0.0038	0.0514

[a] 5 mM each of δ-aminovalerate, cadaverine and pipecolate were added to the medium

for 6 h. Results indicated that addition of three compounds did not affect so strongly the metabolites from lysine and the amounts of radioactive VFA produced were almost similar to those produced in the mixed rumen bacterial suspensions without three compounds (Table 4). At the same time, it was shown that no radioactivities were detected in DAV and cadaverine different from the results of Lewis and Emergy (1962a,b). And the mixed rumen bacteria did not produce pipecolate from lysine unlike rumen ciliate protozoa (Onodera and Kandatsu, 1972). The catabolic pathway of lysine by the mixed rumen bacteria could, therefore, be presumed to coincide with that of an anaerobic bacterium, clostridia, which fermented lysine to acetate, butyrate and ammonia as shown by Stadtman (1963).

Rumen protozoa can produce pipecolate from lysine, as mentioned above (Onodera and Kandatsu, 1969, 1972). Pipecolate was eventually shown to be an end-product in the metabolism of lysine by mixed rumen ciliate protozoa (Onodera and Kandatsu, 1972). There is no study on lysine metabolism by rumen anaerobic fungi. Thus the main substances produced from lysine in the rumen are thought as present to be acetate, butyrate, ammonia and pipecolate. The VFAs are in the fate to be absorbed and utilized by the host animal and ammonia is utilized by bacteria and other organisms. The fate of pipecolate in the rumen and its possible physiological function in the host animal will be discussed in the final item.

2. Lysine synthesis by rumen protozoa and its nutritional role

The discovery of pipecolate has really been a chance for the author to demonstrate the lysine-synethsizing ability of mixed rumen protozoa, not only from free

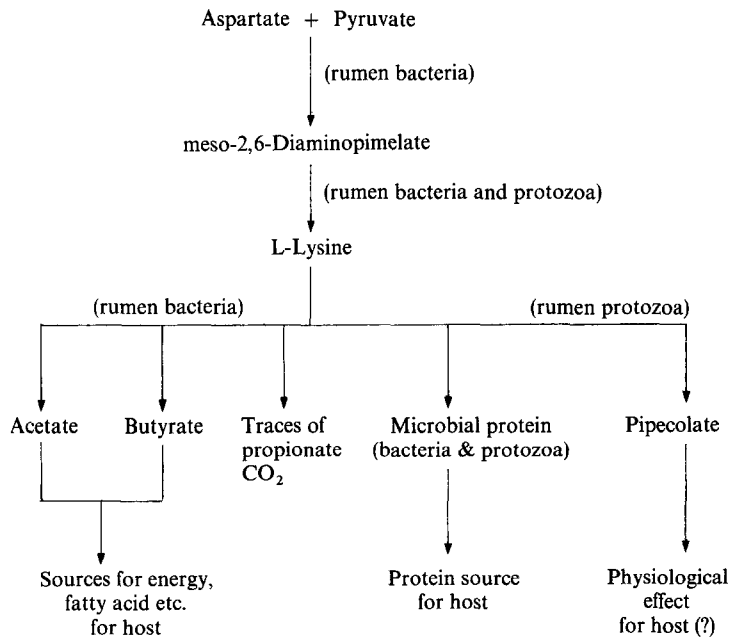

Fig. 3. L-Lysine metabolism in the rumen and roles of its metabolites in host animal

2,6-diaminopimelate (DAP) (Onodera and Kandatsu, 1973, 1974), but also from DAP bound in rumen bacterial cell walls (Onodera et al., 1974). These facts have been confirmed by the Welsh group (Lane and Ling, 1979; Masson and Ling, 1986). However, it is also shown that rumen protozoa have a poor DAP-synthesizing capacity from L-[U-^{14}C]aspartate (an usual precursor in DAP synthesis in bacteria) and [U-^{14}C]acetate (Onodera and Kandatsu, 1974). Thus DAP seemed to be chiefly synthesized by bacteria and incorporated in the peptidoglycan of bacterial cell walls (Ghuysen, 1968). Peptidoglycans are resistant to pepsin and trypsin and bacterial cell walls are thought to be poorly digested in the digestive tract of ruminants (Allison, 1970). DAP is also thought to be undegraded by ruminant animals (Mason and White, 1971). Rumen protozoa, therefore, may contribute to the nutrition of the host animal by producing lysine from the apparently less digestible, DAP-containing peptidoglycan (Onodera et al., 1974). This idea was supported by the fact that in vitro lysine production from L-[U-^{14}C]aspartate by mixed rumen protozoa plus bacteria was shown to be about two-fold higher than that by the mixed rumen bacteria alone. Furthermore, in vitro lysine production by faunated goat rumen contents was 23% higher than that from defaunated animals (Table 5) (Onodera, 1986).

Further, a comparison of the nutritive value of protein of the faunated rumen contents of goats given diets of haycube and concentrate (3:1 and 1:1) with that

Table 5. Amounts of lysine synthesized from radioactive aspartate by four types of rumen microbial suspensions after incubation for 20 min (from Onodera, 1986)

Samples[a]	Lysine synthesized (nmol/ml)[b]			
	FB	FBP	FRC	DRC
Microbial hydrolysate				
Without additives ($n = 2$)	0.265	0.522		
With additives ($n = 3$)	2.130 ± 0.514[c]	3.978 ± 0.269[d]	5.933 ± 0.403[e]	5.511 ± 0.378[e]
Supernatant fluid				
Without additives ($n = 2$)	0.998	1.123		
With additives ($n = 3$)	4.278 ± 0.215[c]	6.078 ± 0.592[d]	5.056 ± 0.386[d]	3.422 ± 0.101[e]
Total				
Without additives ($n = 2$)	1.263	1.645		
With additives ($n = 3$)	6.400 ± 0.191[c]	10.611 ± 0.959[d]	10.989 ± 0.649[d]	8.933 ± 0.310[e]

[a] Additives used were 5 μmol of urea and 5 mg each of rice starch and xylan

[b] FB, bacterial suspension from faunated goat; FBP, mixed suspension of bacteria and protozoa from faunated goat; FRC, rumen contents from faunated goat; DRC, rumen contents from defaunated goat

[c,d,e] Means (± standard error) in the same row with different superscripts differ ($P < 0.05$)

of their defaunated counterparts, revealed that in the faunated goats histidine and methionine tended to be the first and second limiting amino acids (LAA), respectively, while in the defaunated goats lysine tended to be the second LAA (Table 6) (Onodera and Koga, 1987). In these experiments, the nutritive value of protein was evaluated by the partially modified chemical score method (quasi-chemical score, QCS) in which arginine, tryptophan, tryosine and cysteine were not included in the calculation of chemical score and beef muscle protein was used as the reference protein. From these results, it is apparent that lysine content tend to be higher in faunated rumen contents than in defaunated and this is due to the facts that rumen protozoa can synthesize lysine from 2,6-diaminopmelate in bacterial cell walls as described above and protozoal protein has a higher lysine content (Weller, 1957; Purser and Buechler, 1966; Czerkawski, 1976). An information on QCS of abomasum or duodenum contents is now needed.

Table 6. Frequency of the appearance of limiting amino acids (LAA) and the mean quasi-chemical score (QCS) values for faunated (with protozoa in the rumen) and defaunated (without protozoa in the rumen) goats (from Onodera and Koga, 1987)

Presence or absence of protozoa	Ratio of haycube and concentrate in rations					
	3:1			1:1		
	LAA	Appearance (%)	Mean QCS	LAA	Appearance (%)	Mean QCS
First LAA						
Faunated	His	100	64	His	88	59
				Met	12	59
Defaunated	Met	75	66	His	100	65
	His	25	69			
Second LAA						
Faunated	Met	100	76	Met	88	73
				His	12	63
Defaunated	Lys	56	72	Lys	88	71
	His	44	69	Met	12	72
Third LAA						
Faunated	Lys	100	80	Lys	100	86
Defaunated	Lys	42	78	Met	88	75
	Met	29	74	Lys	12	75
	His	29	81			

By the way, in these experiments histidine tended to be the first LAA in both faunated and defaunated rumen contents, in spite of the fact that it was the third LAA in the rations used. This is attributed to that histidine is the first LAA in rumen microbes (QCS: 44 for protozoa and 46 for bacteria, calculated using the data reported by Czerkawski (1976)). Bergen et al. (1968) showed that histidine was the first LAA, when rumen protozoal protein was fed to rats and LAA was determined using the plasma amino acid score method. On the other hand, Nimrick et al. (1970) in sheep and Richardson and Hatfield (1978) in steers

equally demonstrated, on feeding the animals with purified diets containing urea as a sole source of nitrogen, infusing amino acids into the abomasum and measuring the nitrogen retention and plasma amino acid pattern, that the first, second and third LAA of rumen microbes for ruminants were methionine, lysine and threonine, respectively. They also infused histidine and tryptophan into the abomasum, but these amino acids did not improve the nitrogen retention. These speculations led to the conclusion that histidine might not be essential for ruminant animals like adult human beings or its requirement might be so low that it could be met sufficiently by rumen microbes. Bergen et al. (1967) regarded histidine as a non-essential amino acid in their discussion, though they did not mention any reasons. If histidine is not essential for ruminants, lysine apparently tends to be the first LAA in high concentrate-fed defaunated animals. However, Storm and Ørskov (1984) reported that histidine was essential for lambs. Further accurate studies and, if possible, biochemical studies are needed.

3. Issues around the pipecolate

As mentioned above, pipecolate was isolated from the incubation medium of rumen protozoa and crystallized, and its chemical structure was determined and identified as L-(−)-pipecolic acid (Onodera and Kandatsu, 1969). This is also shown to be an end product in lysine metabolism by rumen protozoa (Onodera and Kandatsu, 1972). When pipecolate (10 mmol) was dosed into the goat rumen through rumen fistula immediately after feeding, its concentration in the rumen was 170 μg/g of fresh rumen digesta (FRD) 2 h after dosage, then decreased gradually and reached normal level (around 8 μg/g FRD) 7 h after dosage (Onodera and Tano, 1980). However, recent in vitro experiment showed that added pipecolate (2 mM) was degraded very slowly (up to 0.017 mM/h) and did not affect the concentrations of individual VFA and each free amino acid in both mixed rumen bacterial suspensions and mixed rumen microbial suspension with protozoa during 6 h incubation period (Ohro and Onodera, unpublished). This imino acid, therefore, seemed to be absorbed from the rumen wall and/or to flow into the lower digestive tracts. Little is known regarding the absorption of pipecolate from digestive tracts, but it is thought that it can be transported into blood stream of mouse (Giacobini et al., 1980; Giacobini, 1983).

Pipecolate can also be a good substrate in lysine synthesis in aerobic fungi (Kinzel and Bhattacharjee, 1979) and eugrenids (Rothstein and Saffran, 1963), but there are no reports concerning the lysine synthesis from pipecolate by rumen anaerobic fungi.

One of the most attractive points in physiological roles of pipecolate in the animal body revealed over a past decade is its neurophysiological role in mammalian brain. Giacobini (1983) summarized about physiological activity of pipecolate in the brain that pipecolate physiologically plays a regulatory role on GABAergic neurotransmission by controlling GABA (γ-aminobutyric acid) concentration at the synaptic cleft either by inhibiting its reuptake in both glial cells and endings or by increasing its release (Fig. 4). The latest report (Guiterrez and Delgado-Coello, 1989) indicated that pipecolate was involved in the functioning of GABAergic system and was able to increase the release of GABA stimulated

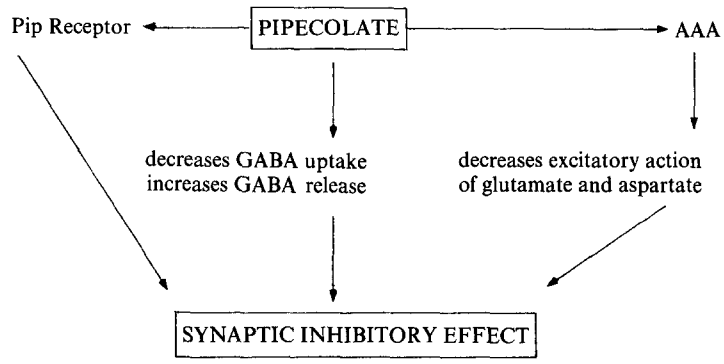

Fig. 4. Proposed alternatives of neuromodulatory action of pipecolate in the central nervous system. *Pip* pipecolate; *AAA* α-aminoadipate; *GABA* γ-aminobutyrate (cited from Giacobini, 1983)

by mild depolarization and inhibit the uptake of GABA in cerebral cortex slices of mouse. Thus they suggested that pipecolate could interact with a probable recognition site of the inactivation GABA system and might be effective amplifier of GABA action at synapses at which GABA is normally released. It is needless to say that GABA is known as an inhibitory neurotransmitter in central nervous system in animals.

Pipecolate is thought to be a major metabolite of lysine in the brain in mammals and birds, while in liver and kidney major pathway of L-lysine catabolism involves saccharopine as an intermediate and the pathway via pipecolate is of minor importance (Giacobini et al., 1980). Giacobini (1983) postulated that pipecolate could cross the blood-brain barrier and enter the brain with an evidence for a brain uptake of pipecolate 1 h after intraperitoneal injection of [^{3}H]pipecolate, and a dietary origin of pipecolate should also be considered in neurophysiology. Intraventricular administration of pipecolate produced hypotonia and sedation accompanied by suppression of flighting behavior in the mouse and caused depression in both electroencephalogram activity and behavior in the cat (Miyata et al., 1973). Effect of pipecolate and hence rumen protozoa on the behaviors of ruminants are not yet examined, though we are just ready to determine the pipecolate in blood and rumen fluid by HPLC (Onodera and Saito, 1992, ready for submission).

L-α-aminoadipate can be produced in small quantity from pipecolate in the brain, and its inhibitory effect on the excitatory properties of glutamate and aspartate in the brain should not be neglected (Giacobini, 1983) (Fig. 4).

A metabolic disorder designated Zellweger syndrome (in other word, a cerebro-hepato-renal syndrome) is characterized by hyperpipecolatemia and mental retardation in infants of both sexes. This is a kind of human genetic disorders. Most cases die within weeks or months and the frequency of the disease has been estimated to be of the order of 1/100,000 births (Giacobini, 1983). Pipecolate accumulates in the body fluids of infants with not only with Zellweger syndrome but also hyperthyroidism, hyperlysinemia, kwashiorkor, neonatal adrenoleukodystrophy and so on. Etiological studies revealed that the peroxisomes were reduced in size and number (Goldfischer et al., 1973; Schut-

gens et al., 1986) and peroxisomal L-pipecolate oxidation was deficient in liver from Zellweger syndrome patients (Mihalik et al., 1989).

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