Formation of Glutamine and Activities of Glutamine Synthetase in Goats

Yu IDE

National Institute of Animal Health, Kodaira-shi, Tokyo 187

Hajime MATSUMOTO

Azabu Veterinary College, Sagami-hara-shi, Kanagawa 229

(Received for publication September 3, 1976)

Abstract. When goats were administered orally with urea in a dose of 0.3 g per kg of body weight, the blood concentration of glutamine-N in the jugular vein increased to about 6 μ g per ml of plasma, reflecting the removal of ammonia by forming glutamine in the animal body. The blood glutamine seemed to undergo a more rapid turnover than the blood urea. Glutamine synthetase catalyzes the formation of glutamine from ammonia and glutamic acid. It was made clear that the activity of the enzyme per gram of tissue was the highest in the spleen, which was followed by the retina, cerebrum, liver, cerebellum and kidney in this regard. The whole tissue activity of the enzyme, however, was higher in the liver than in the spleen. It was considered that the activity of glutamine synthetase in the spleen increased in kids with growth, and that the spleen might play an important role in forming glutamine in adult goats.

Ammonia is produced to a large amount in the rumen by digestion of feed proteins and urea in ruminants [1, 9, 10]. It is known to be readily absorbed from the rumen into the blood stream [1, 10]. In consequence, the removal of this ammonia seems to be of particular importance and interest, as ammonia is very toxic for animals.

Ammonia is considered to be removed by forming urea and glutamine [5]. A few detailed studies, however, have been made on this problem in ruminants. The present studies deal with the activity of glutamine synthetase and the pattern of formation of glutamine in goats administered orally with urea.

Materials and Methods

1. Animals

Adult hybrid Saanen goats weighing 25 to 45 kg were used with their seven kids varying in age. They were fed a ration consisting of hay, wheat bran, corn, and soybean meal, and allowed to have drinking water ad libitum.

2. Procedure of urea administration

The adult goats were administered orally with a dose of 3% solution of urea containing 0.3 g of urea per kg of body weight by the aid of a catheter. After administration, blood samples were collected from them at the jugular vein with a heparinized syringe. They were immediately cooled and centrifuged below 4°C at 600 g for 15 minutes. The resulting plasma was used for subsequent analyses.

3. Assay of glutamine synthetase

The method used was essentially the same as that of Elliot [7]. It was performed in the following conditions: Na-glutamate, 110 mM; hydroxylamine-HCl, 45 mM; Na-ATP (adenosine triphos-

phate), 11 mM; $MgCl_2$, 45 mM; cysteine, 45 mM; and trisaminomethane buffer at pH 7.2, 100 mM. Incubation was carried out at 38°C with 1.75 ml of the substrate solution and 0.5 ml of enzyme preparations. In the course of incubation, 0.45 ml each of the assay solution was withdrawn at 5-minute intervals. The reaction was stopped by the addition of 0.1 ml of a mixture of 24% trichloroacetic acid and 50% HCl (1:1). Zero-time samples were withdrawn for blanks immediately after the addition of the enzyme.

The activity of the enzyme was expressed as micromoles of γ -glutamyl hydroxamic acid (GHA) formed under the assay conditions.

4. Enzyme preparations for assay

The liver, spleen, kidney, cerebrum, cerebellum and retina were removed from each goat which had been killed by cardiac puncture. They were minced and 1 g of each organ was homogenized in 9 ml of ice-cold 0.1 M trisaminomethane buffer at pH 7.2 in a Potter-Elvehjem homogenizer for 3 minutes. The resultant homogenate (1:10 homogenate) was diluted with the same buffer to prepare 1:20, 1:40 and 1:80 homogenates, which were used for assay.

5. Chemical analysis

To a plasma sample was added an equal volume of 20% trichloroacetic acid. The resultant deproteinized solution was used for the determination of ammonia and glutamine.

Ammonia was diffused by the method of Seligson and Seligson [12] and determined with phenol-hypochlorite reagents [13]. Glutamine was determined by estimating the amount of ammonia which had been freed from the deproteinized solution by heating at 70°C for 75 minutes [8, 11]. Urea was determined by the diacetylmonoxime method [6]. In the enzyme assay solution γ -glutamyl hydroxamic acid was colored by the addition of 0.05 ml of ferric chloride reagent [7]. The solution was centrifuged and the supernatant examined for optical density at 500 $m\mu$ with an EPS-3T Hitachi spectrophotometer using microcuvette systems. Synthetic γ -glutamyl hydroxamic acid (Sigma Chemical Co.) was used as standard in this determination.

Results

1. Changes in the blood concentrations of ammonia, glutamine and urea in goats administered with urea

The plasma concentrations of ammonia, glutamine, and urea were found to have been markedly changed by oral urea administration. As shown in Fig. 1, in goat

No. 1 the concentration of ammonia increased rapidly and reached 15 μ g-N per ml of plasma 20 minutes after administration with urea. Then it decreased gradually and returned nearly to the preadministration value 3 hours after administration. The concentration of glutamine was 12 μ g-N before administration with urea. It increased to 18 μ g-N 20 minutes after administration. After that, it decreased to 14 μ g-N, but remained at higher levels than the preadministration value. The concentration of urea increased continuously from 210 to 350 μ g-N after administration with urea.

Goat No. 2 presented essentially the same changes as goat No. 1 in the plasma concentrations of ammonia, glutamine and urea. On the other hand, control goats administered with water exhibited no remarkable changes in the plasma concentration of these substances.

2. The activity of glutamine synthetase in adult goats

The typical results of the assay are shown in Figs. 2a to 2d. Under the assay conditions with 1:40 and 1:80 homogenates of spleen and retina, the enzyme reaction proceeded mostly in proportion to both the concentration of enzyme and the time of incubation for 20 minutes. In the assay

Fig. 1. Changes in the plasma concentration of ammonia, glutamine and urea in the jugular vein after oral administration of urea in goat No. 1

Fig. 2a. Relationship of GHA formation to time and concentration of splenic and retinal homogenates for assay

Remarks.

A, B and C are for the spleen and D, E and F for the retina. Dilution of homogenate: 1:20 for A and D, 1:40 for B and E and 1:80 for C and F.

Fig. 2b. Relationship of GHA formation to time and concentration of cerebral and cerebellar homogenates for assay

Remarks.

A, B and C are for the cerebrum and D, E and F for the cerebellum. Dilution of homogenate: 1:20 for A and D, 1:40 for B and E and 1:80 for C and F.

with 1:20 homogenates, however, the reaction was depressed with the lapse of time. In the liver, the reaction was markedly

Fig. 2c. Relationship of GHA formation to time and concentration of hepatic homogenates for assay

Remarks.

Dilution of homogenate: 1:10 for A, 1:20 for B, 1:40 for C and 1:80 for D.

Fig. 2d. Relationship of GHA formation to time and concentration of renal homogenates for assay

Remarks.

Dilution of homogenate: 1:10 for A and 1:20 for B.

depressed with an increase in the concentration of the homogenate, but was linear up to 10 minutes of incubation. For the cerebrum and cerebellum, the linear reaction was observed to occur with the 1:40 and 1:80 homogenates for 10 to 20 minutes.

The activity of the kidney was relatively low, and an accurate determination was rather difficult. The reaction, however,

Table 1. Activities of glutamine synthetase per gram of wet tissue in adult goats

Goat No.	Sex	Body weight (kg)	Activities ($\mu\text{mol/h/g}$ of tissue)					
			Retina	Spleen	Liver	Cerebrum	Cerebellum	Kidney
1	Female	30	202	308	55	144	60	16
2	..	25	173	413	38	128	75	58
3	..	30	300	480	96	69	43	42
4	..	45	—	324	37	34	14	—
5	..	32	158	464	58	56	26	—
6	..	37	183	408	78	132	41	21
Average			203	400	60	94	43	34
Standard deviation			56	71	23	46	22	19

Table 2. Whole tissue activities of glutamine synthetase per kg of body weight in adult goats

Goat No.	Activities ($\mu\text{mol/h/kg}$ of body weight)*					
	Retina	Spleen	Liver	Cerebrum	Cerebellum	Kidney
1	—	382	825	472	26	50
2	—	578	547	394	36	149
3	—	778	1472	161	17	111
5	—	754	834	117	10	—
6	—	882	1307	321	18	84
Average	—	675	997	293	21	99
Standard deviation	—	197	381	151	10	42

Remarks.

*: Determined by the following formula: (Activities per gram of tissues) $\times$ (Weight of tissues)/Body weight of animals.

Fig. 3. Development of glutamine synthetase in tissues of the kid during growth

proceeded almost in a linear manner with the 1:10 and 1:20 homogenates for 5 minutes of incubation.

The activities of glutamine synthetase determined in six adult goats are shown in Table 1. The activity per gram of tissue

was the highest in the spleen. It was also high in the retina. It was higher in the cerebrum than in the liver, cerebellum and kidney. It was the lowest in the kidney in all the goats examined.

On the basis of body weight, the whole tissue activities of the enzyme were calculated in each animal, as shown in Table 2. They were the highest in the liver. They were higher in the spleen than in the cerebrum. They were lower in the cerebellum and kidney than in the cerebrum.

3. Development of glutamine synthetase activities in kids

The activities of glutamine synthetase were determined on tissues from kids of different age. They were plotted for each animal, as shown in Fig. 3. Generally, they

were lower in the tissues of kids than in those of adult goats, except for the cerebrum. There were no remarkable age differences in the activities of liver or cerebrum among the kids. It seemed, however, that there might be a post-partum increase in the activities of the enzyme in the retina. The high activity of the enzyme seemed to appear in the spleen during growth.

Discussion

The results of the present experiment indicated that the oral administration of urea brought about an increase in the systemic blood concentrations of ammonia, glutamine and urea. The concentration of ammonia increased rapidly and reached a plateau about 20 minutes after the administration of urea. This indicates that the breakdown of urea and the absorption of ammonia from the rumen are considerably rapid. The changes in the concentration of glutamine which occur in response to the administration of urea are a biochemical reflection of the removal of rumen ammonia by forming glutamine in the ruminant. The pattern of changes in the blood concentrations of glutamine and urea implies that the turnover might be more rapid in glutamine than in urea and that nitrogen of glutamine might be catabolized further into urea.

The results of assay of glutamine synthetase indicated that the enzyme activity per gram of tissue was the highest in the spleen of all the organs in adult goats. In rats, this enzyme was found in the retina, liver, brain, kidney, testis and other tissues, of which the retina had its highest activity [3]. In goats, the activity of glutamine synthetase was high in retina, but was higher in the spleen than in the retina.

Buttery [2] determined the glutamine

synthetase activity of the spleen in guinea pigs, mice, rats, pigs, goats, sheep and cattle, of which the ruminants showed the specifically high activity per gram of tissue, as compared with the monogastric animals. From these findings, he pointed out the importance of the role of the spleen in the removal of rumen ammonia by forming glutamine in ruminants. The results of the present investigation are principally compatible with Buttery's findings.

It has been reported that the spleen does not reach the liver, but exceeds the cerebrum in the whole tissue activity of glutamine synthetase. This seems to indicate the importance of the liver and the rank of the spleen which may be next to that of the liver in the formation of glutamine. Ammonia produced in the rumen is known not only to be absorbed into the portal blood stream, but also to pass into the peritoneal fluid and reach the jugular vein without transversing the liver [4]. Probably, the ammonia in the portal blood may be removed to a large amount by the formation of glutamine or urea in the liver, but that in the peritoneal blood may be removed by forming glutamine in many organs, including the spleen.

The results of the present investigation suggest that glutamine synthetase may have developed in the retina and spleen in kids. The development of glutamine synthetase has been reported to occur in the retina and liver in rats after birth and to be subject to the hormonal control [3]. In goats, there may be essentially the same control system for the enzyme in the spleen.

In the ruminant, the function of the rumen develops for several weeks after birth. It is assumed that glutamine synthetase may be produced in the spleen in association with the development of the function of the rumen. Further studies are

needed to verify this assumption.

References

- [1] Annison, E. P. (1956). Nitrogen metabolism in the sheep. Protein digestion in the rumen. *Biochem. J.*, **64**, 705-714.
- [2] Buttery, P. J. (1970). Glutamine synthetase activity in the ruminant spleen. *Biochim. biophys. Acta (Amst.)*, **198**, 616-617.
- [3] Chader, G. J. (1971). Hormonal effects on the neural retina. I. Glutamine synthetase development in the retina and liver of the normal and triiodothyronine-treated rat. *Arch. Biochem. Biophys.*, **144**, 657-662.
- [4] Chalmers, M. I., Jaffray, A. E., and White, F. (1971). Movement of ammonia following intraruminal administration of urea or casein. *Proc. nutr. Soc.*, **30**, 7-17.
- [5] Cohen, P. P., and Brown, G. W., Jr. (1960). Ammonia metabolism and urea biosynthesis. In *Comparative Biochemistry*, vol. 2, Florkin, M., and Mason, H. S., editors, Academic Press Inc., New York, 186-203.
- [6] Coulombe, J. J., and Favreau, L. (1963). A new simple semimicromethod for colorimetric determination of urea. *Clin. Chem.*, **9**, 102-108.
- [7] Elliott, W. H. (1955). Glutamine synthesis. In *Methods in Enzymology*, vol. 2, Colowick, S. P., and Kaplan, N. O., editors, Academic Press Inc., New York, 337-339.
- [8] Harris, M. M. (1943). Studies regarding a glutamine-like substance in blood and spinal fluid, including a method for its quantitative determination. *J. clin. Invest.*, **22**, 569-576.
- [9] Ide, Y. (1975). Quantitative aspect of the endogenous urea cycle in goats. *Jap. J. vet. Sci.*, **37**, 327-333.
- [10] McDonald, I. W. (1952). The role of ammonia in ruminal digestion of protein. *Biochem. J.*, **51**, 86-90.
- [11] Richter, D., and Dawson, R. M. C. (1948). The ammonia and glutamine content of the brain. *J. biol. Chem.*, **176**, 1199-1210.
- [12] Seligson, D., and Seligson, H. (1951). A microdiffusion method for the determination of nitrogen liberated as ammonia. *J. Lab. clin. Med.*, **38**, 324-330.
- [13] Wetherburn, M. W. (1967). Phenol-hypochlorite reaction for determination of ammonia. *Anal. Chem.*, **39**, 971-974.