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Metabolism of L-cysteine in rats fed low and high protein diets.

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Metabolism of L-cysteine in rats fed low and high protein diets.*

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Abstract

L-Cysteine (5.0 mmol per kg of body weight) was intraperitoneally injected into rats fed a 25% casein or 5% casein diet. Concentrations of acidic and neutral amino acids in various tissues were determined 2 h later. In the rats fed the 25% casein diet there was a tendency for tissue amino acid and glutathione levels to be slightly lower than controls. In the 5% casein diet group, however, concentrations of tissue amino acids and glutathione generally increased after L-cysteine administration. S-(2-Hydroxy-2-carboxyethylthio)cysteine (HCETC,3-mercaptolactate-cysteine disulfide), though in trace amounts, was detected in kidney and blood plasma in the 5% casein diet group. Increases in cysteine-glutathione disulfide in liver, kidney and erythrocytes in the 5% casein diet group were considerable. These results indicate that L-cysteine was rapidly metabolized in the 25% casein diet group through the oxidative pathway, while in the 5% casein diet group, in which liver cysteine dioxygenase activity is supposed to be quite low, the oxidative metabolism of L-cysteine decreased and part of the L-cysteine was metabolized through the transaminative pathway. Administration of 15.0 mmol L-cysteine per kg of body weight to rats fed the 25% casein diet resulted in an increase in cysteine-glutathione disulfide in liver, kidney and erythrocytes, and the appearance of HCETC in blood plasma.(ABSTRACT TRUNCATED AT 250 WORDS)

KEYWORDS: cysteine, mercaptolactate-cysteine disulfide, cysteine-glutathione disulfide, oxidative pathway, transaminative pathway

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METABOLISM OF L-CYSTEINE IN RATS FED LOW AND HIGH PROTEIN DIETS

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Abstract. L-Cysteine (5.0 mmol per kg of body weight) was intraperitoneally injected into rats fed a 25 % casein or 5 % casein diet. Concentrations of acidic and neutral amino acids in various tissues were determined 2 h later. In the rats fed the 25 % casein diet there was a tendency for tissue amino acid and glutathione levels to be slightly lower than controls. In the 5 % casein diet group, however, concentrations of tissue amino acids and glutathione generally increased after L-cysteine administration. S-(2-Hydroxy-2-carboxyethylthio)cysteine (HCETC, 3-mercaptolactate-cysteine disulfide), though in trace amounts, was detected in kidney and blood plasma in the 5 % casein diet group. Increases in cysteine-glutathione disulfide in liver, kidney and erythrocytes in the 5 % casein diet group were considerable. These results indicate that L-cysteine was rapidly metabolized in the 25 % casein diet group through the oxidative pathway, while in the 5 % casein diet group, in which liver cysteine dioxygenase activity is supposed to be quite low, the oxidative metabolism of L-cysteine decreased and part of the L-cysteine was metabolized through the transaminative pathway. Administration of 15.0 mmol L-cysteine per kg of body weight to rats fed the 25 % casein diet resulted in an increase in cysteine-glutathione disulfide in liver, kidney and erythrocytes, and the appearance of HCETC in blood plasma. The increase in alanine in these tissues was remarkable. These results indicate that L-cysteine was metabolized actively in these rats. L-Cysteine in such large amounts exceeded the capacity of the oxidative pathway, and it was partly metabolized through the transaminative pathway.

Key words : cysteine, mercaptolactate-cysteine disulfide, cysteine-glutathione disulfide, oxidative pathway, transaminative pathway.

L-Cysteine is mainly metabolized through the pathway in which the sulfur atom is first oxidized by cysteine dioxygenase (EC 1. 13. 11. 20), and the oxidation product, L-alanine 3-sulfinic acid (cysteine sulfinic acid) is further metabolized to sulfate, pyruvate and taurine (1). In the present report this pathway is referred to as the oxidative pathway of cysteine metabolism.

In 1954 Meister *et al.* suggested the presence of the transaminative pathway, an alternative pathway of cysteine metabolism in mammals (2). This is one of pathways in which the sulfur atom is removed from the carbon skelton before oxidation (1, 3). The discovery of a mixed disulfide, S-(2-hydroxy-2-carboxyethylthio)cysteine (HCETC, 3-mercaptolactate-cysteine disulfide) in the urine of

normal human subjects (4) and a patient with β-mercaptolactate-cysteine disulfiduria (5) prompted us to investigate the transaminative pathway in mammalian tissues. Considerable evidence has accumulated which shows that this pathway functions in rat tissues and that HCETC is formed in side reactions of the pathway (3, 6, 7, 8, 12).

In the present study, attempts were made to increase the transaminative metabolism of L-cysteine in order to induce 3-mercaptolactate-cysteine disulfiduria in rats, and to examine the difference in L-cysteine metabolism in rats fed diets having different protein contents.

MATERIALS AND METHODS

Materials. Male rats of the Wistar strain weighing 240-330 g were used. A standard MF diet containing total protein of 24 % and synthetic diets containing 25 % casein (formula B) or 5 % casein (excess casein in formula B was substituted with corn starch) were obtained from Oriental Yeast Co., Ltd., Tokyo, Japan.

L-Cysteine was purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A.

Methods. Rats were fed the MF diet and water ad libitum. Before the administration of L-cysteine, rats of group A were given the 25 % casein diet for 5 days. L-Cysteine (5.0 mmol per kg of body weight) dissolved in water was injected intraperitoneally. Rats were killed by decapitation 2 h after the injection. Preparation of tissue extracts was performed at 0℃. Blood was collected in a beaker containing 0.2 ml of heparin solution (1000 units/ml). Blood plasma was obtained by centrifugation at 1,200 × g for 10 min. Erythrocytes were washed 3 times with 0.9 % sodium chloride-0.1 mM EDTA, pH 7.4 (solution A). White blood cells floated on erythrocytes by centrifugation were eliminated with a pipet. The liver, kidney, heart and whole brain were washed with solution A. Tissues were then homogenized with one volume of solution A and 2 volumes of 6 % sulfosalicylic acid using a Potter-Elvehjem glass homogenizer. The homogenates were centrifuged at 1,200 × g for 20 min. The resulting supernatants were filtered through Whatman No. 1 filter paper. Acidic and neutral amino acids in 0.5 ml of the filtrate were determined with a Hitachi KLA-5 amino acid analyzer using 0.2 N sodium acetate buffer, pH 3.19, at 55℃. Some rats in group A were given an injection of 15.0 mmol L-cysteine per kg of body weight and killed 1, 2 or 3 h later. Tissue amino acid concentrations were determined as above.

Rats of group B were given the 5 % casein diet for 2 days before the administration of L-cysteine. The administration of L-cysteine and amino acid analyses were performed as above.

RESULTS AND DISCUSSION

Table 1 is the summary of amino acid determinations of various tissues 2 h after the intraperitoneal injection of L-cysteine (5.0 mmol per kg of body weight) into rats fed the 25 % casein diet. The table contains values of acidic and neutral amino acids up to valine in the amino acid analysis chart because metabolites of L-cysteine are eluted in this part. Taurine was not calculated because of overlapping with other peaks. As shown in the table, differences between rats with and without L-cysteine administration were not remarkable.

TABLE 1. TISSUE AMINO ACID CONCENTRATIONS AFTER ADMINISTRATION OF L-CYSTEINE (5.0 MMOL PER KG OF BODY WEIGHT) TO RATS FED A 25% CASEIN DIET.[a]

	Asp	Thr + Gln	Ser	Glu	Gly	Ala	Val	Half-cystine	GSH	Cys-SG	HCETC
Liver											
A (4)	0.73 ± 0.07	2.88 ± 0.16	0.47 ± 0.09	1.61 ± 0.29	1.59 ± 0.08	1.67 ± 0.26	0.50 ± 0.09	tr	6.13 ± 0.19	tr	nd
B (2)	0.83 ± 0.09	3.46 ± 0.20	0.26 ± 0.05	2.08 ± 0.53	1.41 ± 0.08	3.56 ± 0.05	0.27 ± 0.01	nd	7.23 ± 0.25	tr	nd
Kidney											
A (4)	1.28 ± 0.11	0.61 ± 0.11	0.63 ± 0.09	2.97 ± 0.08	2.03 ± 0.42	0.72 ± 0.13	0.28 ± 0.04	tr	1.90 ± 0.11	0.07 ± 0.01	nd
B (2)	1.14 ± 0.23	1.02 ± 0.05	0.74 ± 0.07	4.35 ± 0.14	2.44 ± 0.27	0.87 ± 0.01	0.28 ± 0.03	tr	2.37 ± 0.11	tr	nd
Heart											
A (5)	1.13 ± 0.42	3.13 ± 0.29	0.55 ± 0.14	3.38 ± 0.20	0.42 ± 0.05	1.50 ± 0.18	0.15 ± 0.04	nd	1.18 ± 0.03	tr	nd
B (2)	1.12 ± 0.23	4.10 ± 0.07	0.77 ± 0.11	4.13 ± 0.47	0.49 ± 0.05	1.97 ± 0.32	0.14 ± 0.02	nd	1.23 ± 0.16	tr	nd
Brain											
A (5)	2.24 ± 0.37	2.47 ± 0.11	0.59 ± 0.07	5.99 ± 0.30	0.75 ± 0.07	0.55 ± 0.08	tr ~ 0.08	nd	0.93 ± 0.08	tr	nd
B (2)	2.11 ± 0.19	3.04 ± 0.22	0.70 ± 0.02	6.31 ± 0.12	0.85 ± 0.01	0.51 ± 0.05	tr ~ 0.06	nd	1.00 ± 0.02	tr	nd
Erythrocytes											
A (5)	tr ~ 0.09	0.48 ± 0.04	0.22 ± 0.02	0.18 ± 0.07	0.18 ± 0.04	0.25 ± 0.08	nd	nd	*	tr	nd
B (2)	tr ~ 0.04	0.44 ± 0.00	0.20 ± 0.02	0.16 ± 0.05	0.15 ± 0.01	0.25 ± 0.03	nd	nd	#	nd	nd
Blood plasma											
A (4)	tr	0.38 ± 0.07	0.15 ± 0.04	0.04 ± 0.00	0.13 ± 0.02	0.23 ± 0.04	0.12 ± 0.02	0.10 ± 0.05	nd	tr	nd
B (2)	tr	0.42 ± 0.01	0.16 ± 0.03	0.05 ± 0.01	0.15 ± 0.02	0.33 ± 0.01	0.17 ± 0.03	tr	tr	nd	nd

[a] Amino acid concentrations were determined 2 h after an intraperitoneal injection of L-cysteine. Values, expressed as μmol/g wet weight or ml (plasma and packed erythrocytes), are given as the mean ± SD of tissues obtained from different animals. Number of animals are shown in parentheses. A, Cysteine-administered. B, Control. Abbreviations: GSH, glutathione (reduced); Cys-SG, cysteine-glutathione disulfide; HCETC, 3-mercaptolactate-cysteine disulfide; tr, trace; nd, not detected.

*Total glutathione : 1.85 ± 0.40 (estimate); # Total glutathione : 1.55 ± 0.40 (estimate).

There seems, however, to be a tendency for tissue amino acid concentrations of rats injected with L-cysteine to be lower than those of control rats. Decreases in alanine in liver and blood plasma, and glutamate (and possibly glutamine) in liver, kidney and heart were significant.

TABLE 2. TISSUE AMINO ACID CONCENTRATIONS AFTER ADMINISTRATION OF L-CYSTEINE (5.0 MMOL PER KG OF BODY WEIGHT) TO RATS FED A 5% CASEIN DIET.[a]

	Asp	Thr + Gln	Ser	Glu	Gly	Ala	Val	Half-cystine	GSH	Cys-SG	HCETC
Liver											
A (3)	1.29 ± 0.41	3.09 ± 0.94	0.68 ± 0.29	1.50 ± 0.61	2.40 ± 0.35	4.01 ± 1.15	0.28 ± 0.19	tr ~ 0.25	5.88 ± 0.95	0.15 ± 0.06	nd
B (2)	1.04 ± 0.15	3.23 ± 0.03	0.37 ± 0.01	3.28 ± 0.12	1.68 ± 0.49	2.12 ± 0.06	0.15 ± 0.01	tr	3.16 ± 0.21	nd	nd
Kidney											
A (3)	1.60 ± 0.18	0.48 ± 0.04	0.60 ± 0.09	2.58 ± 0.28	1.79 ± 0.27	1.42 ± 0.24	0.16 ± 0.02	0.74 ± 0.25	1.57 ± 0.21	0.16 ± 0.06	tr
B (2)	0.93 ± 0.35	0.53 ± 0.10	0.47 ± 0.06	3.58 ± 0.34	1.86 ± 0.16	0.84 ± 0.34	0.16 ± 0.02	tr	1.77 ± 0.02	0.06 ± 0.03	nd
Heart											
A (3)	0.93 ± 0.09	2.23 ± 0.23	0.36 ± 0.17	2.46 ± 0.32	0.22 ± 0.07	1.41 ± 0.14	0.08 ± 0.03	tr ~ 0.08	1.05 ± 0.08	0.04 ± 0.01	nd
B (2)	0.61 ± 0.19	2.28 ± 0.09	0.30 ± 0.19	2.36 ± 0.32	0.29 ± 0.07	1.38 ± 0.22	0.05 ± 0.02	nd	0.94 ± 0.06	nd	nd
Brain											
A (3)	1.75 ± 0.15	2.14 ± 0.15	0.50 ± 0.07	5.51 ± 0.27	0.75 ± 0.11	0.46 ± 0.05	0.05 ± 0.01	nd	0.96 ± 0.10	tr	nd
B (2)	1.70 ± 0.01	1.92 ± 0.15	0.44 ± 0.03	5.00 ± 0.15	0.77 ± 0.07	0.32 ± 0.02	0.04 ± 0.01	nd	0.75 ± 0.01	tr	nd
Erythrocytes											
A (3)	0.03 ± 0.01	0.76 ± 0.10	0.29 ± 0.04	0.13 ± 0.03	0.14 ± 0.05	0.56 ± 0.16	tr ~ 0.06	tr	*	0.22 ± 0.10	nd
B (2)	0.03 ± 0.02	0.49 ± 0.16	0.17 ± 0.04	0.24 ± 0.01	0.14 ± 0.00	0.30 ± 0.07	tr	nd	#	nd	nd
Blood plasma											
A (3)	tr ~ ± 0.02	0.78 ± 0.10	0.35 ± 0.07	0.08 ± 0.02	0.23 ± 0.08	0.92 ± 0.23	0.15 ± 0.05	1.03 ± 0.25	tr	tr	tr
B (2)	tr	0.53 ± 0.03	0.18 ± 0.02	0.06 ± 0.01	0.22 ± 0.03	0.52 ± 0.10	0.09 ± 0.05	tr	nd	nd	nd

[a]Amino acid concentrations were determined and expressed as in TABLE 1.

A, Cysteine-administered. B, Control. *Total glutathione : 2.06 ± 0.30 (estimate); #Total glutathione : 1.98 ± 0.50 (estimate).

It is notable that half-cystine was not detected in these tissues except for trace amounts in kidney and blood plasma, and that it did not increase in these tissues except for blood plasma even after L-cysteine administration. Glutathione concentrations in liver and kidney decreased slightly after L-cysteine injection.

Cysteine-glutathione disulfide was detected in trace amounts in liver, kidney, heart and brain as reported (9). The concentration of this disulfide increased slightly only in kidney after cysteine administration. HCETC was not detected in tissues of the rats even after L-cysteine administration.

Table 2 summarizes similar amino acid determinations of tissues of rats fed the 5 % casein diet after the administration of 5 mmol L-cysteine per kg of body weight. In contrast to the above results, L-cysteine administration to the rats fed the 5 % casein diet resulted in increases in amino acids and peptides derived from L-cysteine, namely, half-cystine, glutathione, cysteine-glutathione disulfide and alanine in all tissues examined. The increase in alanine and the decrease in glutamate in liver were remarkable.

Cysteine dioxygenase is the key enzyme of the oxidative pathway of cysteine metabolism (10). The activity of this enzyme in rat liver is strongly affected by protein intake (11, 12), and hepatic cysteine dioxygenase activity in rats fed the low protein diet such as 5 % casein diet for 2 days was very low. The activity increased sharply when rats were fed a diet containing protein more than 20 %.

Results obtained with rats fed the 25 % casein diet (Table 1) indicate that the L-cysteine administered was rapidly metabolized through the oxidative pathway. On the other hand, results shown in Table 2 indicate that in rats fed the low protein diet, metabolism of cysteine decreased as indicated by the increase in contents of tissue glutathione, half-cystine and cysteine-glutathione disulfide.

As reported previously, HCETC is formed by side reactions of the transaminative pathway of cysteine metabolism (13, 14). The disulfide was contained in kidney and blood plasma after L-cysteine administration to rats fed the low protein diet (Table 2). However, HCETC was not detected in tissues of rats fed the 25 % casein diet even after L-cysteine administration as shown in Table 1. These results indicate that some part of L-cysteine was metabolized through the transaminative pathway in rats fed the low protein diet. The increase in alanine contents in liver, kidney, brain and erythrocytes and the decrease in glutamate in these tissues appear to support this assumption.

Cysteine-glutathione disulfide has been detected in liver extracts of rats and guinea pigs (15) and in bovine lenses (16). Recently we detected this disulfide in the reaction mixture in which 3-mercaptopyruvate was incubated with rat heart homogenate (13, 14). The disulfide was also detected in liver, kidney, heart and brain (9). Concentrations of this disulfide in these tissues increased after the administration of L-cysteine to rats fed the low protein diet. However, the disulfide did not increase in tissues of rats fed the 25 % casein diet after L-cysteine administration except for kidney, indicating the rapid metabolism of L-cysteine in these rats as described above.

Table 3 is the summary of results when 15.0 mmol L-cysteine per kg of body weight was injected intraperitoneally to rats fed the 25 % casein diet. In these rats, a considerable amount of cysteine-glutathione disulfide was detected in liver, kidney and erythrocytes. HCETC, though in a very small amount, was detected in blood plasma. The increase in alanine in tissues was remarkable. These results indicate that L-cysteine was metabolized actively in these rats. However, the increase in half-cystine and cysteine-glutathione disulfide in tissues and the

appearance of HCETC in blood plasma indicate that 15 mmol L-cysteine per kg of body weight exceeded the capacity of the oxidative pathway, and a portion of the L-cysteine was used for the formation of cysteine-glutathione disulfide and was metabolized through the transaminative pathway.

TABLE 3. TISSUE AMINO ACID CONCENTRATIONS AFTER ADMINISTRATION OF L-CYSTEINE (15.0 MMOL PER KG OF BODY WEIGHT) TO RATS FED A 25% CASEIN DIET.[a]

H	Asp	Thr + Gln	Ser	Glu	Gly	Ala	Val	Half-cystine	GSH	Cys-SG	HCETC
Liver											
1	4.45	2.71	0.82	0.93	1.36	6.10	0.46	0.12	5.72	0.09	nd
2	2.53	3.00	0.83	0.84	1.53	6.24	0.63	0.25	4.26	0.10	nd
3	2.79	4.51	1.58	1.19	1.50	7.60	1.07	0.25	4.10	0.12	nd
Kidney											
1	2.83	0.89	0.76	2.52	1.42	2.04	0.37	1.67	1.67	0.21	nd
2	2.24	0.83	0.71	2.16	1.36	2.64	0.44	1.60	1.06	0.26	nd
3	2.42	1.58	0.90	1.76	1.21	4.05	0.73	1.98	0.84	0.22	nd
Erythrocytes											
1	0.05	0.82	0.37	0.10	0.19	1.26	0.14	0.41	*	0.35	nd
2	0.06	0.93	0.44	0.08	0.31	1.70	0.24	0.41	*	0.40	nd
3	0.06	1.22	0.42	0.04	0.33	2.49	0.38	0.35	*	0.26	nd
Blood plasma											
1	0.26	1.07	0.58	0.21	0.35	1.89	0.43	3.10	nd	tr	tr
2	0.08	1.30	0.64	0.17	0.39	2.66	0.46	3.97	nd	tr	tr
3	0.74	2.44	1.21	0.56	0.54	4.93	1.07	4.02	nd	0.06	tr

[a]Amino acid concentrations were determined 1, 2 and 3h after the intraperitoneal injection of L-cysteine and expressed as in TABLE 1. For controls, see (B) in TABLE 1.
*Could not be calculated exactly because of the overlapping of peaks.

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