

Acta Medica Okayama

Volume 44, Issue 3

1990

Article 2

JUNE 1990

Tissue contents and urinary excretion of taurine after administration of L-cysteine and L-2-oxothiazolidine-4-carboxylate to rats.

Tazuko Taguchi*

Reiko Akagi†

Toshihiko Ubuka‡

*Okayama University,

†Okayama University,

‡Okayama University,

Tissue contents and urinary excretion of taurine after administration of L-cysteine and L-2-oxothiazolidine-4-carboxylate to rats.*

Tazuko Taguchi, Reiko Akagi, and Toshihiko Ubuka

Abstract

Tissue contents and urinary excretion of taurine were studied in rats after the administration of L-cysteine and its derivatives. Average taurine content in the liver of rats fed a 25% casein diet for 7 days increased 2-fold 2h after the intraperitoneal administration of 5 mmol of L-cysteine per kg of body weight, whereas that in rats fed a 5% casein diet for 2 days increased only slightly. The difference in the liver taurine contents between these two groups was discussed in relation to cysteine dioxygenase. Taurine contents in the heart, brain and blood did not differ significantly between these two groups or between the control and the group of rats which received L-cysteine. The increase in liver taurine concentrations after L-cysteine administration was much higher than that after L-cystine administration, suggesting a difference in their absorption. The intraperitoneal administration of 5 mmol/kg of L-2-oxothiazolidine-4-carboxylate (OTCA) resulted in a 3-fold increase in liver taurine content. The average increase in taurine excretion in the 24-h urine after OTCA administration corresponded to about 6.0% and that in the next 24-h urine to about 2.6% of OTCA administered, suggesting that nearly 10% of OTCA was metabolized to taurine and excreted in the urine.

KEYWORDS: taurine, cysteine metabolism, 2-oxothiazolidine-4-carboxylate

*PMID: 2382577 [PubMed - indexed for MEDLINE]

Tissue Contents and Urinary Excretion of Taurine after Administration of L-Cysteine and L-2-Oxothiazolidine-4-Carboxylate to Rats

Tazuko Taguchi^a, Reiko Akagi^b and Toshihiko Ubuka*

Department of Biochemistry, Okayama University Medical School, Okayama 700, Japan

Tissue contents and urinary excretion of taurine were studied in rats after the administration of L-cysteine and its derivatives. Average taurine content in the liver of rats fed a 25 % casein diet for 7 days increased 2-fold 2h after the intraperitoneal administration of 5 mmol of L-cysteine per kg of body weight, whereas that in rats fed a 5 % casein diet for 2 days increased only slightly. The difference in the liver taurine contents between these two groups was discussed in relation to cysteine dioxygenase. Taurine contents in the heart, brain and blood did not differ significantly between these two groups or between the control and the group of rats which received L-cysteine. The increase in liver taurine concentrations after L-cysteine administration was much higher than that after L-cystine administration, suggesting a difference in their absorption. The intraperitoneal administration of 5 mmol/kg of L-2-oxothiazolidine-4-carboxylate (OTCA) resulted in a 3-fold increase in liver taurine content. The average increase in taurine excretion in the 24-h urine after OTCA administration corresponded to about 6.0 % and that in the next 24-h urine to about 2.6 % of OTCA administered, suggesting that nearly 10 % of OTCA was metabolized to taurine and excreted in the urine.

Key words : taurine, cysteine metabolism, 2-oxothiazolidine-4-carboxylate

Taurine is an important end product of cysteine metabolism in mammals (1), and is the second major compound after sulfate among sulfur compounds excreted in the urine (2). Taurine is contained in high concentrations in the central nervous system and muscle, and is

involved in the function of these tissues (3). The present study was undertaken to examine the nutritional status of taurine. Tissue contents and urinary excretion of taurine following the administration of L-cysteine, L-cystine and L-2-oxothiazolidine-4-carboxylate (OTCA), a derivative of L-cysteine (4), are reported here.

*To whom correspondence should be addressed.

a : Present address : Division of Food and Nutrition, Faculty of Junior College, Mimasaka Women's University, Tsuyama 708, Japan.

b : Present address : Faculty of Food and Nutrition, Okayama Prefectural Junior College, 3-1-1 Ishimacho, Okayama 700, Japan.

Materials and Methods

Animals. Male Wistar rats weighing 200-250 g were used. Rats were maintained on a laboratory diet, MF

(protein content, 24%), of Oriental Yeast Co., Ltd., Tokyo, Japan, and water *ad libitum*.

Materials. Casein, corn starch (α), potato starch (α), cellulose powder, a mixture of vitamins and a mixture of minerals were obtained from Oriental Yeast Co., Ltd. Two kinds of synthetic diets, a 25% casein diet and a 5% casein diet, were used in the present study. The 25% casein diet was prepared as reported (5) using the above materials. In the 5% casein diet, 20% casein in the 25% casein diet was replaced with corn starch.

L-Cysteine, free base, was obtained from Sigma Chemical Co., St. Louis, MO, USA. L-Cystine was purchased from Wako Pure Chemical Ind., Ltd., Osaka, Japan. OTCA was obtained from Chemical Dynamics Corp., South Plainfield, NJ, USA.

Feeding of rats and administration of L-cysteine and related compounds. Each rat was housed in a metabolic cage and maintained on the MF diet. L-Cysteine solution (2 mmol/ml), L-cystine suspension (1 mmol/ml), and OTCA solution (2 mmol/ml, neutralized with sodium hydroxide solution) were administered intraperitoneally or into the stomach through a catheter at doses shown below. The latter method of administration is referred to as the intragastric administration. The above solutions of sulfur-containing amino acids were administered to rats fed the MF diet, or rats fed the 25% casein diet for 7 days or the 5% casein diet for 2 days after feeding with the MF diet.

Determination of tissue taurine concentrations. Rats were killed by decapitation 2 h after the administration of sulfur-containing amino acids. When examining changes in the taurine concentration in the blood plasma, rats were killed at various times after the administration. Preparation of tissue extracts was performed at 0–4°C as reported elsewhere (6). Five tenths ml of a tissue extract was applied to a Hitachi KLA-5 amino acid analyzer (Hitachi Seisakusho, Ltd., Tokyo, Japan), which was operated at 55°C using 0.2 N sodium citrate buffer, pH 3.19 at a flow rate of 1.0 ml/min. Taurine was eluted at 36 min. Hypotaurine was not separated from taurine under the present conditions.

Determination of urinary taurine. Twenty-four hour urine was collected in an Erlenmeyer flask containing 5 ml of 50% acetic acid and 5 ml of toluene. Taurine in the collected urine was determined by ion-exchange chromatography and ninhydrin reaction as described (5).

Values are expressed as means \pm SD, and statistical significance was evaluated by Student's *t*-test or by a paired *t*-test.

Results and Discussion

Fig. 1 shows changes in plasma concentrations of taurine and some other amino acids in

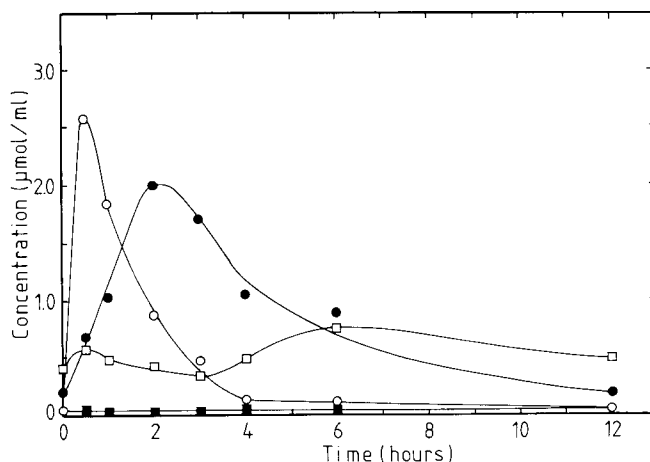


Fig. 1 Plasma concentrations of taurine and some other amino acids after the administration of L-cysteine to rats. L-Cysteine (5 mmol/kg of body weight) was intraperitoneally administered to rats. At the times indicated, the plasma concentration of taurine was determined with an amino acid analyzer. Each value is the mean obtained from 3 animals. Cysteine, \circ — \circ ; taurine plus hypotaurine, \bullet — \bullet ; methionine, \blacksquare — \blacksquare ; alanine, \square — \square .

rats fed MF diet after the intraperitoneal administration of 5.0 mmol/kg body weight of L-cysteine. The cystine concentration increased rapidly following L-cysteine administration and reached the maximum value at 30 min after the administration.

The increase in the taurine concentration followed that of cystine and reached the peak at 2 h after the administration of L-cysteine. As reported previously (7), the blood plasma of the rats after the intraperitoneal administration of L-cysteine contained hypotaurine, which exhibited a maximum value at 2 h after the cysteine loading. The profile of serum hypotaurine exhibited a similar pattern of change as that of serum taurine, and its concentration amounted to about half of that of taurine. In the present study, taurine and hypotaurine were not separately determined, and the values of taurine in this paper represent the total amount of taurine and hypotaurine. According to these data, rats were killed at 2 h after the administration of L-cysteine and other sulfur-containing compounds when tissue taurine contents were determined.

After the L-cysteine loading, a slight increase in alanine occurred after 4 h as shown in Fig. 1. This seems to suggest the formation of alanine from pyruvate, which was formed via a cysteine metabolic pathway other than taurine formation. Fig. 1 also shows that the methionine concentration did not change after the cysteine administration.

Table 1 summarizes taurine contents per g or

ml of tissues after the intraperitoneal administration of 5.0 mmol/kg of body weight of L-cysteine to rats fed the 5 % or 25 % casein diet. The taurine content in the liver of rats fed the 25 % casein diet doubled at 2 h after the L-cysteine loading. The taurine concentration in the blood plasma increased about 3 times. The increase in the taurine contents in the kidney was significantly high. It has been reported that taurine is produced from L-cysteine mainly in the liver in which the activity of cysteine dioxygenase (EC 1.13.11.20) is high (8). The high contents of taurine in the liver after L-cysteine loading seem to agree with these reports. The increase in the taurine concentration in the blood plasma seems to reflect the taurine transport from the liver. It is unknown whether the high contents in the kidney originated from the production in the kidney or the uptake of taurine from serum by this organ, because cysteine dioxygenase is lower in the rat kidney than in the rat liver (8). As mentioned above, hypotaurine increased in the serum after L-cysteine administration to rats, reaching the maximum at 2 h after the administration (7). Therefore, it is possible that hypotaurine is a transport form of the taurine/hypotaurine system.

The average taurine content in the liver of rats fed the 5 % casein diet for 2 days was 66 % of that of rats fed the 25 % casein diet. The taurine concentrations per g or ml of tissues of rats fed the 5 % casein diet did not change significantly

Table 1 Tissue taurine concentrations after the administration of L-cysteine in rats fed the 5 % or 25 % casein diet^a

Tissue	5 % Casein diet group		25 % Casein diet group	
	Control (2)	Cysteine (3)	Control (2)	Cysteine (4)
Liver	6.28 ± 1.57	8.07 ± 0.07	9.45 ± 0.08	20.06 ± 0.63*
Kidney	10.15 ± 0.52	8.76 ± 1.38	10.40 ± 1.60	18.37 ± 1.68**
Heart	20.94 ± 0.98	20.75 ± 1.48	22.96 ± 2.07	21.87 ± 1.91
Brain	3.07 ± 0.46	3.88 ± 0.53	4.04 ± 0.03	4.27 ± 0.43
Blood plasma	0.30 ± 0.01	0.49 ± 0.06***	0.16 ± 0.01	0.52 ± 0.24
Erythrocyte	0.16 ± 0.06	0.12 ± 0.01	0.07 ± 0.05	0.21 ± 0.08

^a : L-Cysteine (5 mmol/kg of body weight) was administered intraperitoneally to rats fed the 5 % or 25 % casein diet. Two hours later, tissue extracts were prepared, and taurine contents were determined with an amino acid analyzer. Numbers of animals are shown in parentheses. Significantly different by Student's *t*-test from the control ; *, *p* < 0.001 ; **, *p* < 0.005 ; ***, *p* < 0.05.

Table 2 Tissue taurine concentrations after the administration of L-cysteine or L-cystine in rats fed the MF diet^a

Tissue	Taurine concentration ($\mu\text{mol/g}$ wet weight or ml)		
	Control (5)	Cysteine (5)	Cystine (5)
Liver	3.68 \pm 1.29	29.66 \pm 2.97*	11.68 \pm 1.20*
Kidney	10.90 \pm 0.80	12.66 \pm 2.08	10.32 \pm 0.40
Blood plasma	0.43 \pm 0.06	1.09 \pm 0.20*	0.53 \pm 0.05**
Erythrocyte	0.28 \pm 0.07	0.23 \pm 0.09	— ^b

a : L-Cysteine (8mmol/kg of body weight) or L-cystine (4mmol/kg of body weight) was administered into the stomach of rats through a catheter. Two hours later, tissue extracts were prepared, and taurine concentrations were determined with an amino acid analyzer. Numbers of animals are shown in parentheses. Significantly different by Student's *t*-test from the control ; *, $p < 0.001$; **, $p < 0.025$.

b : Not determined.

after L-cysteine loading. It has been reported that cysteine dioxygenase in the liver is an inducible enzyme and its activity decreased to a very low level 2 days after the feeding of a low protein diet such as the 5 % casein diet (9). The taurine contents in the rats fed the 5 % casein diet in the present study show that in these rats taurine production was low. The present results also indicate that cysteine dioxygenase is the key enzyme in the formation of taurine from L-cysteine in rats.

Table 2 shows tissue taurine concentrations 2h after the intragastric administration of L-cysteine and L-cystine, 8.0 and 4.0mmol/kg of body weight, respectively, to rats fed the MF diet. The taurine content in the liver increased 8-fold after the L-cysteine administration, and that in the blood plasma increased 2.5-fold. Taurine content in the liver increased after the loading of 4.0mmol of L-cystine (equivalent to 8.0mmol of L-cysteine) per kg of body weight, but the increase was only 3-fold and far less than that after the L-cysteine administration. The increase in the plasma taurine concentration after the L-cystine administration was slight and not significant. These results indicate that L-cysteine was absorbed rapidly and taurine was formed in the liver. The results also seem to suggest that the absorption of L-cystine was slower than that of L-cysteine or the conversion of L-cystine into taurine was less than that of L-cysteine.

Table 3 shows the taurine concentrations in

rat tissues 2h after the intraperitoneal administration of OTCA. The average taurine content in the liver increased significantly to 3 times that of the control.

Fig. 2 shows the excretion of taurine after the intraperitoneal administration of OTCA to rats fed the 25 % casein diet. As shown in the figure, taurine excretion increased significantly in the first and second 24-h urine after OTCA administration. The average increase after 3 administrations was 298.5 and 130.0 $\mu\text{mol/kg}$ of body weight per day in the first and second 24-h urine, respectively, after the OTCA loading. These results indicate that OTCA corresponding to nearly 10 % was metabolized to taurine and excreted in the urine. In the previous study (5), it was shown that L-cysteine administration resulted in the

Table 3 Tissue taurine concentrations in rats after the administration of L-2-oxothiazolidine-4-carboxylate (OTCA)^a

Tissue	Taurine contents ($\mu\text{mol/g}$ wet weight or ml)	
	Control (5)	OTCA (5)
Liver	3.35 \pm 3.24	10.87 \pm 3.94*
Kidney	12.91 \pm 1.94	11.89 \pm 1.51
Blood plasma	0.30 \pm 0.06	0.28 \pm 0.03
Erythrocyte	0.08 \pm 0.01	0.07 \pm 0.02

a : OTCA (5mmol/kg of body weight) was administered intraperitoneally to rats fed the 25 % casein diet. Two hours later, tissue extracts were prepared, and taurine contents were determined with an amino acid analyzer. Numbers of animals are shown in parentheses. Significantly different by Student's *t*-test from the control ; *, $p < 0.02$.

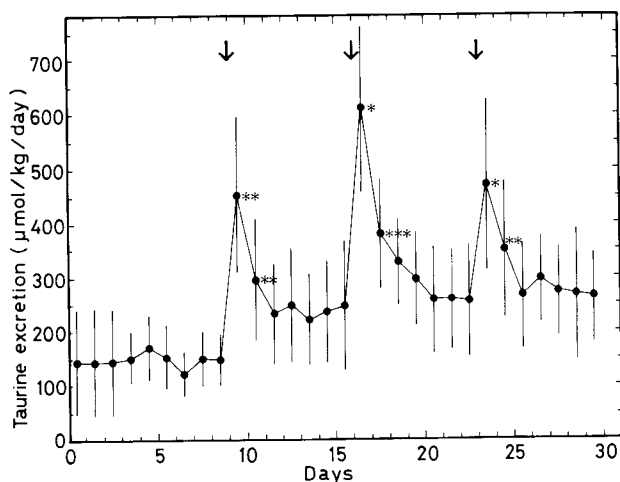


Fig. 2 Urinary excretion of taurine after the administration of L-2-oxothiazolidine-4-carboxylate (OTCA) to rats. Taurine contents in 24-h urine of 5 rats were determined before and after the intraperitoneal administration of OTCA (5 mmol/kg of body weight). Significantly different by a paired *t*-test from the value of the previous day of OTCA administration; *, $p < 0.01$; **, $p < 0.02$; ***, $p < 0.05$.

increase in taurine excretion, corresponding to about 18% of the cysteine loaded.

It has been reported that OTCA was enzymatically hydrolyzed to L-cysteine (4), and that the intraperitoneal administration of OTCA resulted in the increase in the liver glutathione concentration in mice (4, 10) and in guinea pigs (11). The present results show that the administration of OTCA resulted in the increase in tissue contents and excretion of taurine, indicating that OTCA was metabolized via cysteine. Cysteine (4) and cystine (12) are known to be toxic when administered to animals. However, it has been reported that OTCA can protect mice against acetaminophene toxicity (4, 10), and has been suggested that OTCA may be useful as a therapeutic agent for hepatic toxicity. Thus, it seems likely that OTCA may be used as a nutritional alternative for L-cysteine or sulfur-containing amino acids in mammals.

Acknowledgment. The authors are indebted to Dr. Jun Ohta for amino acid analysis and discussion in this study, and to Mrs. Yasuko Tanizaki-Mitsunobu for technical assistance. This work was supported in part by a Grant-in-Aid for Medical Research from the Ministry of Health and Welfare of Japan.

References

1. Griffith OW: Mammalian sulfur amino acid metabolism: An overview; in *Methods in Enzymology*, Jakoby and Griffith eds, Vol 143, Academic Press, New York (1987) pp 366-376.
2. Cavallini D, De Marco C and Mondovi B: Experiments with D-cysteine in the rat. *J Biol Chem* (1958) **230**, 25-30.
3. Hays KC and Sturman JA: Taurine metabolism. *Annu Rev Nutr* (1981) **1**, 401-425.
4. Williamson JM and Meister A: Stimulation of hepatic glutathione formation by administration of L-2-oxothiazolidine-4-carboxylate, a 5-oxo-L-prolinase substrate. *Proc Natl Acad Sci USA* (1981) **78**, 936-939.
5. Yoshida S, Akagi R and Ubuka T: Sulfate and taurine excretion in rats after L-cysteine administration. *Acta Med Okayama* (1989) **43**, 281-288.
6. Hosaki Y, Nishina H and Ubuka T: Free amino acid contents in guinea pig tissues. *Acta Med Okayama* (1985) **39**, 425-429.
7. Yuasa S, Akagi R and Ubuka T: Determination of hypotaurine and taurine in blood plasma of rats after the administration of L-cysteine. *Acta Med Okayama* (1990) **44**, 47-50.
8. Yamaguchi K and Hosokawa Y: Cysteine dioxygenase: in *Methods in Enzymology*, Jakoby and Griffith eds, Vol 143, Academic Press, New York (1987) pp 395-403.
9. Kohashi N, Yamaguchi K, Hosokawa Y, Kori Y, Fujii O and Ueda I: Dietary control of cysteine dioxygenase in rat liver. *J Biochem* (1978) **84**, 159-168.
10. Williamson JM, Boettcher B and Meister A: An intracellular cysteine delivery system that protects against toxicity

- by promoting glutathion synthesis. Proc Natl Acad Sci USA (1982) **79**, 6246-6249.
11. Nishina H, Ohta J and Ubuka T: Effect of L-2-oxothiazolidine-4-carboxylate administration on glutathione and cysteine concentrations in guinea pig liver and kidney. *Physiol Chem Phys Med NMR* (1987) **19**, 9-13.
 12. Earle DP Jr and Victor J: Cirrhosis of the liver caused by excess dietary cystine. *J Exp Med* (1941) **73**, 161-173.

Received February 6, 1990 ; accepted April 3, 1990.