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Production of hypotaurine, taurine and sulfate in rats and mice injected with L-cysteinesulfinate

H. Nakamura, J. Yatsuki, and **T. Ubuka**

Department of Clinical Nutrition, Kawasaki University of Medical Welfare, Kurashiki, Okayama, Japan

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Summary. We studied *in vivo* production of taurine, hypotaurine and sulfate following subcutaneous administration of L-cysteinesulfinate (CSA) to rats and mice. When 5.0 mmol/kg of body weight of CSA was injected to rats, increased urinary excretions of taurine, hypotaurine and sulfate in 24 h urine were 617, 52 and 1,767 μmol/kg, respectively. From these results together with our previous data, sulfate production was calculated to be 1.6 times greater than taurine production. Increased contents (μmol/g of wet tissue) over the control of taurine and hypotaurine in mouse tissues at 60 min after the injection of 5.0 mmol/kg body weight of CSA were: liver, 3.5 and 9.9; kidney, 0.3 and 5.2; heart, 3.7 and 0.2; blood plasma, 0.4 and 0.2, respectively. Upon loading of hypotaurine or taurine, tissue contents of these amino acids in liver and kidney increased greatly. Our results indicate that liver is the most active tissue for taurine production, followed by kidney, and that external CSA, hypotaurine and taurine are easily taken up by these tissues.

Keywords: Amino acids – Cysteine metabolism – Cysteinesulfinate – Hypotaurine – Taurine – Inorganic sulfate

Introduction

Cysteine and methionine, two sulfur-containing amino acids contained in proteins, are taken up by mammals mainly as constituents of dietary proteins. Cysteine is also formed from methionine through transsulfuration pathway in animal tissues. Although methionine is reported to be partly metabolized by transamination reaction (Steele and Benevenga, 1978), main route of methionine metabolism is the transsulfuration pathway, in which methionine sulfur is transferred to cysteine. Cysteine is further metabolized through various pathways (Griffith, 1987). Its final major metabolites are inorganic sulfate and taurine (Griffith, 1987), which are excreted in the urine (Mårtensson, 1982; Nakamura et al., 2002; Tomozawa et al., 1998; Yukihiro et al., 1998). Sulfate is an inorganic sulfur compound which exists in mammals as free (or inorganic) and ester forms. Taurine is an organic sulfonic acid derivative. The oxidation state of sulfur atom of these final metabolites is +6 and that of cysteine is −2. During the transsulfuration process from methionine to cysteine, oxidation state of sulfur does not change. On the other hand, the sulfur atoms of metabolites between cysteine and sulfate or sulfonic acid are in various oxidation states, showing these sulfur atoms are successively oxidized from −2 to +6. It is well known that sulfur is an active element and reacts easily with oxygen, namely, it is easily oxidized. Thus, the metabolites containing sulfur of various oxidation states could act as antioxidants, and thus sulfur-containing metabolites could be considered as endogenous antioxidants in mammalian tissues. Therefore, it is important, from the standpoint of detoxification of reactive oxygen species, to know the metabolic activities of various pathways of cysteine metabolism in mammalian tissues.

The major route of L-cysteine metabolism in mammals is the oxidation pathway, in which L-cysteine is oxidized to L-cysteinesulfinate (CSA) in the reaction catalyzed by L-cysteine dioxygenase [EC 1.13.11.20] (Yamaguchi and Hosokawa, 1987). In this route, cysteine sulfur is oxidized without liberation from the carbon skeleton. CSA is then metabolized through two pathways which are catalyzed by aspartate aminotransferase [EC 2.6.1.1] and CSA decarboxylase [EC 4.1.1.29]. The final metabolites of these pathways are inorganic sulfate and taurine, respectively.

Metabolic pathways of cysteine other than the oxidation pathway may be considered as non-oxidative pathways. The non-oxidative pathways include cysteine metabolism catalyzed by γ-cystathionase [EC 4.4.1.1] (Stipanuk and Beck, 1982) and the pathway initiated by

cysteine transamination (Ubuka et al., 1992). The sulfur compound formed in the γ-cystathionase reaction is hydrogen sulfide. This compound is rapidly oxidized to inorganic sulfate, as shown by experiments using perfused rat tissues (Bartholomew et al., 1980).

We have studied cysteine metabolism initiated by cysteine transamination (Ubuka et al., 1992). The product of this reaction is 3-mercaptopyruvate, and thus, this pathway is called 3-mercaptopyruvate or transamination pathway. We have shown that this pathway is functioning in liver mitochondria and that the final metabolite of cysteine sulfur is inorganic sulfate (Ubuka et al., 1992). We have also reported that administration of L-cysteine and related compounds to rats resulted in rapid excretion of sulfate and taurine (Yoshida et al., 1989; Tomozawa et al., 1998; Yukihiro et al., 1998).

Present paper reports *in vivo* production of taurine studied by determining tissue contents of taurine and hypotaurine when CSA, hypotaurine and taurine were injected to mice. We also studied urinary excretion of taurine and sulfate following CSA administration to rats. In order to conduct the present study, we developed a method for the simultaneous determination of tissue contents of taurine and hypotaurine using reversed phase high-performance liquid chromatography (RP-HPLC) (Nakamura and Ubuka, 2003).

Materials and methods

Animals

Male ICR mice of 8 weeks of age and male Wistar rats of 7 weeks of age were obtained from Japan Clea Corporation (Tokyo, Japan). These animals were maintained on laboratory diet MF of Oriental Yeast Co. (Tokyo, Japan) and water ad lib., and used one or two weeks later.

Materials

CSA, hypotaurine and taurine were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). 4-Dimetylaminoazobenzene-4′-sulfonyl chloride (dabsyl chloride) was the product of Dojin Chemical Laboratory (Kumamoto, Japan). Other chemicals including those for HPLC were analytical grade and purchased from Wako Pure Chemicals Co. (Osaka, Japan).

Administration of CSA, hypotaurine, or taurine to mice and preparation of tissue extracts for the analyses of taurine and hypotaurine

After fasting overnight with free access to water, 0.25 to 0.5 M solutions of CSA (neutralized with sodium hydroxide solution), hypotaurine or taurine at a dose of 5.0 or 10.0 mmol per kg of body weight were injected subcutaneously on the back of mice. The volume of the solution was adjusted to correspond to 2% of body weight.

At 60 min after the injection, mice were anesthetized with ether and blood and tissues were obtained. Blood was taken from dorsal vena cava using a syringe containing EDTA solution as an anticoagulant, and blood plasma was obtained by centrifugation at 3,000 rpm for 10 min at 4 °C. Liver, heart, kidney and skeletal muscle (gastrocnemius muscle) were taken, weighed and stored at minus 60 °C until analysis.

Administration of CSA to rats and collection of urine

CSA solution was injected subcutaneously as above to rats fed laboratory diet and water ad libitum. Each rat was kept in a metabolic cage and 24 h urine was collected in an Erlenmeyer flask containing 1 ml of toluene as antiseptic.

Determination of taurine and hypotaurine

Taurine and hypotaurine were determined by RP-HPLC after derivatization with dabsyl chloride. Previously we developed this method for the simultaneous determination of urinary taurine and hypotaurine (Futani et al., 1994). The previous method was modified for the determination of these metabolites in animal tissues (Nakamura and Ubuka, 2003), and the procedure is briefly described below. Statistical analyses were made by Student's t test.

Preparation of tissue extracts using acetone

An weighed frozen tissue (100–150 mg) was thawed and cut into pieces with scissors and placed in a micro centrifuge tube. Two volumes of 10 mM asparagine (an internal standard) solution and 6 volumes of acetone (minus 20 °C) were added and the mixture was homogenized using Polytron homogenizer at a speed of 3000 rpm for one min, and the resulting homogenate was kept in a freezer at minus 60 °C overnight. After thawing, the homogenate was centrifuged at 10,000 rpm for 10 min at 4 °C, and resulting supernatant was used for derivatization with dabsyl chloride.

Derivatization with dabsyl chloride

To a tissue extract (100 µl) placed in a 20 ml-round bottomed flask, 400 µl of 0.1 M sodium bicarbonate buffer (pH 9.0) and 1.0 ml of 0.2 mM dabsyl chloride solution in acetone (6.5 mg/100 ml). The mixture was heated at 40 °C for 30 min and then evaporated to dryness at 40 °C with a flash evaporator. The resulting residue was dissolved in 300 µl of 70% ethanol, filtered through a 0.2 µm-filter and applied to RP-HPLC.

RP-HPLC

LaChrom chromatography system (Hitachi Seisakusyo, Tokyo, Japan) was used. The system was consisted of a pump L-7100 with a low pressure gradient unit, an UV-VIS detector L-7420 and a data processor D-7500. Column used was TSK-gel ODS-80Ts (4.6 mm ID × 150 mm L) with a guard column TSKguardgel ODS-80Ts (3.2 mm ID × 15 mm L) (Tosoh, Tokyo, Japan) regulated at 20 °C in a column oven 505 (FROM Co, Tokyo, Japan). Solvents used were 50 mM sodium acetate (pH 4.05) (solvent A) and acetonitrile (solvent B). Chromatography was performed with a flow rate of 0.7 ml/min with a gradient of solvent B: 0–3 min, 28% B, 3–23 min, 28–31% B, 23–47 min, 31–32% B.

Determination of urinary taurine and hypotaurine

Urinary taurine and hypotaurine were determined according to the previous method (Futani et al., 1994) with a slight modification: homoserine was used as an internal standard. Chromatography was performed as above.

Results

Taurine and hypotaurine contents in rat and mouse tissues

Table 1 is the summary of taurine and hypotaurine contents in rat and mouse tissues. Heart and skeletal muscles contained high concentrations of taurine both in rats and mice as reported (Jacobsen and Smith, 1968). Taurine con-

Table 1. Taurine and hypotaurine contents in rat and mouse tissues (mean ± SD, μmol/g of wet tissue; n = 5, rats and 6, mice)

Tissue	Taurine		Hypotaurine	
	Rat	Mouse	Rat	Mouse
Liver	1.58 ± 0.50	10.83 ± 1.59	0.18 ± 0.13 (11.4*)	0.33 ± 0.10 (2.8*)
Kidney	10.71 ± 1.18	10.65 ± 1.21	0.36 ± 0.11 (3.4*)	0.45 ± 0.15 (4.7*)
Heart	27.52 ± 3.03	35.03 ± 2.74	0.11 ± 0.06 (0.4*)	0.38 ± 0.07 (1.1*)
Brain	6.34 ± 0.80	–	nd	–
Skeletal muscle	27.99 ± 2.84	42.59 ± 3.89	[a]	[b]
Blood plasma	N	0.31 ± 0.06[c]	N	nd

[a] Trace amount of hypotaurine was detected, but could not be determined exactly
[b] Not detected or trace amount was detected
[c] μmol/ml
[nd] Not detected
[N] Not determined
* % of taurine

tent in the kidney followed those in muscle tissues. Mouse liver contained almost the same concentration of taurine as the kidney does, but taurine concentration in rat liver was much less than that of mouse liver.

In the present study, hypotaurine concentration was determined at the same time with taurine. Hypotaurine contents in mouse liver and kidney and in rat kidney were about 3 to 5% of taurine, but in the rat liver it was more than 10% of taurine. On the other hand, hypotaurine was not detected or negligibly low in rat brain, skeletal muscle of rats and mice and blood plasma of mice.

Contents of taurine, hypotaurine, free and ester sulfate in rat urine after CSA administration

Table 2 is the summary of the excretion (μmol/kg of body weight per 24 h) of taurine, hypotaurine, free and ester sulfate in 24 h urine of rats after subcutaneous administration of 5 mmol of CSA/kg of body weight. In the first 24 h urine after the administration, the increase in the excretion of total taurine (taurine plus hypotaurine) over the control was 669 and that of total sulfate (free plus ester) was 1767. In the next 24 h urine, the increased excretion of total taurine was 46.3, but sulfate excretion did not increase. The ratio of increased excretion of total sulfate and total taurine in the first 24 h urine was 2.64.

It is noteworthy that 55.7 of hypotaurine was excreted in the urine. This seems to indicate that production of taurine occurred actively through hypotaurine formation and that hypotaurine was rapidly oxidized to taurine. As for the sulfate excretion, most of the increased excretion was due to the increase in free sulfate, and the excretion of ester sulfate was unchanged, indicating that detoxification process by sulfate conjugation occurred at a constant rate in the body.

Table 2. Excretion of taurine and hypotaurine in rat urine after L-cysteinesulfinate (CSA) administration (mean ± SD, μmol/24 h per kg of body weight; n = 7)*

	0–24 h urine		24–48 h urine	
	Taurine	Hypotaurine	Taurine	Hypotaurine
Control (A)	354.5 ± 114.6	3.4 ± 4.3	398.3 ± 66.6	5.2 ± 3.9
CSA (B)	971.3 ± 107.6[a]	55.7 ± 11.4[a]	443.8 ± 141.6	5.3 ± 3.8
B–A	616.8	52.3	45.5	0.1
	Free sulfate	Ester sulfate	Free sulfate	Ester sulfate
Control (C)	877.0 ± 157.2	215.9 ± 71.9	797.9 ± 234.9	195.5 ± 38.1
CSA (D)	2,610.5 ± 498.8[a]	249.3 ± 68.5	788.7 ± 140.3	190.4 ± 29.3
D–C	1,733.5	33.4	−9.2	−5.1

* Five mmol of CSA per kg of body weight was injected subcutaneously to each rat, and 24 h urine was collected for 2 days
[a] Significantly different from the control ($p < 0.01$)

Table 3. Taurine and hypotaurine contents in mouse tissues at 60 min after subcutaneous administration of L-cysteinesulfinate (CSA) (mean ± SD, μmol/g of wet tissue)

CSA dose (mmol/kg of body weight)		0	5	10
Number of animals		6	6	6
Liver	Taurine	10.83 ± 1.59	14.34 ± 3.68	21.18 ± 4.30[a,b]
	Hypotaurine	0.33 ± 0.10	10.21 ± 2.75[a]	13.30 ± 1.89[a,b]
Kidney	Taurine	10.65 ± 1.21	10.91 ± 1.32	9.35 ± 1.69
	Hypotaurine	0.45 ± 0.15	5.66 ± 0.80[a]	8.06 ± 1.80[a,b]
Heart	Taurine	35.03 ± 2.74	38.79 ± 3.18	37.14 ± 1.21
	Hypotaurine	0.38 ± 0.07	0.61 ± 0.13[a]	0.58 ± 0.17[a]
Skeletal muscle	Taurine	42.59 ± 3.89	40.14 ± 1.91	44.82 ± 3.68[b]
	Hypotaurine	tr	tr	tr
Blood plasma*	Taurine	0.31 ± 0.06	0.67 ± 0.18[a]	0.80 ± 0.24[a]
	Hypotaurine	nd	0.20 ± 0.11	0.31 ± 0.12

* μmol/ml
[tr] Trace amount of hypotaurine was detected, but could not be determined exactly
[nd] Not detected
[a] Significantly different from the control ($p < 0.01$)
[b] Significantly different from the group received 5 mmol of CSA ($p < 0.05$)

Taurine and hypotaurine contents in mouse tissues after the administration of CSA

As shown in Table 3, changes in taurine and hypotaurine contents were determined in mouse tissues at 60 min after the subcutaneous administration of 5 or 10 mmol of CSA per kg of body weight. In the liver, taurine increased to 1.4 and 2 times the control, respectively. At the same time hypotaurine increased greatly: 30 and 40 times the control values, respectively. In the kidney, hypotaurine increased as in the liver: 11 and 16 times the control. On the other hand, taurine contents in the kidney did not change by CSA administration.

In heart and skeletal muscle both taurine and hypotaurine contents did not change by the administration of CSA.

Taurine content in blood plasma increased 2 and 3 times the control by the administration of 5 or 10 mmol of CSA per kg of body weight, respectively. Likewise, 0.2 and 0.3 μmol/ml of hypotaurine were detected in blood plasma.

Table 4. Taurine and hypotaurine contents in mouse tissues at 60 min after subcutaneous administration of hypotaurine (mean ± SD, μmol/g of wet tissue)

Hypotaurine dose (mmol/kg of body weight)		0	5	10
Number of animals		6	6	6
Liver	Taurine	10.83 ± 1.59	9.48 ± 3.05	9.83 ± 1.64
	Hypotaurine	0.33 ± 0.10	18.75 ± 3.96[a]	28.27 ± 3.72[a,b]
Kidney	Taurine	10.65 ± 1.21	12.96 ± 0.78[a]	12.17 ± 1.34
	Hypotaurine	0.45 ± 0.15	12.13 ± 2.14[a]	24.00 ± 2.09[a,b]
Heart	Taurine	35.03 ± 2.74	35.14 ± 1.77	36.78 ± 1.28
	Hypotaurine	0.38 ± 0.07	0.81 ± 0.23[a]	1.33 ± 0.29[a,b]
Skeletal Muscle	Taurine	42.59 ± 3.89	40.44 ± 1.86	43.57 ± 2.72[b]
	Hypotaurine	tr	tr	tr
Blood plasma*	Taurine	0.31 ± 0.0	1.12 ± 0.10[a]	1.72 ± 0.52[a,b]
	Hypotaurine	nd	1.40 ± 0.43	4.77 ± 1.45[b]

* μmol/ml
[tr] Trace amount of hypotaurine was detected, but could not be determined exactly
[nd] Not detected
[a] Significantly different from the control ($p < 0.01$)
[b] Significantly different from the group received 5 mmol of hypotaurine ($p < 0.05$)

Table 5. Taurine and hypotaurine contents in mouse tissues at 60 min after subcutaneous administration of taurine (mean ± SD, μmol/g of wet tissue)

Taurine dose (mmol/kg of body weight)		0	5	10
Number of animals		6	6	6
Liver	Taurine	10.83 ± 1.59	32.48 ± 4.41[a]	42.09 ± 5.20[a,b]
	Hypotaurine	0.33 ± 0.10	0.03 ± 0.08[a]	0.56 ± 0.20[b]
Kidney	Taurine	10.65 ± 1.21	23.62 ± 1.35[a]	32.60 ± 2.19[a,b]
	Hypotaurine	0.45 ± 0.15	0.46 ± 0.07	0.57 ± 0.06[b]
Heart	Taurine	35.03 ± 2.74	36.50 ± 2.57	39.59 ± 5.99
	Hypotaurine	0.38 ± 0.07	0.38 ± 0.16	0.55 ± 0.11
Skeletal muscle	Taurine	42.59 ± 3.89	45.68 ± 3.25	44.20 ± 0.26
	Hypotaurine	tr	tr	tr
Blood plasma*	Taurine	0.31 ± 0.06	1.46 ± 0.22[a]	4.57 ± 0.71[a,b]
	Hypotaurine	nd	nd	nd

* μmol/ml
[tr] Trace amount of hypotaurine was detected, but could not be determined exactly
[nd] Not detected
[a] Significantly different from the control ($p < 0.01$)
[b] Significantly different from the group received 5 mmol of taurine ($p < 0.05$)

Taurine and hypotaurine contents in mouse tissues after the administration of hypotaurine

In liver and kidney tissues, hypotaurine contents increased greatly after the administration of 5 or 10 mmol of hypotaurine/kg of body weight as shown in Table 4. On the other hand, taurine contents did not increase as hypotaurine in these tissues. In the heart, there was a slight increase in hypotaurine contents, but taurine did not increase as that in liver and kidney. In skeletal muscle taurine and hypotaurine contents did not change. In blood plasma, appreciable amounts of increase in hypotaurine and taurine were observed.

Taurine and hypotaurine contents in mouse tissues after the administration of taurine

As shown in Table 5, taurine contents in liver increased 3 and 4 times the control by the administration of 5 or 10 mmol/kg of taurine. Two and 3 times increase in taurine contents in kidney and 5 and 16 times increase in blood plasma were also observed. On the other hand, in heart and skeletal muscle, taurine contents did not change. Hypotaurine contents also did not change in these tissues.

Discussion

Taurine is one of the important metabolites of L-cysteine in mammals, which is contained in high concentrations in various mammalian tissues (Jacobsen and Smith, 1968). Numerous studies have been performed on the physiological role(s) of taurine. Only clearly established function of taurine is formation of taurobile acids (Hayes and Sturman, 1981), which act for the intestinal digestion and absorption of lipids. Taurine has been assumed to be a candidate for a neurotransmitter, but it has not been established (Wright et al., 1986). In the muscle such as heart and skeletal muscles, taurine contents are especially high as reported in many studies (Jacobsen and Smith, 1968) and also in this report. The mechanism of taurine function in these actively contracting tissues is still unclear.

As for the formation of taurine in mammalian tissues, it has been established that the main pathway is that via CSA which is produced in the oxidation pathway of cysteine metabolism. It has also been established that CSA is metabolized through two pathways, of which initial reactions are transamination and decarboxylation and the final products are sulfate and taurine, respectively.

Griffith studied the metabolic partitioning of CSA between transamination and decarboxylation in mice using L-[1-^{14}C] and L-[3-^{14}C]CSA (Griffith, 1983), and reported that approximately 85% of injected CSA was converted to taurine. Yamaguchi et al. studied the partitioning of cysteine metabolism between taurine and pyruvate pathways in rats using DL-[1-^{14}C], DL-[3-^{14}C] and DL-[U-^{14}C] cysteine (Yamaguchi et al., 1973). They reported that 69% of injected cysteine was metabolized via taurine pathway and 31% via pyruvate pathway when trace amount

of labeled cysteine was administered. They also reported that, upon loading of 50 or 100 mg of L-cysteine per 100 g of body weight, namely 4.13 or 8.25 mmol of L-cysteine per kg of body weight, cysteine metabolism of both pathways were enhanced markedly, but the cysteine metabolism via taurine pathway did not change, namely, 70.8 and 67.5% of L-cysteine was metabolized via this pathway. These studies suggested that taurine formation is greater than sulfate formation in mice and rats.

Ubuka et al. (1992) have accumulated data in rats of urinary excretion of taurine and sulfate following administration of cysteine and related compounds, and have reported that the ratio of taurine and sulfate excreted in the urine is approximately 1: 3–4 (Yoshida et al., 1989; Tomozawa et al., 1998; Yukihiro et al., 1998).

Present study has been performed in order to know the metabolic partition of CSA in rats and to know the participation of mammalian tissues in taurine production from CSA *in vivo*. As shown in Table 2, upon loading of 5 mmol of CSA per kg of body weight, increased excretion of taurine, hypotaurine and sulfate over the control were 617, 52 and 1,767 μmol/kg of body weight per 24 h following CSA administration.

Griffith (1983) reported that in mice about 90% of hypotaurine formed by decarboxylation of CSA was oxidized to taurine and 10% was oxidized to CO_2, suggesting that the latter could be metabolized to sulfate. Eldjahn et al. (1956) reported that in rats 42% of urinary S^{35} following S^{35}-hypotaurine injection was detected as total sulfate. Fujiwara et al. (1995) reported that, upon loading of 5 mmol of hypotaurine per kg of body weight to rats, increased excretion of taurine, hypotaurine and sulfate (mmol per kg of body weight per 24 h) over the value before the loading were 1.5, 1.4 and 0.6, respectively, showing that the sum of increased excretion of taurine and hypotaurine constitute 83% and that of sulfate 17%, and that the ratio of taurine and sulfate production from loaded hypotaurine was 2.5:1, i.e., sulfate: 71.4% and taurine: 28.6%.

From the present data, namely, the excretion ratio of taurine to sulfate was 1:2.64 and the previous data, namely, 28.6% of hypotaurine was converted to sulfate, the rate of taurine and sulfate production from CSA is calculated as 1:1.6. This means sulfate production from CSA calculated by urinary excretion was 1.6 times the taurine production, namely, sulfate production is greater than taurine production at least under the present experimental conditions.

As mentioned above, cysteine is metabolized through non-oxidative pathways besides oxidation pathway. Sulfur atom of cysteine metabolized by these pathways is finally converted to sulfate. Therefore, it is reasonable that the ratio of taurine and sulfate excretion in rats loaded with L-cysteine and cysteine derivatives was 1: 3–4 (Yoshida et al., 1989; Tomozawa et al., 1998; Yukihiro et al., 1998). Thus, it is concluded that sulfate formation is predominant than taurine formation in cysteine metabolism at least estimated from the urinary excretion in rats.

As shown in Table 1, appreciable amount of hypotaurine was detected in the liver and kidney of rats and mice, which corresponded to 2.8 to 1.4% of taurine in these tissues. This indicates that taurine production through hypotaurine proceeds in these tissues.

Loading of CSA to mice resulted in great increase in hypotaurine contents in liver and kidney and taurine contents in the liver (Table 3). Increased contents (μmol/g of wet tissue) over the control of taurine and hypotaurine in mouse tissues ($n = 6$) at 60 min after the injection of 5.0 mmol/kg body weight of CSA were: liver, 3.5 and 9.9; kidney, 0.3 and 5.2; heart, 3.7 and 0.2; blood plasma, 0.4 and 0.2; skeletal muscle, negligibly low, respectively. These results indicate that liver is the most active tissue for taurine production, followed by kidney. This assumption seems to be in accordance with relatively high hypotaurine contents in these tissues as mentioned above. The reason why the taurine content in the kidney did not change as that in the liver is unknown, but it might be explained, at least partly, by a rapid removal of taurine in this tissue or a mechanism to maintain a constant level of tissue taurine.

Loading of hypotaurine to mice resulted in great increase in hypotaurine contents in the liver and kidney, but taurine contents increased only moderately in the kidney and did not increase in the liver (Table 4). The reason of the low level of increase in taurine contents in these tissues is unknown, but it may be explained, at least in part, by the transport of produced taurine into blood as shown in the increase in taurine contents in blood.

Upon loading of taurine to mice (Table 5), taurine contents in the liver and kidney increased greatly, but not in skeletal muscle and heart. These results indicate that taurine is easily transported to liver and kidney. In muscle tissues, it seems that a relatively constant taurine level was maintained.

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Authors' address: Toshihiko Ubuka, MD, PhD, Department of Clinical Nutrition, Kawasaki University of Medical Welfare, 288 Matsushima, Kurashiki, Okayama 701-0193, Japan,
Fax: +81-86-462-1193, E-mail: ubukatos@mw.kawasaki-m.ac.jp