Amino Acids (2002) 23: 447–452
DOI 10.1007/s00726-002-0214-y

Amino
Acids
Printed in Austria

Effect of dietary sulfur amino acids on the taurine content of rat tissues

H. Satsu, Y. Kobayashi, T. Yokoyama, E. Terasawa, and **M. Shimizu**

Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo, Japan

Received January 8, 2002
Accepted January 18, 2002
Published online August 20, 2002;

Summary. The effect of dietary sulfur amino acids on the taurine content of rat blood and tissues was investigated. Three types of diet were prepared for this study: a low-taurine diet (LTD), normal taurine diet (NTD; LTD + 0.5% Met), and high-taurine diet (HTD; LTD + 0.5% Met + 3% taurine). These diets had no differing effect on the growth of the rats. The concentration of taurine in the blood from the HTD- and NTD-fed rats was respectively 1,200% and 200% more than that from LTD-. In such rat tissues as the liver, the taurine content was significantly affected by dietary sulfur amino acids, resulting in a higher content with HTD and lower content with LTD. However, little or no effect on taurine content was apparent in the heart or eye. The activity for taurine uptake by the small intestine was not affected by dietary sulfur amino acids. The expression level of taurine transporter mRNA was altered only in the kidney under these dietary conditions: a higher expression level with LTD and lower expression level with HTD.

Keywords: Taurine – Sulfur amino acid – Transporter – Adaptive response

Abbreviations: HTD, high-taurine diet; NTD, normal taurine diet; LTD, low-taurine diet; TAUT, taurine transporter; CSA, cysteine sulfinate; CDO, cysteine dioxygenase; CSAD, cysteine sulfinate decarboxylase; PBS, phosphate-buffered saline; DIDS, 4,4′-diisothiocyanostilbene-2′,2′-disulfonic acid

Introduction

Taurine is one of the most abundant free amino acids in mammals and is required for a number of biological processes, including antioxidation, osmoregulation, and detoxification (Huxtable, 1992; Wright et al., 1986). Taurine is especially essential to the fetus and newborn for their development (Sturman, 1993). The nutritional and physiological requirements for taurine in mammals are partly supplied by dietary sources and partly by biosynthesis. Dietary taurine is transported into the animal body by the taurine transporter (TAUT) in the intestinal epithelial cells, further transported to various tissues by the blood, and absorbed into the cells by TAUT expressed in the tissues. TAUT has been cloned and characterized from various tissues such as the brain (Smith et al., 1992; Liu et al., 1992), kidney (Uchida et al., 1992), thyroid (Jhiang et al., 1993), and placenta (Ramamoorthy et al., 1994). We have previously characterized human intestinal TAUT by using the Caco-2 cell line as a model of human intestinal epithelial cells (Satsu et al., 1997; Shimizu and Satsu, 2000). TAUT is understood to be regulated by dietary taurine or sulfur amino acids in the rat kidney (Chesney et al., 1985; Han et al., 1997) and by the concentration of extracellular taurine in culture cell lines derived from various tissues (Satsu et al., 1997; Jones et al., 1991; Jayanthi et al., 1995).

On the other hand, taurine biosynthesis has been found to occur most in the liver, brain, and kidney, and the taurine biosynthetic pathway has already been determined (Tsuboyama-Kasaoka et al., 1999; Wu et al., 1998); Cys is converted to cysteine sulfinate (CSA) by cysteine dioxygenase (CDO), and CSA is converted to hypotaurine by cysteine sulfinate dacarboxylase (CSAD), hypotaurine finally being non-enzymatically converted to taurine. Sulfur amino acids such as Met and Cys are therefore necessary materials for taurine biosynthesis. It has also been reported that such taurine biosynthetic enzymes as CDO and CSAD in the rat liver were regulated by dietary sulfur amino acids (Ide et al., 1998).

Therefore, both the TAUT and taurine biosynthetic enzymes are likely to be regulated by dietary sulfur amino acids like taurine, Met, and Cys in animal

Table 1. Composition of the HTD, NTD, and LTD diets

Ingredient	LTD	NTD	HTD
		%	
Corn starch (wt/vol)	67.0	66.5	63.5
Corn oil (wt/vol)	5.0	5.0	5.0
Casein	20.0	20.0	20.0
Salt mix plus vitamin mix	8.0	8.0	8.0
Methionine (wt/wt)	0	0.5	0.5
Taurine (wt/wt)	0	0	3.0

The LTD, NTD, and HTD diets were specially prepared by Oriental Yeast Industries, Tokyo, Japan
The composition of these diets was based mainly on the results of Chesney's work (Chesney, et al., 1986)

bodies. However, it has not yet been revealed whether dietary sulfur amino acids actually affect the taurine content in various tissues *in vivo* or not.

In the present investigation, we examined the effect of a chronic treatment with rat diets containing different amounts of sulfur amino acids on the growth of rats, the concentration of free amino acids in the blood, and the taurine content in various tissues. The effect of this dietary treatment on the TAUT activity in the small intestine and on the expression level of TAUT mRNA in several tissues was also investigated.

Material and methods

Animals and dietary treatments

Male Wistar rats at 3 weeks of age were purchased from Japan Material Center (Tokyo, Japan). The rats received *ad libitum* a laboratory diet (MF; Oriental Yeast Industries, Tokyo, Japan) and water for one week. The rats were then assigned to three taurine groups of 6 rats each. The three taurine groups were respectively fed *ad libitum* for 3 weeks on the low-taurine diet (LTD), normal taurine diet (NTD), and high-taurine diet (HTD). Each group had free access to water. LTD, NTD, and HTD were specially prepared by Oriental Yeast Industries to the compositions shown in Table 1 on the basis of Chesney's work (Chesney et al., 1986).

Materials

[1,2-^{3}H] taurine (specific radioactivity of 29 Ci/mmol) was purchased from Amersham (Little Chalfont, UK), phosphate-buffered saline (PBS) was purchased from Nissui Pharmaceuticals (Tokyo, Japan), and Taq DNA polymerase was from Sigma (St. Louis, MO, U.S.A.). All the other chemicals used were of reagent grade.

Measurement of free amino acids in the blood of the rats

Freshly drawn blood was separated by centrifugation for 5 min at 500 × g, and the serum was collected. The serum was mixed with an equal volume of 10% trichloroacetic acid and centrifuged for 10 min at 10,000 × g. The amino acid content of the supernatant was measured with an L-8500 high-speed amino acid analyzer (Hitachi, Japan).

Measurement of the taurine content in various rat tissues

Six rats in each group were sacrificed and the rat tissues were dissected out, washed with ice-cold PBS, immediately frozen with liquid N_2 and stored at −80°C until needed for analysis. Each frozen tissue sample was homogenized in PBS, and the homogenate was mixed with an equal volume of 10% trichloroacetic acid and centrifuged for 10 min at 10,000 × g. The taurine content of the supernatant was measured with the L-8500 high-speed amino acid analyzer.

Uptake experiment by everted sacs from the rat small intestine

Everted sacs were prepared by a modification of a previously described method (Himukai and Hoshi, 1980; Lostao et al., 1998). Six rats were sacrificed, and the entire small intestine was quickly dissected out. The intestinal tract was washed with ice-cold PBS, and then sacs, each approximately 3 cm long, were prepared from the everted intestine (ten sacs were prepared from each rat). The sacs were filled with an ice-cold potassium phosphate buffer (pH 7.4) consisting of 140 mM potassium gluconate, 1.5 mM calcium gluconate, 1 mM magnesium gluconate and 5 mM glucose (K buffer) for use in the taurine uptake experiment. The uptake experiment was performed at 37°C in 3 ml of the 3 nM [^{3}H] taurine-containing uptake buffer, K buffer or Na buffer, the latter containing 140 mM sodium chloride instead of 140 mM potassium gluconate. At the end of the incubation period, the everted sacs were carefully washed with the ice-cold K buffer, and the radioactivity of the total sac was measured by a scintillation counter. The uptake activity was estimated by comparing the uptake of labeled taurine in two kinds of uptake buffer, the K buffer and Na buffer.

RT-PCR experiments

Total RNA was extracted from rat small intestine, liver, and kidney by using an Isogen RNA isolation kit (Nippon Gene, Japan). Poly(A)$^+$ RNA was further purified from total RNA with an Oligotex dT(30) poly(A)$^+$ RNA purification kit (Takara, Japan). A 1 μg amount of poly(A)$^+$ RNA was reverse-transcribed with $(dT)_{18}$ primers by using a first-strand cDNA synthesis kit (Pharmacia, U.S.A.), and the polymerase chain reaction (PCR) was performed with Taq DNA polymerase. The PCR primers were designed on the basis of the sequence of rat TAUT (Smith et al., 1992): forward primer, 5′-ccaccaaggagaagcttcaatgt-3′; reverse primer, 5′-agcagtgaagtagacaaccttgc-3′; and of rat β-actin: forward primer, 5′-atggatgacgatatcgctg-3′; reverse primer, 5′-atggtagtctgtcaggt-3′. The program consisted of 30 cycles of 1 min at 94°C, 1 min at 61°C, and finally 1 min at 72°C. The PCR product was subcloned into the pGEM vector (Promega, Japan), and its nucleotide sequence was determined and compared with the sequence of rat TAUT and rat β-actin. The PCR product was electrophoresed, photographed, and quantified with Image Quant (v.1.11, Molecular Dynamics, U.S.A.).

Statistical analysis

Each value is expressed as the mean ± S.E., Student's *t*-test being used to compare the means and ranges.

Results

Comparison of the body weight, diet consumption, and water consumption of the rats fed with HTD, NTD, and LTD

The body weight of the rats respectively fed with HTD, NTD, and LTD for 3 weeks was measured. Figure 1 shows that there was no significant difference in body weight among the HTD-, NTD-, and LTD-fed rats. This suggests that the dietary sulfur amino acids have no differing effect on the growth of the rats. Furthermore, neither the diet intake nor water drunk varied among the rats fed with HTD, NTD, or LTD for 3 weeks (data not shown).

Concentration of amino acids in blood from the rats fed with HTD, NTD, and LTD

The concentration of free amino acids in blood from the HTD-, NTD-, and LTD-fed rats was measured and compared. As shown in Fig. 2, the concentration of taurine differed greatly among the HTD, NTD, and LTD rats: the respective concentration of taurine in the HTD and NTD groups was 1,200% and 200% higher than that in the LTD group. The concentration of Met differed similarly to that of taurine, but to a lesser extent, although the concentration of other amino acids was not significantly different among the three taurine groups. This result shows that only the taurine concentration was strongly affected by the dietary sulfur amino acids.

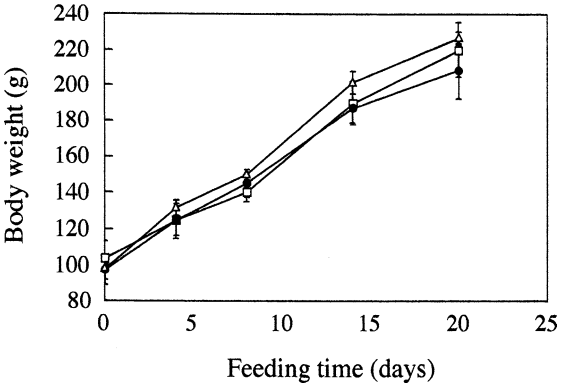

Fig. 1. Effect of dietary sulfur amino acids on the body weight of rats. The body weight of the rats was measured 4, 9, 14, and 20 days after dietary administration of HTD (□), NTD (●), and LTD (△). Each value is the mean ± S.E. (n = 6 different rats)

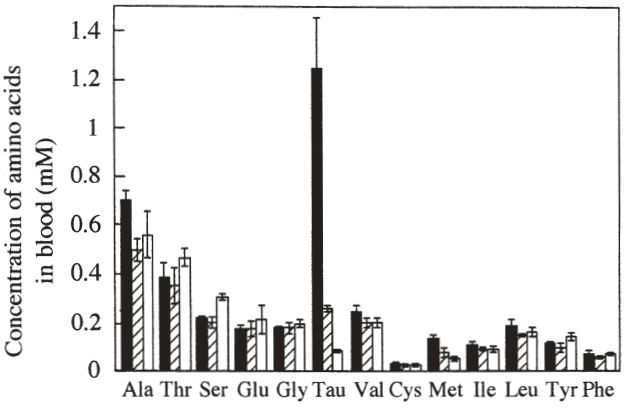

Fig. 2. Concentration of free amino acids in the blood from rats fed with HTD, NTD, and LTD. Rats were fed with HTD, NTD, or LTD for 3 weeks, before blood was withdrawn from the HTD- (■), NTD- (▨), and LTD- (□) fed rats. The concentration of amino acids in the blood was measured as described in the Materials & methods section. Each value is the mean ± S.E. (n = 6 different rats)

Effect of dietary sulfur amino acids on the taurine content of rat tissues

The small intestine, liver, kidney, muscle, brain, heart, lung, spleen, and eye were dissected from rats in the HTD, NTD, and LTD groups, and the taurine content in each of these tissues was measured. The taurine content of the liver was markedly different among the groups: in the HTD- and NTD-fed rats, the taurine content was about 1,300% and 500% more than that in the LTD-fed rats, respectively (Fig. 3A). A significant difference in the taurine content was also observed in the small intestine, kidney, and muscle of the three groups (Figs. 3B, C and D). The taurine content of the brain, lung, spleen, and heart also differed similarly among the groups, although to a lesser extent (Figs. 3E, F, G and H). No difference between groups was apparent with the eye (Fig. 3I).

Effect of dietary sulfur amino acids on the activity of taurine uptake by everted sacs from the rat small intestine

We measured the uptake activity of taurine by using everted sacs from the HTD-, NTD-, and LTD-fed rats to reveal whether or not the dietary sulfur amino acids affected the taurine uptake activity in the small intestine. First, the time dependence of taurine uptake was evaluated, the uptake activity being linear for at least 10 min under these experimental conditions (Fig. 4). Thereafter, we determined that the uptake of taurine

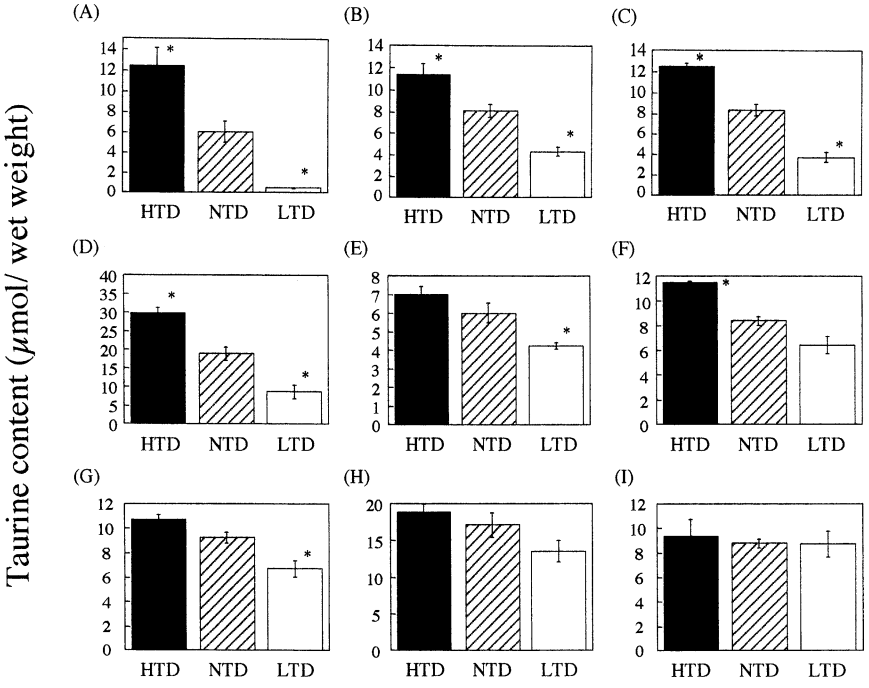

Fig. 3. Effect of dietary sulfur amino acids on the taurine content in various rat tissues. The taurine content in various tissues from the HTD- (■), NTD- (▨), and LTD- (□) fed rats was measured as described in the Materials & methods section. Each value is the mean ± S.E. (n = 6 different rats). Rat tissues are shown as follows: (**A**) liver, (**B**) small intestine; (**C**) kidney, (**D**) muscle, (**E**) brain, (**F**) lung, (**G**) spleen, (**H**) heart, and (**I**) eye. *Significantly different ($p < 0.01$) from the value for NTD

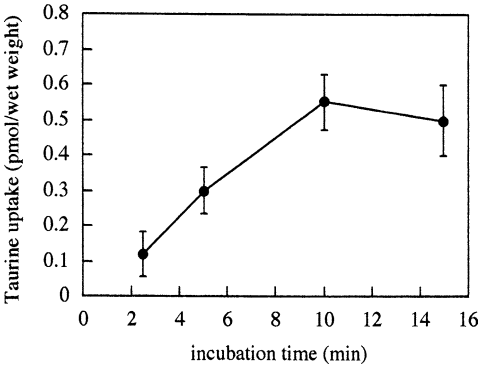

Fig. 4. Time dependence of the taurine uptake in everted sacs from the rat small intestine. Everted sacs were prepared from the rat small intestine as described in the Materials & methods section. These everted sacs were used for measuring the activity of taurine uptake with different incubation times (2.5, 5, 10, and 15 min, respectively). Each value is the mean ± S.E. (n = 6 different rats). The value for one rat is the mean from 7 everted sacs

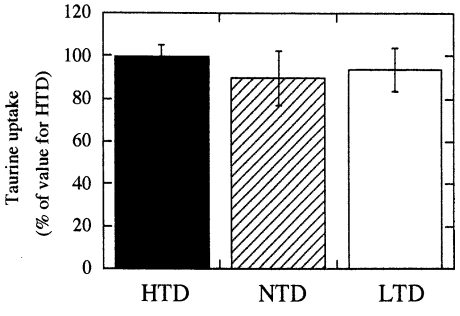

Fig. 5. Effect of dietary sulfur amino acids on the activity for taurine uptake by everted sacs. Everted sacs were prepared from the small intestine of HTD- (■), NTD- (▨), and LTD- (□) fed rats, and uptake experiments were performed at 37°C for 8 min as described in the Materials & methods section. Each value is a percentage of the HTD value and is the mean ± S.E. (n = 6 different rats). The value for one rat is the mean from 7 everted sacs

by the everted sacs would be measured after 8 min of incubation. The activity of taurine uptake was then measured in everted sacs from the HTD-, NTD-, and LTD-fed rats, but no significant difference was apparent (Fig. 5).

Expression level of TAUT mRNA in the small intestine, liver, and kidney of rats fed with HTD, NTD, and LTD

The effect of dietary sulfur amino acids on the expression level of TAUT mRNA in the small intestine, liver, and kidney was also examined. Figure 6 shows that the expression level of TAUT mRNA was not significantly affected by the different diets in the small intestine and in the liver (Figs. 6A and B). On the other hand, the expression level of TAUT mRNA in the kidney was affected by dietary sulfur amino acids,

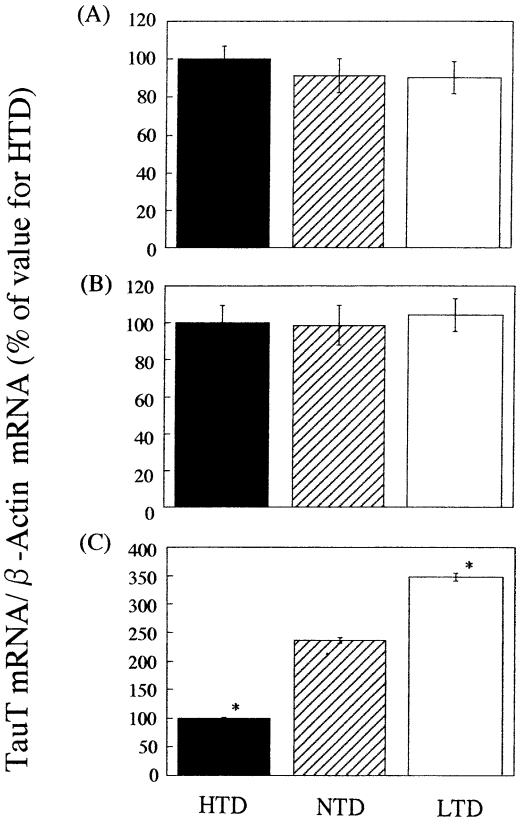

Fig. 6. Effect of dietary sulfur amino acids on the expression level of TAUT mRNA in the small intestine, liver, and kidney. Total RNA was extracted from the small intestine (**A**), liver (**B**), and kidney (**C**) of rats that had been fed with HTD (■), NTD (▨), or LTD (□) and used for an RT-PCR experiment as described in the Materials & methods section. The expression level of TAUT mRNA has been corrected by the expression level of β-actin mRNA. Each value is a percentage of the HTD value and is the mean ± S.E. (n = 6 different rats). *Significantly different ($p < 0.01$) from the value for NTD

giving a lower expression level with HTD, and higher expression level with LTD than that with NTD (Fig. 6C), in agreement with Han's work (Han et al., 1997).

Discussion

The present study examined the effect of dietary sulfur amino acids on the taurine content in rat blood and tissues. The effect of this dietary supplement on TAUT was also investigated.

The effect of the dietary supplement on the taurine content was different among the rat tissues as shown in Fig. 3. The effect on the taurine content by dietary supplementation was most marked in the liver, at least among the tissues examined in this study. On the other hand, little or no effect was apparent in the heart or eye. It remains to be elucidated why the effect of dietary supplementation on the taurine content had this variable effect on different tissues, although this result may suggest the physiological significance and importance of taurine in the eye and heart. It has been reported that taurine is of great importance in the eye for maintaining the structure and function of the photoreceptors on the retina and in the heart for modulating myocardiac muscle contraction by regulating the calcium concentration (Huxtable, 1992). It is thus presumed that the taurine content in those tissues for which taurine has the more vital functions is regulated so as not to be affected by a change of extracellular conditions such as dietary supplementation.

In the liver, the taurine content was extremely changed by dietary supplementation, the similar phenomenone being also reported by Sturman (Sturman, 1973). Sturman also reported that feeding a vitamin B-6-deficient diet resulted in a significantly decrease of taurine content in rat liver and kidney. So it may be hypothesized that the taurine content in the liver is most affected by a change of extracellular conditions such as dietary condition.

To reveal the different response to dietary sulfur amino acids in various tissues, a more detailed investigation at the cellular level is necessary. At the cellular level, the intracellular taurine content is likely to be determined by three factors: the first is taurine absorption into the cells by TAUT; the second is taurine biosynthesis from Met and Cys by such taurine biosynthetic enzymes as CDO and CSAD; and the third is taurine efflux from within the cells. TAUT has already been cloned and characterized, and the taurine biosynthetic pathway has already been identified as already described. However, the taurine efflux pathway has not yet been identified. One candidate molecule involved in taurine efflux is the chloride channel. Sanchez-Olea et al. (Sanchez-Olea et al., 1996) have reported that the efflux of taurine was inhibited by 4,4′-diisothiocyanostilbene-2′,2′-disulfonic acid (DIDS), a blocker of the chloride channel. We have also examined the effect of DIDS on taurine efflux by using human hepatoma HepG2 cell lines. The taurine efflux in HepG2 cells was partly inhibited by DIDS, but not completely (unpublished data). Similar results have also been reported (Sanchez-Olea et al., 1993), suggesting that the chloride channel was involved in taurine efflux, but other unknown molecules are also involved in taurine efflux. It is thus necessary to identify the taurine efflux pathway to

reveal the mechanism for regulating the intracellular taurine content, and further the mechanism for regulating the taurine content in tissues.

Han et al. (Han et al., 1997) have reported that the activity of TAUT in the kidney was regulated by dietary sulfur amino acids. The expression level of TAUT mRNA and protein was also regulated, this same regulation also being shown in our results (Fig. 6-C). In these results, renal epithelial TAUT, which is involved in the reabsorption of taurine, was up-regulated by LTD and down-regulated by HTD. This adaptive response seems to serve to maintain the amount of taurine in the whole body of rats. We therefore hypothesize that intestinal epithelial TAUT also shows a similar adaptive response to that of renal epithelial TAUT. However, the activity of taurine uptake in the small intestine was not altered by dietary supplementation (Fig. 5), nor was the expression level of intestinal TAUT mRNA changed (Fig. 6-A). It is therefore likely that the amount of taurine in the whole body of rats is maintained by the adaptive response of renal TAUT.

In conclusion, we found that dietary sulfur amino acids affected the taurine content in the blood and in several tissues, the response to dietary supplementation being very different among the tissues. This tissue specificity to dietary supplementation with sulfur amino acids may suggest the physiological significance and importance of taurine in each tissue.

References

Chesney RW, Gusowski N, Dabbagh S (1985) Renal cortex taurine content regulates renal adaptive response to altered dietary intake of sulfur amino acids. J Clin Invest 76: 2213–2221

Chesney RW, Lippincott S, Gusowski N, Padilla M, Zelikovic I (1986) Studies on renal adaptation to altered dietary amino acids intake: tissue taurine responses in nursing and adult rats. J Nutr 116: 1965–1976

Han X, Budreau AM, Chesney RW (1997) Functional expression of rat renal cortex taurine transporter in *Xenopus laevis* oocytes: adaptive regulation by dietary manipulation. Pediatr Res 41: 624–631

Himukai M, Hoshi T (1980) Mechanisms of glycyl-L-leucine uptake by guinea-pig small intestine: relative importance of intact-peptide transport. J Physiol 302: 155–169

Huxtable RJ (1992) Physiological actions of taurine. Physiol Rev 72: 101–163

Ide T (1998) Dietary regulation of hepatic enzymes in taurine biosynthesis in rats. Nutr Biochem 9: 99–105

Jayanthi LD, Ramamoorthy S, Mahesh VB, Leibach FH, Ganapathy V (1995) Substrate-specific regulation of the taurine transporter in human placental choriocarcinoma cells (JAR). Biochim Biophys Acta 1235: 351–360

Jhiang SM, Fithian L, Smanik P, McGill J, Tong Q, Mazzaferri EL (1993) Cloning of the human taurine transporter and characterization of taurine uptake in thyroid cells. FEBS Lett 318: 139–144

Jones DP, Miller LA, Dowling C, Chesney RW (1991) Regulation of taurine transporter activity in LLC-PK1 cells: role of protein synthesis and protein kinase C activation. J Am Soc Nephrol 2: 1021–1029

Liu Q-R, Lopez-Corcuera B, Nelson H, Mandiyan S, Nelson N (1992) Cloning and expression of a cDNA encoding the transporter of taurine and β-alanine in mouse brain. Proc Natl Acad Sci USA 89: 12145–12149

Lostao MP, Urdaneta E, Martinez-Anso E, Barber A, Martinez JA (1998) Presence of leptin receptors in rat small intestine and leptin effect on sugar absorption. FEBS Lett 423: 302–306

Ramamoorthy S, Leibach FH, Mahesh VB, Han H, Yang-Feng T, Blakely RD, Ganapathy V (1994) Functional characterization and chromosomal localization of a cloned taurine transporter from human placenta. Biochem J 300: 893–900

Sanchez-Olea R, Pena C, Moran J, Pasantes-Morales H (1993) Inhibition of volume regulation and efflux osmoregulatory amino acids by blockers of Cl^- transport in cultured astrocytes. Neurosci Lett 156: 141–144

Sanchez-Olea R, Morales M, Garcia O, Pasantes-Morales H (1996) Cl channel blockers inhibit the volume-activated efflux of Cl and taurine in cultured neurons. Am J Physiol 270 (Cell Physiol 39): C1703–C1708

Satsu H, Watanabe H, Arai S, Shimizu M (1997) Characterization and regulation of taurine transport in Caco-2, human intestinal cells. J Biochem 121: 1082–1087

Shimizu M, Satsu H (2000) Physiological significance of taurine and the taurine transporter in intestinal epithelial cells. Amino Acids 19: 605–614

Smith KE, Borden LA, Wang C-HD, Hartig PR, Branchek TA, Weinshank RL (1992) Cloning and expression of a high affinity taurine transporter from rat brain. Mol Pharmacol 42: 563–569

Sturman JA (1973) Taurine pool sizes in the rat: effects of vitamin B-6 deficiency and high taurine diet. 103: 1566–1580

Sturman JA (1993) Taurine in development. Physiol Rev 73: 119–147

Tsuboyama-Kasaoka N, Hosokawa Y, Kodama H, Matsumoto A, Oka J, Totani M (1999) Human cysteine dioxygenase gene: structural organization, tissue-specific expression and downregulation by phorbol 12-myristate 13-acetate. Biosci Biotechnol Biochem 63: 1017–1024

Uchida S, Kwon HM, Yamauchi A, Preston AS, Marumo F, Handler JS (1992) Molecular cloning of the cDNA for an MDCK cell Na^+- and Cl^--dependent taurine transporter that is regulated by hypertonicity. Proc Natl Acad Sci USA 89: 8230–8234

Wright CE, Tallan HH, Lin YY (1986) Taurine: biological update. Annu Rev Biochem 55: 427–453

Wu JY, Tang XW, Schloss JV, Faiman MD (1998) Regulation of taurine biosynthesis and its physiological significance in the brain. Adv Exp Med and Biol 442: 339–345

Authors' address: Dr. Hideo Satsu, Laboratory of Food Chemistry, Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan, Fax: +81-3-5841-8026 E-mail: asatsu@mail.ecc.u-tokyo.ac.jp