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Physiological significance of taurine and the taurine transporter in intestinal epithelial cells

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Summary. Taurine transport in human intestinal epithelial Caco-2 cells was down-regulated by culturing the cells in taurine-containing media and was up-regulated in a taurine-free medium. This adaptive regulation was associated with changes in both the Vmax and Km values of taurine transport. A change in the mRNA level of the taurine transporter (TAUT) in this regulation was also observed. The presence of such a regulatory mechanism for maintaining the intracellular taurine content at a certain level suggests that taurine plays an important role in the intestinal cell functions. The intracellular taurine content was increased when Caco-2 cells were exposed to a hypertonic stress. TAUT was up-regulated via the increased expression of TAUT mRNA in the hypertonic cells, suggesting that taurine serves as an osmolyte and protects the cells from osmotic stress. Similar up-regulation of TAUT was observed in the small intestine of water-deprived rats.

Keywords: Amino acids – Taurine – Transporter – Osmoregulation – Small intestine – Caco-2

Introduction

Taurine is one of the most abundant free amino acids in intestinal epithelial cells and is thought to play important roles in the intestinal functions (O'Flaherty, 1997). The cellular taurine content in various tissues is thought to be regulated by a specific transporter (TAUT), as well as by the synthesis of taurine from cysteine. However, the biosynthetic capacity for taurine in humans, and particularly in newborns, is not sufficiently developed (Sturman, 1993). The intestinal absorption of taurine from the diet via TAUT is therefore very important. TAUT is known to be dependent on Na^+ and Cl^- ions, and is a high affinity/low capacity type of transporter (Miyamoto, 1988). The cloning of TAUT has been reported by using several tissues, including

brain (Smith et al., 1992; Liu et al., 1992), thyroid (Jhiang et al., 1993), placenta (Ramamoorthy et al., 1994) and kidney (Uchida et al., 1992). However, intestinal epithelial TAUT has not previously been cloned and its properties are not well understood.

We have recently cloned cDNA of intestinal TAUT for the first time from human intestinal epithelial Caco-2 cells by the RT-PCR method (Satsu et al., 1997), using PCR primers designed on the basis of the sequence of the taurine transporter from human placenta. The taurine transporter cloned from Caco-2 cells has an almost identical sequence to that reported for human placenta. The deduced amino acid sequence suggests that TAUT is a membrane protein with twelve transmembrane domains. This paper discusses the properties of this transporter in terms of its regulation by extracellular conditions.

Materials and methods

Cell culture

Caco-2 cells were cultured with a culture medium consisting of Dulbecco's modified Eagle's medium (DMEM), 10% fetal calf serum (FCS), 1% non-essential amino acids, 2% glutamine, 100 U/ml of penicillin, 100 μg/ml of streptomycin and an appropriate amount of sodium bicarbonate. The cells were incubated at 37°C under a humidified atmosphere of 5% CO_2 in air. Uptake experiments involved Caco-2 cells being cultured in 24-well plates that had been precoated with collagen at a density of 1.4×10^5 cells/well. Cell monolayers for the uptake experiments were used after 14 days of culture.

Uptake experiments

[^{3}H]Taurine uptake experiments were performed in the absence (total uptake) or presence (non-specific uptake) of 50 mM unlabelled taurine, which allowed the specific uptake to be calculated by subtraction. The Caco-2 monolayers were washed twice with 700 μl of phosphate-buffered saline (PBS) for 5 min and once for 15 min with 300 μl of Hank's balanced salt solution (HBSS) containing 4 mM sodium bicarbonate and 10 mM HEPES, the pH being adjusted to 7.4 with KOH (the uptake buffer). The cells were next incubated with 0.3 μCi of [^{3}H]taurine in 300 μl of the uptake buffer, with or without 50 mM taurine, at 37°C for 10 min. At the end of the incubation period, the sample solution was removed, and each monolayer was carefully washed three times with 700 μl of ice-cold PBS containing 0.05% sodium azide for 5 min. To each well was then added 250 μl of 0.1% Triton X-100, and the cell lysate was taken into 3 ml of a scintillation cocktail. The tritium content of each monolayer was finally determined with an LSC 5100 liquid scintillation analyzer (Aloka, Tokyo, Japan).

Measurement of the intracellular content of amino acids

Caco-2 cells were rinsed with PBS and rendered soluble with 0.1% Triton X-100. The cell homogenate was mixed with an equal volume of 10% trichloroacetic acid and centrifuged at 10,000 $\times$ g for 10 min. The amino acid content of the supernatant was measured with an L-8500 amino acid analyzer (Hitachi, Japan).

Animals and diets

Male Wistar rats at 6 weeks of age were purchased from Japan Biological Material Center (Tokyo, Japan). The rats received *ad libitum* a laboratory diet (MF; Oriental Yeast Indust., Tokyo, Japan) and tap water for one week. The rats were then fed on the MF diet with or without water for 72 h.

Uptake experiment by an everted sac from the rat small intestine

The rats were killed and the entire small intestine was quickly removed from each. The intestinal tract was washed with PBS, and then sacs, each approximately 3 cm long, were prepared from the everted intestine (ten sacs from one rat). A sac was filled with a potassium phosphate buffer (pH 7.4) containing 140 mM potassium gluconate, 1.5 mM calcium gluconate, 1 mM magnesium gluconate and 5 mM glucose (the K buffer) for use in the taurine uptake experiment. The uptake experiment was performed at 37°C in 3 ml of the uptake buffer containing an appropriate concentration of [^{3}H]taurine. After incubating for 8 min, the everted sac was thoroughly washed with the 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 labelled taurine in two kinds of uptake buffer, the K buffer and Na buffer, the latter containing 140 mM NaCl instead of 140 mM potassium gluconate.

Northern blot analysis

Poly(A)$^+$ RNA (5 μg) fractionated on 1% agarose gel containing 2.2 M formaldehyde was transferred to a nylon filter (Hybond-N, Amersham) according to the manufacturer's instructions. TAUT mRNA was detected in the Caco-2 cells by hybridizing the filter with human TAUT cDNA that had previously been cloned from human retinal pigment epithelium (Miyamoto et al., 1996) and then labelling by random priming with a [α-^{32}P]dCTP labelling kit (Multiprime, Amersham). Rat TAUT cDNA that had been prepared by RT-PCR was used for hybridization to detect TAUT mRNA in the rat small intestinal epithelium. After treating in a hybridization solution (Rapid, Amersham) at 65°C for 3 h, the filter was washed in 0.1 $\times$ NaCl/Cit containing 0.1% SDS at 65°C.

Statistical analysis

All the values are expressed as means $\pm$ S.E. Student's *t*-test was used to compare means and ranges.

Results and discussion

Regulation of TAUT by extracellular taurine

It is well known that the activity of many nutrient transporters is subject to adaptive regulation. Miyamoto et al. (1993) have reported that the intestinal glucose transporters, SGLT1 and GLUT2, were up-regulated by a high concentration of dietary glucose. Increased expression of the GLUT5 mRNA level by dietary fructose has also been observed (Miyamoto et al., 1993; Burant and Saxena, 1994). We have observed that some of the amino acid transport systems are also subject to adaptive regulation by using an intestinal

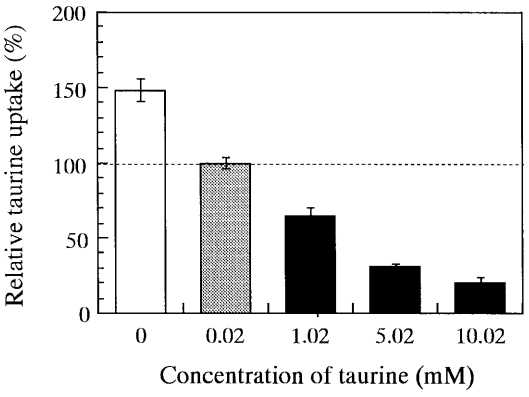

Fig. 1. Relationship between the taurine uptake activity of Caco-2 cells and the concentration of taurine in the culture medium. Cells were precultured for 24 h in a medium containing various concentrations of taurine. The taurine-deficient medium (0 mM taurine) was an FCS-free medium. The medium with 0.02 mM taurine was normal DMEM with 10% FCS. The media with 1.02, 5.02 and 10.02 mM taurine were prepared by adding taurine to normal DMEM with FCS. Each value is the mean ± S.E. ($n = 4$). The values at 0, 1.02, 5.02 and 10.02 mM taurine are all significantly different ($p < 0.01$) from the value at 0.02 mM taurine (control medium)

epithelial cell line (Satsu et al., 1998). These results suggest that intestinal cells possess a mechanism for regulating the transporter activity, thereby controlling the intestinal uptake of dietary nurtrients.

To reveal the regulatory mechanism for taurine transport in intestinal cells, the effect of taurine concentration in the culture medium on the taurine uptake activity of Caco-2 cells was first investigated. The cells were cultured in a serum-free medium (Hashimoto and Shimizu, 1993) without taurine for 24 h, and then the taurine uptake activity of the cells was measured. The results show that the activity was increased to approximately 150% of the control level by this treatment (Fig. 1). On the other hand, when taurine was added to the medium to give a final taurine concentration of 1, 5 or 10 mM, the taurine uptake activity was markedly decreased in a concentration-dependent manner (Fig. 1). Similar down-regulation of TAUT in kidney LLC-PK1 cells and placental JAR cells has been reported by Jones et al. (1991) and Jayanthi et al. (1995), respectively. However, the taurine concentration required to induce the down-regulation in Caco-2 cells was much higher than that in JAR cells. A 70% suppression of the taurine uptake activity was observed in JAR cells that had been treated with only 250 μM taurine for 24 h, while treatment with 10 mM taurine for 24 h was required to give a similar effect on Caco-2 cells, suggesting the operation of different regulatory mechanisms. This down-regulation of TAUT in Caco-2 cells was only observed with taurine and hypotaurine, although β-alanine induced the down-regulation of TAUT to a small degree (data not shown). Interestingly, γ-aminobutyric acid (GABA) did not induce such down-regulation of TAUT, although GABA is also a substrate of this transporter.

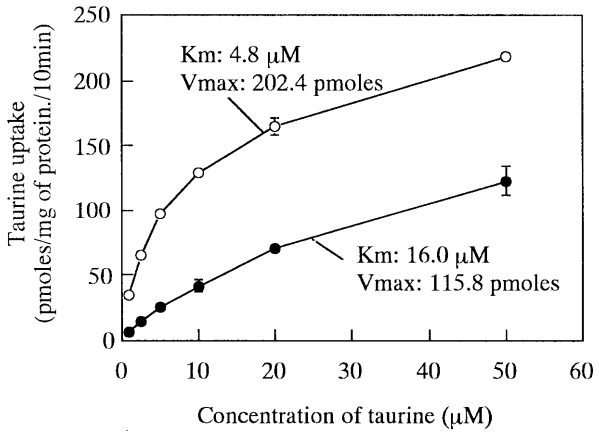

Fig. 2. Kinetic analysis of the taurine uptake by control and taurine-treated cells. Cells were precultured in DMEM with 10% FCS (○) or in the same medium containing 10 mM taurine (●) for 24 h. Each value is the mean ± S.E (n = 4)

Kinetic analyses of the taurine transport in Caco-2 control cells and taurine-treated cells were performed. The down-regulation of TAUT caused by a high concentration of taurine was accompanied by an increase in Km value and decrease in Vmax value (Fig. 2), suggesting that the affinity of the transporter had been reduced and that the number of transporters available on the cell surface had decreased. To determine whether or not the down-regulation was accompanied by a change in the expression level of TAUT, Northern blot analysis was performed. The results show that the mRNA level was markedly lower in those cells that had been cultured with 10 mM taurine for 24 h than in the control cells (data not shown). This low level for the transcripts indicates that the down-regulation occurred at least at the transcriptional level. To examine whether the protein synthesis of TAUT was involved in this down-regulation, the effect of cycloheximide (CHX) was also studied. As shown in Fig. 3, incubation with 100 mM CHX did not affect the taurine uptake activity in the control cells, but it significantly reduced the degree of taurine-induced down-regulation, suggesting that the synthesis of a protein, probably a regulatory protein that decreased the affinity of TAUT, was involved in this down-regulation. It appears important to determine whether such a regulatory protein truly exists or not, but we have not yet succeeded in identifying the protein.

Regulation of TAUT by hypertonic stress

One of the cellular functions of taurine is thought to be osmoregulation. Changes in the activity of TAUT in intestinal Caco-2 cells cultured under hypertonic conditions were therefore studied (Satsu et al., 1999). Human intestinal Caco-2 cells were incubated with different concentrations of raffinose for 2 days and then the taurine uptake activity was measured. As

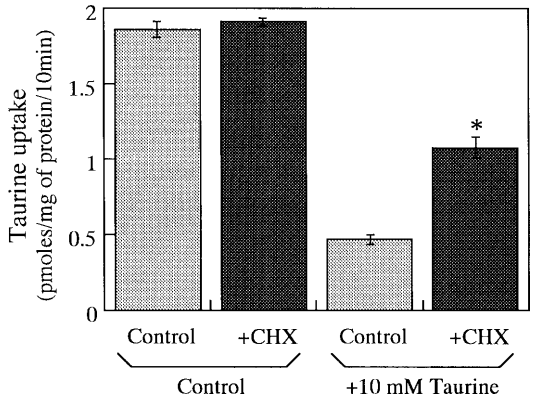

Fig. 3. Effect of cycloheximide (*CHX*) on the taurine-induced down-regulation of taurine uptake. Caco-2 monolayers were preincubated with 100 mM CHX for 3 h. The medium was then changed to a fresh culture medium containing 10 mM taurine and 100 mM CHX. Taurine uptake was measured after 24 h. Each value is the mean ± S.E (n = 4). *Significantly different ($p < 0.01$) from the control (without CHX treatment)

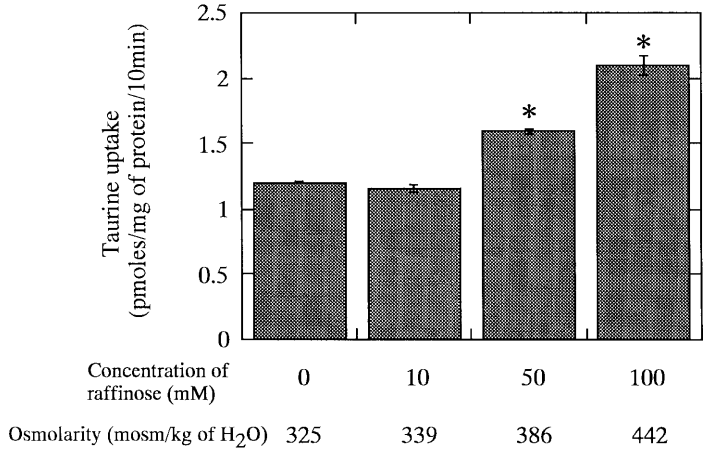

Fig. 4. Concentration dependence of the osmotic regulation in Caco-2 monolayers. Cells were precultured for 24 h in a medium containing 10, 50 or 200 mM raffinose. Each value is the mean ± S.E (n = 4). *Significantly different ($p < 0.01$) from the control (isotonic condition)

shown in Fig. 4, the TAUT activity was markedly increased by increasing the osmolarity of the culture medium.

This up-regulation caused by hypertonic stress was specifically observed with TAUT, while the uptake of other amino acids such as Leu, Lys and Glu did not change under the same hypertonic condition (Fig. 5). The peptide (Gly-Gln) uptake activity of Caco-2 cells rather decreased, although the reason of this decrease has not been revealed. To confirm the specific response of TAUT to hyperosmotic stress, the intracellular amino acid concentrations were measured before and after a hypertonic treatment

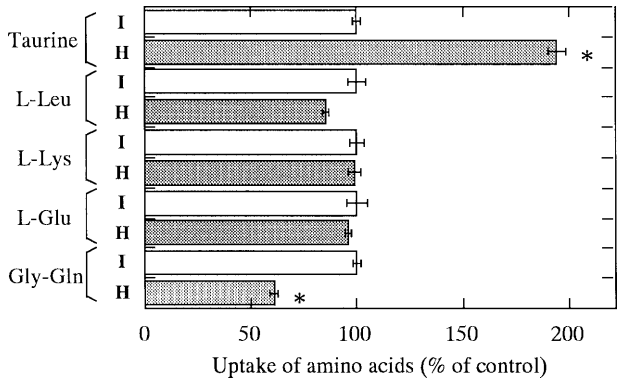

Fig. 5. Effect of hypertensive and isotonic media on the uptake of various amino acids and peptide. Cells were preincubated for 48 h in a medium with 100 mM raffinose (*H*) or without (*I*). Each value is the mean ± S.E (n = 4). *Significantly different ($p < 0.01$) from the control (isotonic condition)

(100 mM raffinose for 48 h). While the intracellular concentrations of other amino acids did not change, apart from the concentrations of Met and Thr being slightly increased, the taurine concentration was markedly increased (to more than 200% of the control value), indicating that the up-regulation of TAUT occurred in a specific manner.

Kinetic analyses indicated that Vmax for the taurine uptake increased in the hypertonic cells, while the affinity of TAUT was not significantly different from that in the control cells (data not shown), suggesting that this up-regulation was accompanied by an increase in the number of transporters. Osmoregulation involving an increase in the maximal velocity of taurine transport without any change in the affinity of the transporter has previously been observed in kidney cells (Uchida et al., 1991), and this was true with our results as well. The expression level of TAUT mRNA was then examined by Nothern blot analysis. The mRNA level was markedly higher in those cells cultured with the hypertonic medium (100 mM raffinose for 24 h) than in the control cells, suggesting that this up-regulation occurred at least at the transcriptional level. We have tried to determine the amount of transporter proteins by using specific antibodies, but this has so far not been successful, probably because of the generally low expression level of the TAUT protein in Caco-2 cells.

Although our results suggest that the hypertonically induced up-regulation of TAUT was at least partly due to the increased transcription of the transported gene, the mechanism for this increase is still not understood. In studies on the response of yeast cells to hypertonic conditions, the involvement of a certain MAP kinase (MAPK) in the osmosis-related signal transduction has been observed (Han et al., 1994). Furthermore, Denkert (1998) has recently reported that the MAPK family protein, p38, was involved in the hypertonically induced up-regulation of such other osmolyte transporters as betaine and myoinositol transporters in the human monocyte.

However, the involvement of MAPK in the hypertonically induced up-regulation of TAUT in Caco-2 cells has not been observed in our experiments. Further studies on the mechanism are in progress.

Osmoregulation of TAUT in the rat intestine

Male Wistar rats at 7 weeks of age were kept without water for 72 h, and the osmotic pressure of the blood serum was then measured. The osmotic pressure was increased from 376.7 ± 11.2 to 444.8 ± 16.8 (mOsm/kg of H_2O) by this treatment, indicating that the water deprivation induced a hypertonic condition in the rats. The taurine uptake activity in the intestinal mucosa was significantly higher in the water-deprived rats (Fig. 6). The expression level of TAUT mRNA in rat intestinal mucosa was examined by a Northern blot analysis. As shown in Fig. 7, the mRNA level was markedly higher in the intestine obtained from the water-deprived rats, whereas β-actin transcripts

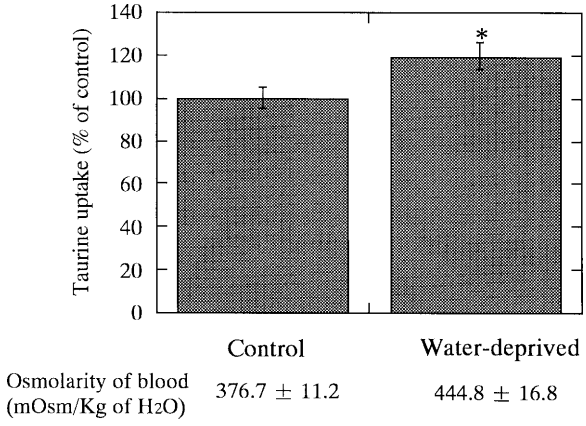

Fig. 6. Taurine uptake in the small intestine from control and water-deprived rats. Each value is the mean ± S.E from six different rats, the value from one rat being the mean of 7 everted sacs. *Significantly different ($p < 0.05$) from the control value

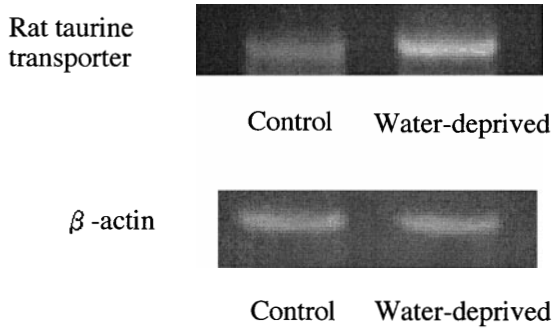

Fig. 7. Expression level of rat taurine transporter mRNA in the small intestine from control and water-deprived rats

did not affect the expression level. Moyer et al. (1988) have reported that the rat is not an appropriate animal for studying the intestinal taurine uptake because the Na^+- and Cl^--dependent taurine uptake was very low in adult rats (e.g., 7 weeks of age). However, our study shows that 7-week-old rats expressed sufficiently high activity of Na^+- and Cl^--dependent taurine uptake. Furthermore, the present results demonstrate that TAUT in the rat small intestine is an osmoregulatable transporter, in good agreement with the response of TAUT in human intestinal Caco-2 cells to hypertonic stress.

Although the mechanism for the osmoregulation of TAUT in intestinal cells is still equivocal, it is obvious that intestinal epithelial cells possess a sophisticated system to regulate the activity of taurine uptake. This means that intracellular taurine plays a crucial role in protecting the intestinal cells and in maintaining the cell functions, and therefore its concentration is always monitored and regulated.

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