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Hypertonicity stimulates taurine uptake and transporter gene expression in Caco-2 cells

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Abstract

The osmoregulation of taurine transport in intestinal epithelial cells was investigated using human intestinal Caco-2 cells. The activity of taurine transport in the Caco-2 cells was increased by hypertonic conditions. This hypertonicity-induced up-regulation was dependent on both the culturing time and the osmotic pressure. Hypertonicity did not affect the activity of L-leucine, L-lysine, or L-glutamic acid transport, suggesting that osmoregulation was specific to taurine transport. The intracellular taurine content of Caco-2 cells was also increased by culturing in a hypertonic medium. These hypertonicity-induced changes in the intracellular taurine content and transport activity were reversible. A kinetic analysis of taurine transport in the control and hypertonic cells suggested that the up-regulation was associated with an increase in the amount of the taurine transporter. The mRNA level of the taurine transporter in hypertonic cells was markedly higher than that in the control cells, indicating that this osmotic regulation was due to the increased expression of the taurine transporter gene.

Keywords: Taurine; Caco-2; Small intestine; Transporter; Osmoregulation

1. Introduction

Organisms as diverse as bacteria and mammals accumulate intracellular organic osmolytes when confronted with an increase in extracellular osmolarity [1]. In mammalian cells especially, the extracellular hypertonicity is balanced by maintaining a high intracellular content of non-perturbing osmolytes like betaine, *myo*-inositol, glycerophosphorylcholine (GPC), sorbitol, and taurine [1,2]. The intracellular accumulation of such of these osmolytes as betaine, *myo*-inositol, and taurine is performed by membrane transporters [3]. It has been found in many cells that hypertonicity induced the activity of these osmolyte transporters [3], this osmotic response being performed to maintain the cell volume against exposure to the hypertonic environment.

The osmotic regulation of osmolyte transporters has been studied in several tissues [3]. Since kidney medulla is the only tissue that normally becomes hypertonic as part of the urinary concentrating

Abbreviations: DMEM, Dulbecco's modified Eagle's medium; NEAA, non-essential amino acids; FCS, fetal calf serum; PBS, phosphate-buffered saline; HBSS, Hanks' balanced salt solution; HEPES, 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; BGT-1, betaine transporter from Madin-Darby canine kidney cells; AR, aldose reductase; TONE, tonicity-responsive element; ORE, osmotic-response element

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mechanism, osmoregulation of the osmolyte transporters has been extensively investigated using kidney cells [2,4,5]. However, the osmotic response in the small intestine has not previously been investigated. Intestinal epithelial cells are thought to have an opportunity of being exposed under hypertonic conditions when the digested food substances come into the luminal tract of the small intestine. In fact, it has been reported that the volume of intact crypts isolated from the guinea pig small intestine was changed by exposure to hypertonicity [6]. However, it is not known whether some of the organic osmolytes work to maintain the volume of intestinal epithelial cells or not. Among the osmolyte transporters, the taurine transporter is present in the small intestine [7], whereas the betaine transporter (BGT-1) and *myo*-inositol transporter are not expressed [3,8,28]. Taurine is therefore presumed to behave as an osmolyte in the small intestine, and the activity of taurine transport in small intestinal epithelial cells could be regulated by extracellular osmolarity.

In the present investigation, the hypertonicity-induced change in the activity of taurine transport was studied using Caco-2, the human intestinal epithelial cell line. This cell line spontaneously differentiates and exhibits various enterocytic characteristics, including brush-border membrane enzymes and nutrient transporters [9]. We have previously reported that Caco-2 expressed a taurine transporter and that the resulting transport activity was subject to adaptive regulation [7]. Caco-2 is therefore thought to be a good model for studying the osmotic regulation of taurine transport in human intestinal epithelial cells.

2. Materials and methods

2.1. Materials

The Caco-2 cell line was obtained from American Type Culture Collection (Rockville, MD, USA). Dulbecco's modified Eagle's medium (DMEM) was purchased from Nissui Pharmaceuticals (Tokyo, Japan). Fetal calf serum (FCS), L-glutamine, and penicillin-streptomycin (10 000 U/ml and 10 mg/ml in 0.9% sodium chloride, respectively) were purchased from Gibco (Gaithersburg, MD, USA), and non-essential amino acids (NEAA) were purchased from Cosmobio (Tokyo, Japan). [1,2-^{3}H]Taurine (specific radioactivity, 29 Ci/mmol), L-[4,5-^{3}H]leucine (specific radioactivity, 155 Ci/mmol), L-[4,5-^{3}H]lysine monohydrochloride (specific radioactivity, 85.0 Ci/mmol), L-[G-^{3}H]glutamic acid (specific radioactivity, 49.0 Ci/mmol) and [α-^{32}P]dCTP were all from Amersham (Little Chalfont, UK). All the other chemicals used were of reagent grade.

2.2. Cell culture

Caco-2 cells were cultured in 78.5 cm^2 plastic dishes with a culture medium consisting of DMEM, 10% FCS, 1% NEAA, 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 in a humidified atmosphere of 5% CO_2 in air, the culture medium being renewed on alternate days. After they had reached confluence, the cells were sub-cultured (1:2) after trypsinization with 0.1% trypsin and 0.02% EDTA in phosphate-buffered saline (PBS). All the cells used in this study were between passages 38 and 70. Uptake experiments used Caco-2 cells cultured in 24-well plates that had been precoated with collagen at a density of 1.4×10^5 cells/well. The cell monolayers for the uptake experiments were used after 14 days of culture.

2.3. Uptake experiments

[^{3}H]Taurine uptake experiments were performed in the absence (total uptake) or presence (non-specific uptake) of 50 mM unlabelled taurine, this allowing the specific uptake to be calculated by subtraction.

The Caco-2 monolayers were washed twice with 700 μl of PBS for 5 min, and then once with 300 μl of Hanks' balanced salt solution (HBSS) containing 4 mM sodium bicarbonate and 10 mM HEPES, the pH value being adjusted to 7.4 with KOH (uptake buffer), for 15 min. The cells were next incubated with 0.3 μCi of [^{3}H]taurine in 300 μl of the uptake buffer, with or without excess (50 mM) taurine, at 37°C for 10 min. At the end of the incubation period the buffer 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, before the dissolved cells were 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).

2.4. Measurement of the intracellular content of amino acids

The cells were rinsed twice 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 × *g* for 10 min. The amino acid content of the supernatant was measured with an L-8500 high speed amino acid analyzer (Hitachi, Japan).

2.5. Northernblot analysis

Poly(A)$^+$ RNA (5μg) 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. The filter was hybridized with human taurine transporter cDNA that had previously been cloned from human retinal pigment epithelium [10] and labelled by random priming with a [α-^{32}P]dCTP labelling kit (Multiprime, Amersham). After treating in a hybridization solution (Rapid, Amersham) at 65°C for 3 h, the filter was washed in 0.1 × NaCl/Cit containing 0.1% SDS at 65°C.

3. Results

3.1. Osmoregulation of the taurine uptake by Caco-2 cells under various hypertonic conditions

After culturing for 14 days, the Caco-2 cells were incubated with 100 mM raffinose, sucrose, sorbitol, mannitol or urea for 48 h and then the taurine uptake was measured. The activity of taurine uptake was up-regulated by culturing with raffinose, sucrose, sorbitol and mannitol, reaching nearly 200% of the control value in the case of raffinose (Fig. 1). Pretreatment with urea, which can permeate into cells and cannot produce hypertonic conditions, had no effect on the taurine uptake by Caco-2 (Fig. 1).

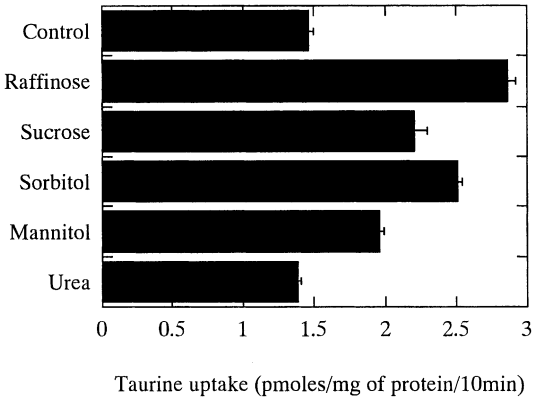

Fig. 1. Osmoregulation of the taurine uptake by Caco-2 monolayers. Cells were precultured in a medium containing 100 mM raffinose, sucrose, sorbitol, mannitol, or urea for 48 h. The taurine uptake was then measured. Each value is the mean ± S.E.M. ($n = 4$).

3.2. Time and dose dependence of the hypertonicity-induced up-regulation of taurine uptake by Caco-2 cells

The culture medium was changed to one containing 100 mM raffinose. The monolayers were incubated for 12–96 h and then uptake experiments were performed. As shown in Fig. 2, the transport activity increased with increasing time of incubation with 100 mM raffinose.

Caco-2 cells were also pretreated with 0, 10, 50, 100 or 200 mM raffinose (osmolarity of 325, 339, 386, 442, or 547 mosm/kg of H_2O, respectively) for 48 h, and then the activity of taurine uptake was

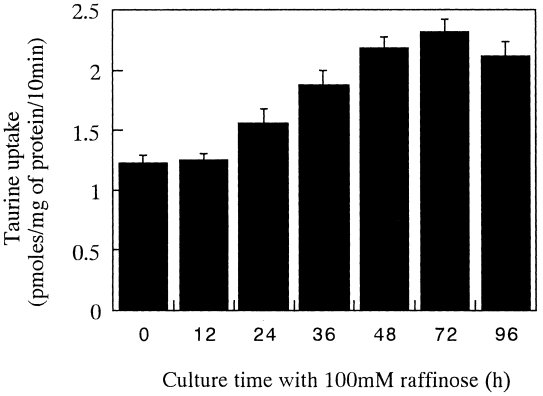

Fig. 2. Effect of the culture time with 100 mM raffinose on the taurine uptake by Caco-2 monolayers. Cells were precultured in the medium with 100 mM raffinose for various times (0–96 h), uptake experiments then being performed as described in Section 2. Each value is the mean ± S.E.M. ($n = 4$).

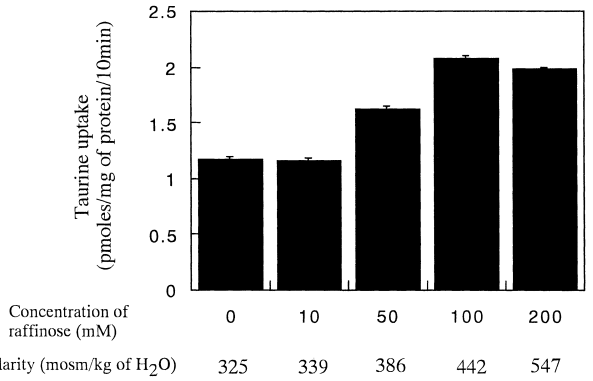

Fig. 3. Concentration dependence of the osmotic regulation in Caco-2 monolayers. Cells were precultured for 24 h in a medium containing 10, 50, 100, or 200 mM raffinose. Uptake experiments were then performed as described in Section 2. Each value is the mean ± S.E.M. ($n = 4$). The osmolarity values of the media containing various concentration of raffinose (0–200 mM) were measured with an osmotron (Orion Riken).

determined (Fig. 3). The results show that the up-regulation of taurine transport was dependent on the osmotic pressure.

3.3. Specificity of the taurine uptake due to hypertonicity

In addition to taurine, some of the amino acids are known to behave as osmolytes [11]. We therefore investigated the uptake activity of L-leucine (Leu), L-lysine (Lys), and L-glutamic acid (Glu) by Caco-2

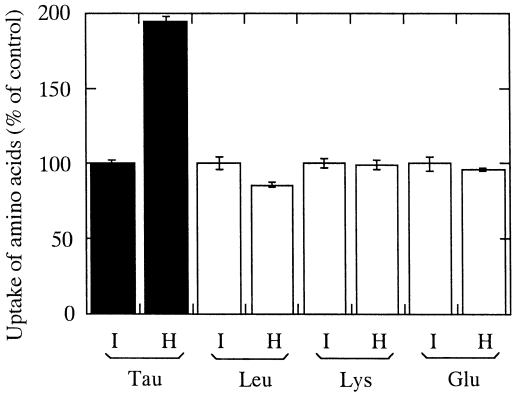

Fig. 4. Effect of hypertonic and isotonic media on the uptake of various amino acids. Cells were precultured for 48 h in a medium with 100 mM raffinose (H) or without (I). Uptake experiments were then performed as described in Section 2. Each value is the mean ± S.E.M. ($n = 4$).

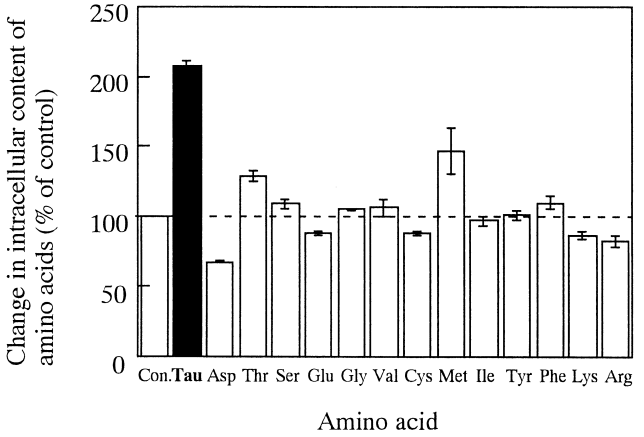

Fig. 5. Changes in the intracellular amino acid contents of Caco-2 monolayers cultured with 100 mM raffinose. Cells were precultured for 48 h in a medium containing 100 mM raffinose. The intracellular content of each amino acid was measured as described in Section 2. The value of each amino acid in hypertonic cells represents the relative amount to that in isotonic cells. Each value is the mean ± S.E.M. ($n = 6$).

cultured in a hypertonic medium. Fig. 4 shows that the uptake activity of Leu, Lys and Glu was not influenced at all, and that only the taurine uptake was up-regulated in Caco-2 cells.

3.4. Change of the intracellular amino acid content in Caco-2 cells cultured with 100 mM raffinose

If taurine really functions as an osmolyte, its concentration should be elevated in hypertonic cells. The intracellular amino acid content of hypertonic and isotonic cells was therefore measured. Among 14 amino acids, only the taurine content markedly increased in hypertonic cells, reaching almost 200% of the control value (Fig. 5). This result suggests that taurine acted as an osmolyte in Caco-2 cells.

3.5. Reversibility of the hypertonicity-induced increase in taurine uptake and intracellular taurine content

The taurine uptake activity and the intracellular content of taurine were measured after substituting the hypertonic medium with an isotonic medium. Fig. 6A shows that the uptake activity of taurine decreased after changing the hypertonic medium to an isotonic one. The intracellular content of taurine also decreased, nearly to the control level, 72 h after changing the medium (Fig. 6B).

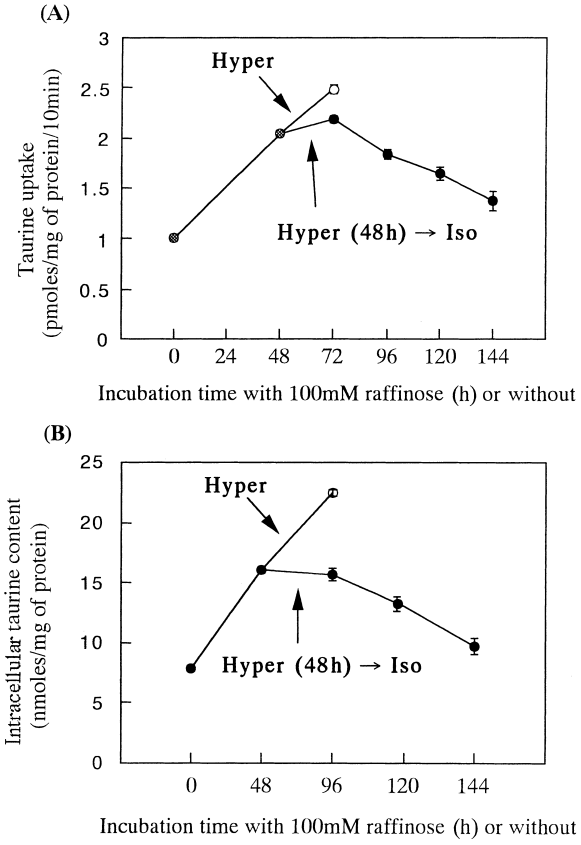

Fig. 6. Taurine uptake activity and intracellular taurine content after substituting the hypertonic medium with an isotonic one. After cells had been precultured in the medium with 100 mM raffinose for 48 h, the medium was changed to an isotonic one and the incubation was conducted. Uptake experiments were then performed and the taurine content measured as described in Section 2. Each value is the mean ± S.E.M. ($n = 4$).

3.6. *Kinetics of taurine uptake in Caco-2 cells cultured under hypertonic conditions*

A kinetic analysis of the taurine transport activity was performed on cells cultured in hypertonic and isotonic media. The V_{max} value for the isotonic cells was 259 pmol/mg of protein/10 min and the K_m value was 5.9 μM. On the other hand, the V_{max} and K_m values for the cells due to hypertonicity were 418 pmol/mg of protein/10 min and 7.1 μM, respectively (Table 1). These results indicate that the osmoregulation of taurine uptake was associated with an increase in the maximal velocity of taurine transport, but without any change in the affinity of the taurine transporter.

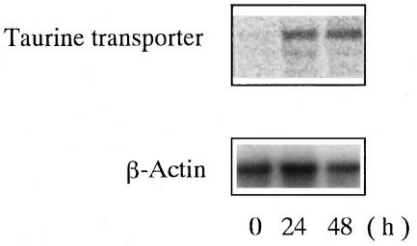

Fig. 7. Northern blot analysis of mRNA from Caco-2 cells cultured with the hypertonic medium. 5 μg of poly(A)$^+$ RNA isolated from Caco-2 cells cultured with 100 mM raffinose for different times (0, 24 or 48 h) was subjected to a Northern blot analysis. β-Actin cDNA was used as the control.

3.7. *Expression level of taurine transporter mRNA in Caco-2 cells cultured under hypertonic conditions*

To determine whether or not the osmotic regulation was accompanied by a change in the expression level of taurine transporter mRNA, a Northern blot analysis was performed. Taurine transporter cDNA cloned from human retinal pigment epithelial cells was used as a probe to perform the Northern-blot analysis. 5 μg of poly(A)$^+$ RNA extracted from Caco-2 cells that had been cultured in a hypertonic medium for 24 and 48 h was used. Fig. 7 shows that the mRNA level was markedly higher in those cells cultured with the hypertonic medium than in the control cells, whereas β-actin transcripts did not affect the expression level. The high mRNA level for the transcripts suggests that this up-regulation of the taurine transporter occurred at least at the transcriptional level.

Table 1
Kinetics of taurine transport in Caco-2 cells cultured with hypertonic and isotonic media

	Isotonicity	Hypertonicity
V_{max} (pmol/mg of protein/10 min)	259	418
K_m (μM)	5.87	7.18

Caco-2 cells were precultured in a medium with 100 mM raffinose or without for 48 h. The taurine uptake was then measured over the concentration range of 1–50 μM. Eadie-Hofstee plots were made to calculate the V_{max} and K_m values for taurine transport.

4. Discussion

We investigated the osmoregulation of taurine transport in human intestinal Caco-2 cells and found that hypertonicity up-regulated the taurine transporter activity in the cells, this regulation being associated with increased expression of the taurine transporter gene.

The osmotic regulation of the three organic osmolyte (betaine, *myo*-inositol, and taurine) transporters has previously been studied in many tissues [3]. However the expression of each differed according to the tissue. In kidney, for example, all three osmolytes are known to work [2,3]. On the other hand, taurine and *myo*-inositol behaved as osmolytes in the eye and brain [12,13]. In the small intestine, only the taurine transporter was detectable among the osmolyte transporters [7,8,28]. The presence of the taurine transporter in Caco-2 cells and its regulation by hyperosmolarity also suggest that taurine uniquely plays the role of an osmolyte in the small intestine.

It is known that some of the amino acids also serve as osmolytes and that their transport systems are subject to osmoregulation [11]. For example, system A for neutral amino acids and system X_{AG}^- for anionic amino acids participated in osmotic regulation in an NBL-1 bovine renal epithelial cell line [14,15]. The induction of system X_{AG}^- by hypertonic stress was accompanied by an increase in the EAAC1 (one of the glutamate transporter clones belonging to system X_{AG}^-) mRNA level [15]. However, as shown in Figs. 4 and 5, hypertonicity had little effect on the Leu, Lys and Glu uptake by Caco-2 cells or on the intracellular content of amino acids other than taurine. This suggests that other amino acids would not serve as osmolytes and that only taurine participated in the osmotic response of Caco-2 cells.

Physiological regulation of the taurine transport system has been reported to occur by two mechanisms. One is adaptive regulation which depends on the intracellular or extracellular concentration of taurine [7,16], i.e. the activity of taurine transport would be down-regulated by culturing with a high concentration of taurine. In placental [16] and intestinal cell lines [7] this type of regulation has been reported to be associated with a decrease in the maximal velocity of taurine transport and also with a decrease in the affinity of the transporter. The other mechanism is osmoregulation as indicated in this study. Osmoregulation involving an increase in the maximal velocity of taurine transport without any change in the affinity of the transporter has previously been observed [4,17] and this was true with our results as well (Table 1). The different kinetic behavior of these two regulation mechanisms indicates that the two types of taurine transport regulation would independently act.

The mechanism for the osmoregulation of transporters is mostly at the transcriptional level; for example, it is known that hyperosmolarity induced the BGT-1 and *myo*-inositol transporter at the transcriptional level [8,18,25,26]. The osmoregulation of the taurine transporter, however, has been reported to occur by two different regulatory mechanisms. Regulation has been reported to be associated with the increased abundance of mRNA in the kidney [17] and hepatoma [19], suggesting regulation at the transcriptional level to be the main mechanism. In contrast, Miyamoto et al. have reported that osmoregulation of the taurine transporter in human retinal pigment epithelial cells occurred at the translational level [20]. The results observed with Caco-2 cells (Fig. 7) suggest that, like the former case, osmoregulation of the taurine transporter in the human intestinal epithelium occurs at the transcriptional level.

Two possibilities are proposed for the regulatory mechanism that changes the expression of the taurine transporter gene (Fig. 7): one is an increase in the transcription of the taurine transporter gene; the other is an increase in the stability of taurine transporter mRNA. To elucidate this regulatory mechanism, more experiments such as nuclear run-on studies are needed. However, osmoregulation of other osmolyte transporters such as betaine and *myo*-inositol is known to be due to an increase in the transcription of the transporter gene [25,26]. Furthermore, the osmoregulation of aldose reductase (AR), which participates in the synthesis of sorbitol from glucose, is also accompanied by an increase in the transcription of the aldose reductase gene [27]. This information suggests that the increased mRNA abundance of the taurine transporter in hypertonicity is due to the increased transcription of the transporter gene, like the case of other osmolyte transporters and AR.

The transcriptional regulation of BGT-1 has been

extensively investigated. The promoter region of the BGT-1 gene has been identified [21], and Takenaka et al. have determined the tonicity-sensitive element (tonicity-responsive element, TONE) in the promoter region [22]. Furthermore, an electrophoretic mobility shift assay has shown that the transcriptional factor which specially interacted with TONE existed in nuclear extracts from MDCK cells and that this factor was induced by hypertonicity [22].

The promoter region of the AR has also been identified [23], and its essential tonicity-sensitive element has been determined [24] as well as TONE. The osmotic response element (ORE) determined from the promoter region of AR was, however, not identical with TONE, although the two elements were somewhat similar. We hypothesize that the promoter region of the taurine transporter also possesses a hypertonicity-responsive element similar to TONE or ORE.

In conclusion, the present study has shown that the activity of the taurine transporter in the human intestinal epithelium was subject to osmoregulation and that this induction was due to the increased expression of the taurine transporter gene. This finding indicates that an osmotic-responsive element like TONE or ORE may exist in the promoter region of the taurine transporter. Therefore, we are now trying to determine the promoter of the human taurine transporter from the human genomic library, and to determine the osmoresponsive element in the promoter region of the human taurine transporter.

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