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Characterization and Regulation of Taurine Transport in Caco-2, Human Intestinal Cells

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We characterized the taurine transport system in human intestinal Caco-2 cells and showed that it is subject to adaptive regulation. The activity of taurine transport in Caco-2 cells was evaluated by means of an Na^+- and Cl^--dependent high-affinity transport system, the characteristics of which were similar to those of the β-amino acid-specific taurine transport system previously described for various tissues. The activity of taurine transport was down-regulated on culturing in taurine-containing medium. This taurine-induced down-regulation was dependent on both the incubation time with taurine and the concentration of taurine. Hypotaurine and β-alanine were also capable of inducing this adaptive regulation, whereas α-amino acids and γ-aminoisobutyric acid were not. Kinetic analysis of control and taurine-treated cells suggested that the down-regulation was associated with a decrease in the maximal velocity of taurine transport and also with a decrease in the affinity of the taurine transporter. Cycloheximide treatment weakened the taurine-induced down-regulation. The mRNA level of the taurine transporter (HTAU type) in taurine-treated cells was markedly decreased compared with in control cells. These results indicate that a complex regulatory mechanism is involved in this down-regulation.

Key words: adaptive regulation, Caco-2, human small intestine, taurine, transporter.

Taurine (2-ethaneaminosulfonic acid) is one of the major intracellular β-amino acids in mammals and is required for a number of biological processes, including osmoregulation, antioxidation, and detoxification (*1, 2*). Taurine is especially essential to the fetus and newborn for their development. The nutritional and physiological requirements for taurine in mammals are partly met by dietary sources and partly by biosynthesis. The fetus and newborn, however, have little biosynthetic capacity (*3*), the fetus obtaining taurine through transplacental transport from the maternal blood, and the newborn through intestinal absorption from the diet. It is understood that the transplacental and intestinal transport of taurine occurs *via* a specific transport system (*4, 5*). The cloning and characterization of the taurine transporters from the brain (*6, 7*), kidney (*8*), thyroid (*9*), placenta (*10*), and retina (*11*) have recently been reported, although the intestinal taurine transporter has not yet been cloned.

Some amino acid transport systems, such as A, L, N^m, and X_{AG}^-, have been reported to be subject to adaptive regulation (*12-16*). With this regulation, cells maintain their physiological states by changing the amino acid transport in response to the extracellular conditions. This type of regulation has also been observed for taurine transport in the kidney (*17*) and placenta (*18*). However, the regulation of taurine transport in the small intestine has not yet been investigated. The adaptive regulation of taurine transport in the small intestine, if any, is of great importance, because taurine absorption in the small intestine influences the supply of dietary taurine to the animal body.

Caco-2, a human intestinal epithelial cell line, has frequently been used to study the characteristics and regulation of nutrient and drug absorption in the small intestine at the cellular level (*19-22, 24, 30*), because this cell line spontaneously differentiates and exhibits various enterocytic characteristics (*23*). Brandsch *et al.* (*24*) recently reported that Caco-2 expressed a taurine transporter and that the transport activity was regulated by protein kinase C. In the present investigation, we characterize the taurine transport system in Caco-2 in more detail and show that the taurine transport is subject to adaptive regulation. We found that the taurine transport activity was down-regulated on culturing under taurine-rich conditions. The mechanism underlying this regulation is also discussed in this paper.

MATERIALS AND METHODS

Materials—The Caco-2 cell line was obtained from the American Type Culture Collection (Rockville, MD, USA). Dulbecco's modified Eagle's medium (DMEM) was purchased from Nissui Pharmaceuticals (Tokyo), and fetal calf serum (FCS), L-glutamine, and penicillin-streptomycin

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Abbreviations: DMEM, Dulbecco's modified Eagle's medium; NEAA, non-essential amino acids; FCS, fetal calf serum; PBS, phosphate-buffered saline; HBSS, Hank's balanced salt solution; HTAU, taurine transporter from human placenta; GABA, γ-aminoisobutyric acid; CHX, cycloheximide; COL, colchicine.

(10,000 units/ml and 10 mg/ml in 0.9% sodium chloride, respectively) were purchased from Gibco (Gaithersburg, MD, USA). Non-essential amino acids (NEAA) were purchased from Cosmobio (Tokyo), and [1,2-^{3}H]taurine (specific radioactivity, 29 Ci/mmol) and [α-^{32}P]dCTP from Amersham (Little Chalfont, England). All the other chemicals used were of reagent grade.

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 under 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. For uptake experiments, Caco-2 cells were 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.

Uptake Experiments—[^{3}H]Taurine uptake experiments were performed in the absence (total uptake) or presence (non-specific uptake) of 50 mM unlabeled taurine, which allowed 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 Hank's balanced salt solution (HBSS) containing 4 mM sodium bicarbonate and 10 mM HEPES, the pH 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 washed carefully 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 then the solubilized 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).

RT-PCR—Total RNA was extracted from the cultured Caco-2 cells with an Isogen RNA isolation kit (Nippon Gene, Tokyo) according to the manufacturer's instructions. Poly(A)$^+$ RNA purified by affinity chromatography on oligo(dT)-cellulose (Pharmacia, USA) was reverse-transcribed with random hexamers using a first-strand cDNA synthesis kit (Pharmacia). The polymerase chain reaction (PCR) was performed with Taq DNA polymerase, the PCR primers being designed on the basis of the sequence of HTAU: forward primer, 5′-atggccaccaaggagaagctg-3′; reverse primer, 5′-agagagctcacatcatggtctccacaatga-3′. The program consisted of 30 cycles of 1 min at 94°C, 2 min at 55°C, and finally 2 min at 72°C. The PCR product was subcloned into pUC 18, and its nucleotide sequence was determined with a dye terminator cycle sequencing kit (Cetus; Perkin Elmer).

Northern-Blot Analysis—Twenty micrograms of total RNA fractionated on a 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 the PCR product derived from Caco-2 that had been labeled by random priming with [α-^{32}P]dCTP (Multiprime labeling kit, Amersham). Hybridization was performed in a hybridization solution (Rapid, Amersham) at 65°C for 3 h, the filter then being washed in 0.1 × NaCl/Cit containing 0.1% SDS at 65°C.

RESULTS

Kinetics and Ion Dependence of the Taurine Uptake by Caco-2 Monolayers—The kinetic parameters for taurine uptake (34.5 nM) were determined. The activity of taurine uptake was measured at 2.5, 5, 15, 30, 45, 60, and 120 min, the results showing that the uptake was linear for at least up to 120 min (data not shown). The uptake experiments were therefore performed for 10 min. The dose dependence of taurine uptake was also determined, the uptake activity being measured with 1, 2.5, 5, 10, 20, and 50 μM taurine. The results indicated Michaelis-Menten saturation kinetics; the K_m value was 4.8 μM and the V_m value 202.4 pmol/mg of protein/10 min (Fig. 1). Since the activity of taurine transport in various tissues has been reported to depend on the Na^+ and Cl^- concentrations (*4, 5, 25, 26*), the Na^+ and Cl^- dependence of taurine uptake by Caco-2 monolayers was examined. The taurine uptake by Caco-2 monolayers was found to be clearly dependent on the Na^+ and Cl^- concentrations (Fig. 2). The low K_m value, and Na^+ and Cl^- dependence suggest that a high-affinity taurine transport system exists in this cell line.

Specificity of the Taurine Transport System in Caco-2 Monolayers—The competition for taurine uptake by amino acids is shown in Fig. 3. It is well known that amino acids are basically transported by various amino acid transport systems, whereas taurine has been reported to be transported by a system which mainly accepts β-amino acids (system β) (*4, 5, 25*). At the concentration of 5 mM, β-amino acids, which are specific to system β, almost completely inhibited taurine uptake, while γ-aminoisobutyric acid (GABA) also inhibited the uptake. In contrast, the uptake of labeled taurine was not markedly inhibited by α-amino acids.

Taurine-Induced Down-Regulation of Taurine Uptake by Caco-2 Monolayers—In a preliminary experiment, we found that the taurine uptake decreased on culturing of the

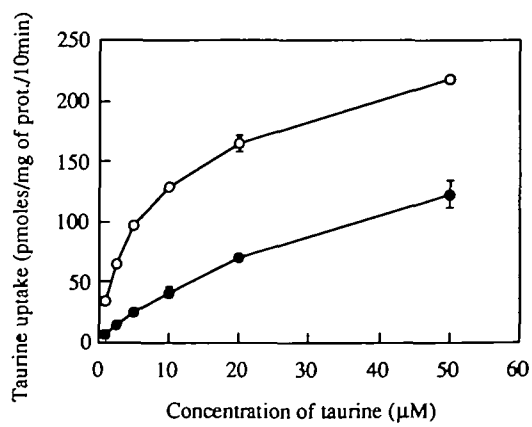

Fig. 1. Kinetic analysis of the taurine uptake by control and taurine-treated cells. Cells were precultured in a medium containing 10 mM taurine for 24 h. The taurine uptake was then measured over the concentration range of 1-50 μM for taurine-treated (●) and control (○) cells. Each value is the mean ± SEM ($n = 4$).

cells with taurine, so the time dependence of this taurine-induced down-regulation was investigated. After culturing of cells for 14 days, the medium was changed to one containing 10 mM taurine. The monolayers were incubated for various times, and then uptake experiments were performed. As shown in Fig. 4, the transport activity decreased as the time of incubation with 10 mM taurine increased.

Caco-2 cells were also pretreated with various concentrations (0.05-10 mM) of taurine for 24 h, and then the activity of taurine uptake was determined (Fig. 5). The results showed that the down-regulation resulting on pretreatment with taurine was dose-dependent. The activity of taurine uptake decreased by up to 30% of the initial value on incubation with 10 mM taurine.

Down-Regulation of Taurine Uptake by Other Amino Acids—In order to determine whether other β-amino acids were capable of regulating the taurine transport activity or not, Caco-2 cells were pretreated with other amino acids. Figure 6 shows that the taurine uptake by cells cultured with 10 mM hypotaurine and β-alanine for 24 h decreased by up to about 30 and 65% of the control values, respectively. Pretreatment with α-amino acids, which are not substrates for taurine transport, had no effect on the taurine uptake. GABA also had no effect on the transport activity.

Concentration-Dependence of Taurine Uptake by Taurine-Treated Cells—Kinetic analysis of taurine transport activity was performed on taurine-treated and control cells. For the control cells, the K_m value was 4.8 μM and the V_m value 202.4 pmol/mg of protein/10 min. The K_m and V_m values for the taurine-treated cells were 16.0 μM and 115.8 pmol/mg of protein/10 min, respectively (Fig. 1). These results show that the taurine-induced down-regulation was associated with a decrease in the affinity of the taurine

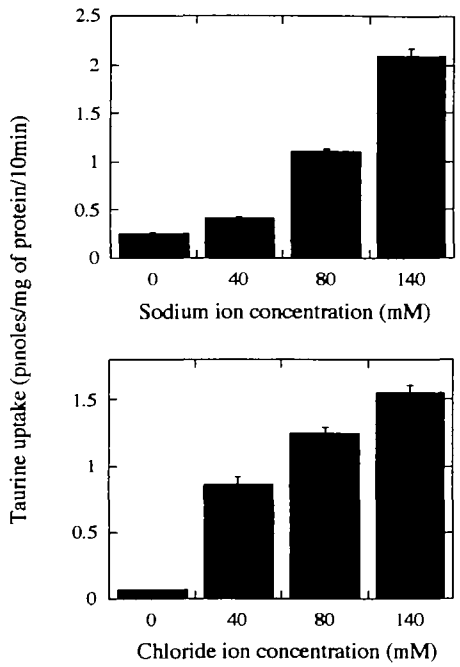

Fig. 2. **Sodium and chloride ion concentration-dependence of the specific taurine uptake by Caco-2 monolayers.** The uptake of taurine was measured at different sodium and chloride concentrations (0-140 mM). Each value is the mean±SEM (n=4).

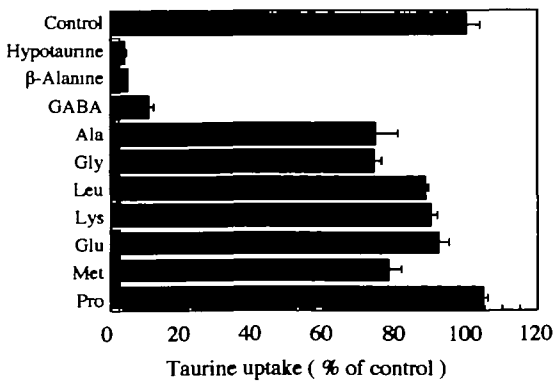

Fig. 3. **Competition for taurine uptake by other amino acids.** Taurine uptake was measured in the presence and absence of 5 mM amino acids. Each value is the mean±SEM (n=4).

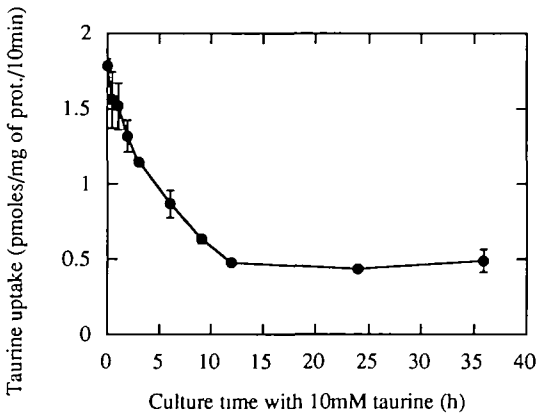

Fig. 4. **Effect of the culture time with 10 mM taurine on the taurine uptake by Caco-2 monolayers.** Cells were precultured in the medium for various times (0.5-36 h), uptake experiments then being performed as described under "MATERIALS AND METHODS." Each value is the mean±SEM (n=4).

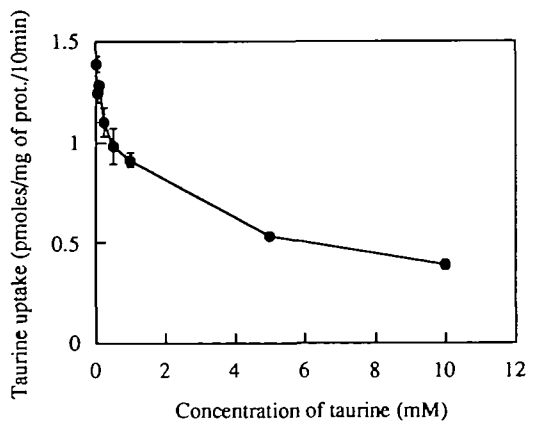

Fig. 5. **Concentration dependence of the taurine-induced down-regulation in Caco-2 monolayers.** Cells were precultured for 24 h in a medium containing various concentrations of taurine. Uptake experiments were then performed as described under "MATERIALS AND METHODS." Each value is the mean±SEM (n=4).

transporter and also with a decrease in the maximal velocity of taurine transport.

Effects of Cycloheximide and Colchicine on the Taurine-Induced Down-Regulation of Taurine Uptake—To examine the involvement of protein synthesis and intracellular translocation of the taurine transporter in this down-regulation, the effects of cycloheximide (CHX), an inhibitor of protein synthesis, and colchicine (COL), an inhibitor of the microtubular network, were studied. Incubation with 100 μM CHX did not affect the taurine uptake by Caco-2 cells cultured in the normal medium. However, in the case of Caco-2 cells cultured with 10 mM taurine for 24 h, CHX treatment weakened the taurine-induced down-regulation. Taurine uptake by cells cultured with 10 mM taurine decreased by up to 30% of the control value, while that by cells cultured with 10 mM taurine+100 μM CHX decreased by up to 65% of the control value (Fig. 7). These results suggest that protein synthesis was involved in this taurine-induced down-regulation.

The inhibition of intracellular translocation by 100 μM COL decreased the taurine uptake by Caco-2 monolayers cultured in the normal medium to 60% of the control value. In cells cultured with 10 mM taurine, the taurine uptake was decreased to a similar extent by COL treatment (Fig. 8). Intracellular translocation was therefore unlikely to have participated in this taurine-induced down-regulation.

Expression Level of Taurine Transporter mRNA in Caco-2 Cells Cultured with Taurine—To determine whether or not the down-regulation was accompanied by a change in the expression level of taurine transporter mRNA, Northern analysis was performed. As the taurine transporter in the small intestine had not yet been cloned, we performed RT-PCR and determined its sequence, the PCR primers being designed on the basis of the sequence of human placenta HTAU. The sequence of the PCR product derived from Caco-2 cells was similar to that of HTAU, although it contained 5 different nucleotides (3 amino acids) from the reported ones. Using this PCR product as a probe, Northern analysis was performed. Total RNA extracted from Caco-2 cells cultured in medium containing 10 mM

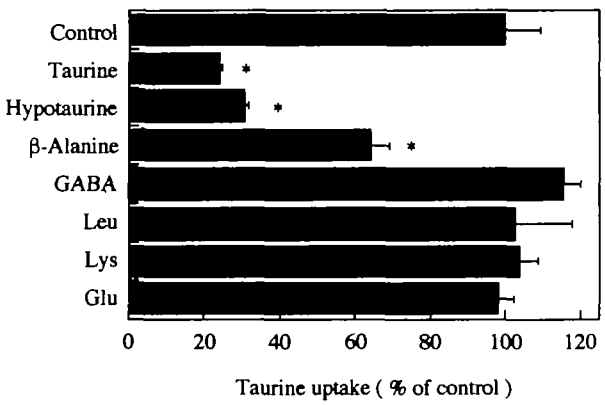

Fig. 6. **Down-regulation of taurine uptake by various amino acids.** Cells were precultured for 24 h in a medium containing 10 mM various amino acids. Uptake experiments were then performed as described under "MATERIALS AND METHODS." Each value is the mean±SEM ($n=4$). *Significantly different from the control value ($p<0.05$).

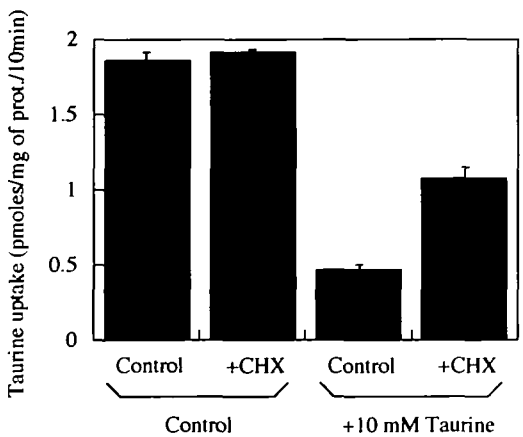

Fig. 7. **Effect of cycloheximide (CHX) on the taurine-induced down-regulation of taurine uptake.** Caco-2 monolayers were preincubated with CHX (100 μM) for 3 h. The medium was then changed to fresh culture medium containing 10 mM taurine and 100 μM CHX. After 24 h, the uptake of taurine was measured. Each value is the mean±SEM ($n=4$).

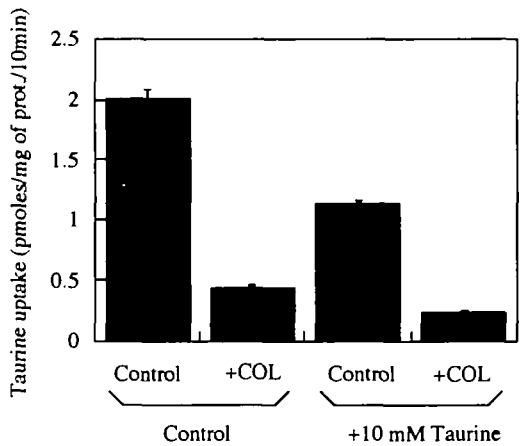

Fig. 8. **Effect of colchicine (COL) on the taurine-induced down-regulation of taurine uptake.** Caco-2 monolayers were preincubated with COL (100 μM) for 3 h. The medium was then changed to fresh culture medium containing 10 mM taurine and 100 μM COL. After 24 h, the uptake of taurine was measured. Each value is the mean±SEM ($n=4$).

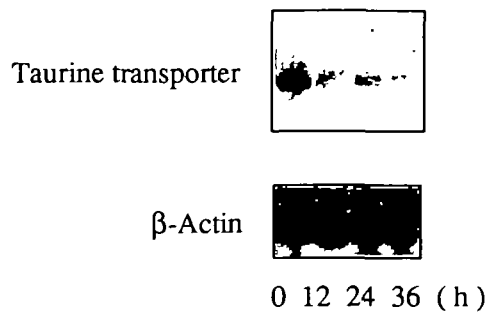

Fig. 9. **Northern analysis of mRNA from Caco-2 cells cultured with 10 mM taurine for different times.** Total RNA isolated from Caco-2 cells cultured with 10 mM taurine for different times (0, 12, 24, and 36 h) was subjected to Northern blot analysis. β-Actin cDNA was used as a control.

taurine for 12, 24, or 36 h was used. Figure 9 clearly shows that the mRNA level was markedly decreased in cells cultured with 10 mM taurine when compared with in control cells, whereas β-actin transcripts did not change the expression level. This low level for the transcripts indicates that down-regulation occurred at least at the transcriptional level.

DISCUSSION

We studied the characteristics of the intestinal taurine transport system in detail, it being found that this system was subject to adaptive regulation in a human intestinal cell line Caco-2.

The activity of taurine transport in Caco-2 cells was examined by means of an Na^+- and Cl^--dependent high-affinity transport system, the characteristics of which were similar to those of the β-amino acid-specific taurine transport system previously described for various tissues, including rabbit and rat small intestine (*4, 26*). Taurine transporters have been so far cloned from the brain (*6, 7*), kidney (*8*), thyroid (*9*), placenta (*10*), and retina (*11*), but not from the small intestine. The human taurine transporters, cloned from the thyroid (*9*), placenta (*10*), and retina (*11*), are highly homologous but not completely identical. Jayanthi *et al.* (*18*) reported that the mRNA level of the taurine transporter (HTAU) was markedly decreased in the human placental JAR cell line cultured with taurine. Therefore we designed PCR primers on the basis of the sequence of HTAU, and performed RT-PCR. The amino acid sequence deduced from the sequence of the PCR product derived from Caco-2 cells was highly homologous, but not completely identical, to that of HTAU. The difference appears to be due to microheterogeneity or mistakes in the Taq DNA polymerase reaction during PCR.

The down-regulation of taurine uptake caused by the culturing of cells with a high concentration of taurine has also been studied in renal (*17*) and placental cell lines (*18*). However, the characteristics of this regulation in Caco-2 cells were different in some respects from those reported for the renal and placental cell lines. In the JAR cell line, for example, down-regulation was examined with very low concentrations of taurine; the maximal decrease in uptake (30% of the control value) resulting on incubation of the cells at a taurine concentration of 250 μM for 24 h, whereas in Caco-2 cells, the taurine uptake decreased to the maximal extent with 10 mM taurine (30% of the control value). Furthermore, although the taurine transport activity decreased on treatment of cells with β-alanine for 24 h in both cell lines, the degree of the down-regulation differed; in JAR cells, the taurine uptake decreased to 38% of the control value on treatment with 250 μM β-alanine, while the taurine uptake by Caco-2 cells decreased to only 60% of the control value on treatment with 10 mM β-alanine. It should be emphasized that the concentration of taurine necessary to cause down-regulation was greatly different between Caco-2 and JAR cells. This difference may have been caused by a different post-transcriptional modification or by the action of an unknown regulatory factor such as protein, the existence of which Jones *et al.* (*17*) have suggested. From the viewpoint of significance, this low sensitivity to adaptive regulation of the intestinal Caco-2 cell line may result from that the small intestine is the first possible regulating tissue to be affected by changes in external conditions. However, the adaptive regulation of amino acid transport in the small intestine has never been investigated, and the significance of such adaptive regulation is not yet understood.

As shown in Fig. 6, down-regulation was induced not only by taurine, but also by other substrates for taurine transport. Interestingly, GABA had no ability to induce down-regulation, although GABA is thought to be a substrate for taurine transport, as shown by the results of competition studies (Fig. 3), in which GABA strongly inhibited taurine uptake. This phenomenon may be of great importance when investigating the cellular mechanism underlying this down-regulation.

With respect to the relationship between the level of mRNA and the change in transport activity with adaptive regulation, two different results have been reported. In the NBL-1 bovine renal epithelial cell line, the glutamate transport activity was up-regulated by amino acid deprivation, whereas the mRNA level of the EAAC1 glutamate transporter was decreased, after which it was later restored to the control level (*16*). Therefore, post-transcriptional events are thought to have been primarily responsible for this adaptive regulation. On the other hand, the decrease of taurine transport activity in JAR cells cultured with taurine was associated with a markedly low level of specific mRNA for the taurine transporter (*18*). In this study, the taurine-induced down-regulation in Caco-2 cells was found to be correlated with a low mRNA level of the taurine transporter (Fig. 9), similar to in the case of taurine transport in the JAR cell line.

In most cases of adaptive regulation by amino acids, a change in transporter activity is only associated with a decrease in the maximal velocity (*12, 15, 16*). However, in the case of taurine, the adaptive response of the transporter was also associated with a decrease in the affinity of the transporter, this phenomenon being observed in both the present study and in that involving JAR cells (*18*). This result is similar to in the case of the regulation of GLUT-1 in 3T3-L1 cells (*27*); the up-regulation of this transporter by cycloheximide was accompanied by an increase in the affinity of the transporter, but not by an increase in the maximal velocity. The existence of a regulatory protein participating in the down-regulation of GLUT-1 has been suggested (*28*); this regulatory protein, which may be rapidly metabolized, has the ability to decrease the affinity of GLUT-1. It is plausible that such a regulatory protein, which decreases the affinity of a transporter and whose function is blocked by cycloheximide, exists in some nutrient transport systems. To our knowledge, there has been only one report on the identification of a regulatory protein; McCormick and Johnstone (*29*) reported that the transport activity of system A was regulated by the α_3-subunit of integrins. However, the α_3-subunit of integrins participated in the up-regulation of system A, not in its down-regulation. It appears to be most important to determine whether such a regulatory protein truly exists or not, because this would provide more information on the cellular mechanism regulating nutrient transporters.

The present finding that the activity of the taurine transporter is subject to adaptive regulation in human intestinal Caco-2 cells raises the possibility that the taurine transporter in the human small intestine is also regulated

by external conditions. We are now trying to determine whether adaptive regulation can be observed *in vivo* or not. In studies on the adaptive regulation of glucose and fructose transport, the adaptive response observed in cultured cell lines was also observed in *in vivo* studies on rats (*30-32*). The adaptive regulation of taurine transport could, therefore, be expected to be observed in *in vivo* studies as well as with the Caco-2 human intestinal culture cell line.

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