Functional characterization and regulation of the taurine transporter and cysteine dioxygenase in human hepatoblastoma HepG2 cells

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We investigated the characterization and the regulation of TAUT (taurine transporter) and CDO (cysteine dioxygenase), one of the key enzymes of taurine biosynthesis, in human hepatoblastoma HepG2 cells. The activity of TAUT in the HepG2 cells was evaluated by means of a sodium- and chloride-dependent high-affinity transport system, the characteristics of which were similar to those of the β amino-acid-specific taurine transport system described previously for various tissues [Uchida, Kwon, Yamauchi, Preston, Marumo and Handler (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 8230–8234; Ramamoorthy, Leibach, Mahesh, Han, Yang-Feng, Blakely and Ganapathy (1994) Biochem. J. **300**, 893–900; and Satsu, Watanabe, Arai and Shimizu (1997) J. Biochem. (Tokyo) **121**, 1082–1087]. By culturing in a hypertonic medium, the intracellular taurine content of HepG2 cells was markedly increased. Under hypertonic conditions, the activity of TAUT was up-regulated, and the results of the kinetic analysis suggested that this up-regulation was associated with an increase in the amount of TAUT. The expression level of TAUT mRNA was markedly higher than that of the control cells. The expression level of CDO mRNA was also up-regulated under the hypertonic conditions. Culturing the cells in a taurine-rich medium resulted in both the activity of TAUT and the expression level of TAUT mRNA being down-regulated in HepG2 cells. On the other hand, the expression level of CDO mRNA was not affected under a taurine-rich condition. The present results show that both TAUT and CDO were co-operatively regulated in response to hypertonicity, but did not co-operatively respond to the change in extracellular taurine concentration. Generally, the TAUT and taurine biosynthetic enzymes have independent regulatory systems, but under certain conditions, they could be regulated in harmony with each other.

Key words: adaptive regulation, cysteine dioxygenase, hepatocyte, osmolyte, taurine, transporter.

INTRODUCTION

Taurine (2-aminoethanesulphonic acid) is one of the major free β amino acids, which is normally present in a high concentration in many mammalian tissues. It is believed that taurine has various physiological functions, including conjugation with bile acids, and maintenance of osmolarity, antioxidation, detoxification and membrane stabilization [1,2]. Taurine is essential for the development of the foetus and the newborn [3]. Extracellular taurine is transported into the cells by the TAUT (taurine transporter). Cloning and characterization of TAUT have been reported from brain [4,5], kidney [6], thyroid [7] and placenta [8], and it is known that TAUT is expressed in almost all tissues [4–8]. The regulation of TAUT has also been studied in kidney, placenta and small intestine [6,9–14]; e.g. TAUT has been shown to be up-regulated in a hypertonic environment to increase the content of intracellular taurine and maintain the cell volume [6,9–11]. Another reported function of TAUT is adaptive regulation; taurine induced the down-regulation of TAUT [12–14]. The regulation or modulation of TAUT by food-derived substances and cytokines has also been reported in human intestinal epithelial cells [15–17]. However, there is very little information available on the regulation of TAUT in human liver cells.

Taurine is known to be endogenously synthesized from methionine and cysteine [18]. The taurine biosynthetic pathway has been determined: cysteine is converted into CSA (cysteine sulphinic acid) by CDO (cysteine dioxygenase), CSA is converted into hypotaurine by CSA decarboxylase and hypotaurine is non-enzymically converted into taurine. CDO is understood to be one of the key enzymes in this biosynthetic pathway. Taurine synthesis mainly occurs in the liver, brain and kidney [18]. Taurine is provided in these tissues not only by its uptake from external cells, but also by endogenous synthesis. Therefore it is important to determine the regulation of taurine biosynthetic enzymes as well as that of TAUT. Bitoun and Tappaz [19,20] and Bitoun et al. [21] have recently reported the gene expression of TAUT and taurine biosynthetic enzymes in rat brain, kidney and astrocytes. However, there has been no report focusing on the regulation of both TAUT and taurine biosynthetic enzymes in human hepatic cells. In the hepatocyte, Timbrell et al. [22] have reported that taurine had a cytoprotective effect on the hepatotoxicity induced by chemical compounds such as hydrazine, 1,4-naphthoquinone and carbon tetrachloride. It has also been reported that bacterial lipopolysaccharide-induced hepatotoxicity was protected by taurine [23]. Accordingly, taurine has an important role to play against cytotoxicity in the hepatocyte, and it is therefore necessary to investigate the regulation of the TAUT and taurine biosynthetic enzymes that participate in the production of taurine.

In the present study, we focus on the regulation of both the TAUT and CDO, one of the key enzymes of taurine synthesis in liver cells. We first characterize the taurine transport system in the human hepatic HepG2 cell line, and then investigate the regulation of TAUT and CDO in HepG2 cells under high osmotic conditions and taurine-rich conditions respectively.

Abbreviations used: CDO, cysteine dioxygenase; CSA, cysteine sulphinic acid; TAUT, taurine transporter; TonE, tonicity-responsive enhancer; TREE, taurine-responsive element.

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MATERIALS AND METHODS

Materials

The HepG2 cell line was purchased from A.T.C.C. (Rockville, MD, U.S.A.) and Dulbecco's modified Eagle's medium was from Sigma. Foetal calf serum was purchased from Asahi Technoglass (Chiba, Japan) and penicillin/streptomycin (10 000 units/ml and 10 mg/ml in 0.9 % sodium chloride) were purchased from Gibco (Gaithersburg, MD, U.S.A.). [1,2-^{3}H]Taurine (specific radioactivity 29.0 Ci/mmol) and [α-^{32}P]dCTP were from Amersham Biosciences (Little Chalfont, Bucks., U.K.). All the other chemicals used were of reagent grade.

Cell culture

HepG2 cells were cultured in 78.5 cm^2 plastic dishes with a culture medium consisting of Dulbecco's modified Eagle's medium, 10 % (v/v) foetal calf serum, 2 % (w/v) glutamine, 100 units/ml penicillin, 100 μg/ml 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 trypsinized with 0.1 % trypsin and 0.02 % EDTA in PBS and then subcultured. HepG2 cells for uptake experiments were cultured in 24-well plates precoated with collagen at a density of 2×10^4 cells/well, the cells being used after 4 days of culture.

[^{3}H]Taurine uptake experiments

Experiments were performed in the absence (total uptake) or presence (non-specific uptake) of 50 mM unlabelled taurine, thus allowing the specific uptake to be calculated by subtraction. The HepG2 cells were washed twice with 700 μl of PBS and then once with 300 μl of Hanks balanced salt solution containing 4 mM sodium bicarbonate and 10 mM Hepes, the pH value being adjusted to 7.4 with potassium hydroxide (uptake buffer). The cells were next incubated at 37 °C for 10 min with 0.3 μCi of [^{3}H]taurine in 300 μl of the uptake buffer, with or without 50 mM taurine. At the end of the incubation period, the buffer was removed, and each cell was carefully washed three times with 700 μl of ice-cold PBS containing 0.05 % sodium azide. The dissolved cells were taken into 3 ml of a scintillation cocktail after the addition of 250 μl of 0.1 % Triton X-100 to each well. The tritium content of each well was measured with an LSC 5100 liquid scintillation analyzer (Aloka, Tokyo, Japan).

Measurement of the intracellular content of amino acids in HepG2 cells

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 % (w/v) 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, Tokyo, Japan).

Northern-blot analysis

To detect the expression level of TAUT mRNA, 10 μg of polyadenylated RNA from HepG2 cells, fractionated on 1 % agarose gel containing 2.2 M formaldehyde, was transferred to a Hybond-N nylon filter (Amersham Biosciences) according to the manufacturer's instructions. The filter was hybridized with the PCR product of human TAUT that had been labelled by random priming with [α-^{32}P]dCTP (Multiprime labelling kit, Amersham Biosciences). Hybridization was performed in a rapid hybridization solution (Amersham Biosciences) at 65 °C for 3 h and the filter was then washed at 65 °C in 0.1×SSC (1.665 mM sodium chloride/1.665 mM sodium citrate) containing 0.1 % SDS.

To detect the expression level of CDO mRNA, 20 μg of total RNA from HepG2 cells was used. Human CDO cDNA [24] was labelled by random priming with [α-^{32}P]dCTP and used as a probe for hybridization, the other procedures being performed as already described.

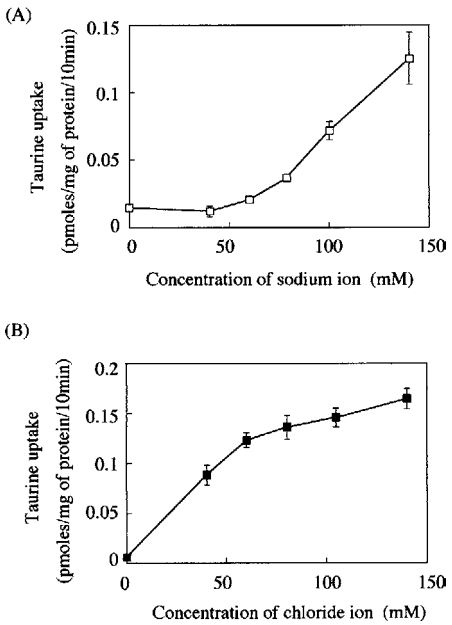

Figure 1 Sodium and chloride dependence of the taurine uptake by HepG2 cells

Uptake of taurine was measured at various sodium (**A**) and chloride (**B**) concentrations (0–140 mM). When the sodium concentration was changed, the chloride concentration was kept constant at 140 mM by adding choline chloride. In contrast, the sodium concentration was kept constant at 140 mM by adding sodium acetate when the chloride concentration was changed. Uptake experiments were then performed as described in the Materials and methods section. Each value represents the means ± S.E.M. ($n = 6$).

Statistical analysis

Each value is expressed as the means ± S.E.M., and Student's t test was used to compare the means and ranges.

RESULTS

Characteristics of the taurine uptake by HepG2 cells

The uptake activity for taurine was measured at 2.5, 5, 15, 30 and 45 min. Since the results show that the taurine uptake was linear at least up to 45 min (results not shown), the experiments were performed for 10 min in the present study.

As the uptake activity for taurine by various tissues has been reported to be dependent on the sodium and chloride concentrations [8,14], the sodium and chloride dependence of the taurine uptake by HepG2 cells was examined. Figure 1 shows that the taurine uptake by HepG2 cells was clearly dependent on the sodium and chloride concentrations. As the sodium concentration increased, the taurine uptake increased exponentially (Figure 1A). On the other hand, the taurine uptake logarithmically increased as

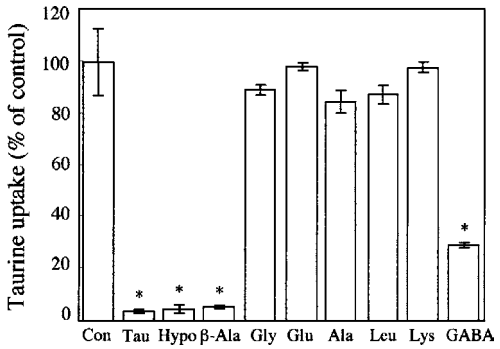

Figure 2 Substrate specificity of the taurine uptake by HepG2 cells

Taurine uptake (34.5 nM) was measured in the presence or absence of 1 mM amino acids. Each value represents the means ± S.E.M. ($n = 6$). Significantly different from the control value (*$P < 0.01$): Hypo, hypotaurine.

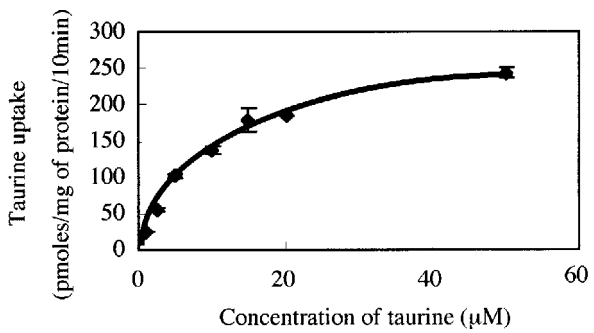

Figure 3 Kinetic analysis of taurine uptake by HepG2 cell line

Taurine uptake was measured over the concentration range 1–50 μM taurine. Each value is the means ± S.E.M. ($n = 6$). Eadie–Hofstee plots were constructed to calculate the V_{max} and K_m values for taurine uptake. Correlation coefficient $r = 0.996$ for Eadie–Hofstee plots.

the chloride concentration increased (Figure 1B). Miyamoto et al. [25] reported that one taurine molecule was transported with two or three sodium ions and one chloride ion by TAUT. Therefore this difference may be due to the number of ions co-transported with taurine by TAUT.

Substrate specificity of the taurine uptake by HepG2 cells was then examined. It is well known that amino acids are transported by various amino acid transport systems. Taurine has been reported to be transported by the system, which mainly accepts β amino acids, called system β. Hypotaurine and β-alanine at a concentration of 1 mM almost completely inhibited the taurine uptake (34.5 nM) (Figure 2). γ-Aminobutyric acid, which is the substrate for system β, also significantly inhibited the taurine uptake. In contrast, taurine uptake was not drastically inhibited by other α amino acids.

A kinetic analysis of the taurine uptake activity was then performed, the uptake activity being measured with 1, 2.5, 5, 10, 20 or 50 μM taurine. The results indicate Michaelis–Menten saturation kinetics (Figure 3); the K_m and V_{max} values were 10.5 μM and 292.5 pmol · (mg of protein)$^{-1}$ · (10 min)$^{-1}$. This suggests that the taurine uptake by HepG2 cells involved a high-affinity single-transport system.

The sodium and chloride dependence, specific inhibition by β amino acids and the kinetic data for taurine uptake all suggest that the high-affinity taurine transport system, described previously in several tissues, also operates in this HepG2 cell line.

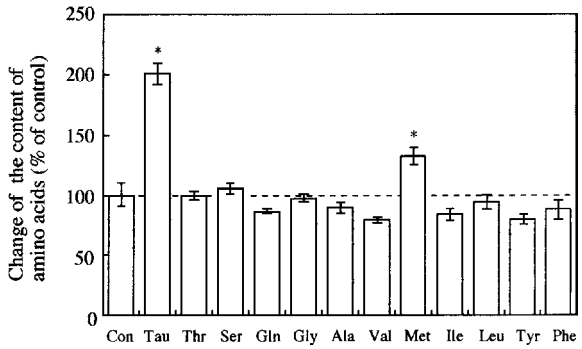

Figure 4 Changes in the intracellular amino acid content of HepG2 cells cultured with 50 mM sodium chloride

HepG2 cells were precultured for 24 h in a medium containing 50 mM sodium chloride. The intracellular content of each amino acid was measured as described in the Materials and methods section. The content of each amino acid in hypertonic cells represents the amount relative to that in iso-osmotic cells. Each value represents the means ± S.E.M. ($n = 6$). *Significantly different from the control value ($P < 0.01$).

Change of the intracellular amino acid content in HepG2 cells cultured under hypertonic conditions

Since taurine is well known to behave as an osmolyte in several tissues [26,27], we examined whether taurine behaves similarly in human hepatoma HepG2 cells. If taurine really functions as an osmolyte, the intracellular concentration of taurine should be increased. Therefore the intracellular amino acid content of hypertonic and iso-osmotic cells was measured. Among the 12 amino acids tested, taurine content significantly increased in hypertonic cells, reaching almost 200 % of the control value (Figure 4). Concentration of methionine also increased significantly in hypertonic cells. This result suggests that taurine mainly acted as an osmolyte in HepG2 cells.

Time and concentration dependence of the hypertonicity-induced up-regulation of taurine uptake by HepG2 cells

HepG2 cells were incubated with a culture medium containing 50 mM sodium chloride for 6, 12 and 24 h, and then uptake experiments were performed. HepG2 cells were also pretreated with 0, 10, 20, 35 and 50 mM sodium chloride for 24 h, and then the activity of taurine uptake was determined. The results show that the up-regulation of taurine uptake was dependent on the concentration of sodium chloride (Figure 5A). Furthermore, Figure 5(B) shows that the uptake activity increased with increase in the time of incubation for 50 mM sodium chloride.

Kinetic analysis of the taurine uptake by HepG2 cells cultured under hypertonic conditions

A kinetic analysis of the taurine uptake activity was performed on cells that had been cultured in hypertonic and iso-osmotic media. When cultured in an iso-osmotic medium, the V_{max} and K_m values were 308.7 pmol · (mg of protein)$^{-1}$ · (10 min)$^{-1}$ and 8.4 μM respectively. However, after being cultured in a hypertonic medium, the V_{max} value increased to 918.8 pmol · (mg of protein)$^{-1}$ · (10 min)$^{-1}$, whereas the K_m value was 11.1 μM (Figure 6). These results indicate that the up-regulation of taurine uptake resulted from the increased number of expressed TAUT, and not from the change in affinity of each TAUT.

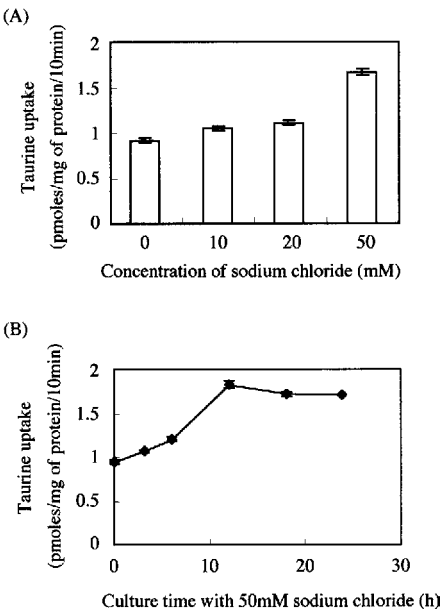

Figure 5 Hypertonicity-induced up-regulation of the taurine uptake by HepG2 cells

(**A**) HepG2 cells were precultured for 24 h in a medium containing 0, 10, 20 or 50 mM sodium chloride. Uptake experiments were then performed as described in the Materials and methods section. (**B**) Cells were precultured in the medium with 50 mM sodium chloride for different times (0–24 h), and uptake experiments were then performed. Each value represents the means ± S.E.M. ($n = 6$).

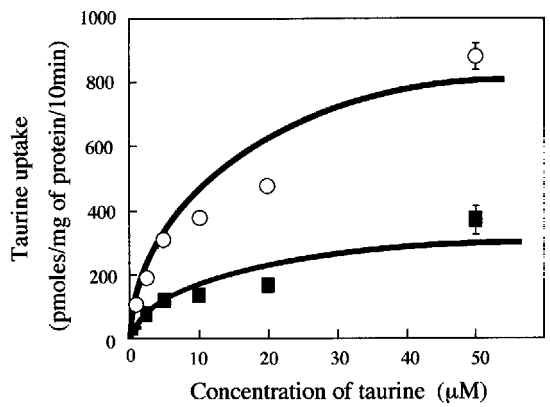

Figure 6 Kinetics of the taurine uptake by HepG2 cells cultured with hypertonic and iso-osmotic media

HepG2 cells were precultured for 24 h in a medium with (○) or without (■) 50 mM sodium chloride. The taurine uptake was then measured over the concentration range 1–50 μM taurine. Eadie–Hofstee plots were constructed to calculate the V_{max} and K_m values for taurine uptake. Correlation coefficient $r = 0.997$ for Eadie–Hofstee plots.

Expression level of TAUT and CDO mRNA in HepG2 cells cultured in a hypertonic medium

Northern-blot analysis was performed to determine whether or not the osmotic regulation was accompanied by a change in the expression level of TAUT mRNA. We determined the amount of TAUT mRNA extracted from HepG2 cells that had been cultured in a hypertonic medium for 6, 12 and 24 h. The results show that the expression level of TAUT mRNA under hypertonic conditions was higher than that of the control (Figure 7A), suggesting that the up-regulation of TAUT occurred at least at the transcriptional level.

The CDO mRNA levels were also determined by Northern-blot analysis. As shown in Figure 7(B), the CDO mRNA levels increased with increasing culture time under hypertonic conditions. This result suggests that CDO was also up-regulated under the hypertonic conditions and may have contributed to the increased content of intracellular taurine.

Taurine-induced down-regulation of the taurine uptake by HepG2 cells

We found in our previous study that the taurine uptake decreased on culturing with a taurine-containing medium in human intestinal Caco-2 cells [14]. Therefore we examined whether or not similar regulation would occur in human liver HepG2 cells. Uptake experiments were performed after the HepG2 cells had been cultured with various concentrations of taurine for 24 h. Figure 8(A) shows that the activity of taurine uptake was significantly decreased with increasing concentration of taurine in the medium. Before uptake experiments were performed, the HepG2 cells were also incubated with the medium containing 50 mM taurine for different times. As shown in Figure 8(B), the uptake activity decreased as the time of incubation with 50 mM taurine was increased.

Kinetic analysis of the taurine uptake by HepG2 cells cultured under taurine-rich conditions

A kinetic analysis of the taurine uptake activity was performed on cells cultured in taurine-rich and control media. Figure 9 shows that the V_{max} and K_m values were 327 pmol · (mg of protein)$^{-1}$ · (10 min)$^{-1}$ and 7.4 μM respectively in the control medium. However, after being cultured with 50 mM taurine, V_{max} decreased to 153 pmol · (mg of protein)$^{-1}$ · (10 min)$^{-1}$ and K_m increased to 28.8 μM. These results indicate that taurine-induced down-regulation of taurine uptake resulted not only from the decrease in the number of expressed TAUT but also from the decrease in their affinity.

Expression level of TAUT and CDO mRNA in HepG2 cells cultured under taurine-rich conditions

Northern-blot analysis was performed to determine whether or not the down-regulation was accompanied by a change in the expression level of TAUT mRNA. mRNA isolated from the HepG2 cells cultured with 50 mM taurine for different time periods was used. The results show that the level of TAUT mRNA decreased as the culture time with taurine increased (Figure 10A), indicating that the down-regulation of taurine uptake was accompanied by a decreased expression of TAUT mRNA.

We also investigated whether or not the expression level of CDO mRNA would be regulated under taurine-rich conditions. In contrast with the expression level under the hypertonic conditions, the expression level of CDO mRNA was not significantly changed (Figure 10B). This suggests that CDO was not affected by the extracellular concentration of taurine.

DISCUSSION

In the present study, we have characterized the taurine uptake system in human hepatic HepG2 cells and investigated the regulation of TAUT and CDO under hypertonic and taurine-rich conditions.

In general, when cells are exposed to hypertonic conditions, water is rapidly expelled from the cells and the cell volume decreases in a few seconds. Soon after the cell volume recovered, water and electrolytic ions were incorporated into the cells in

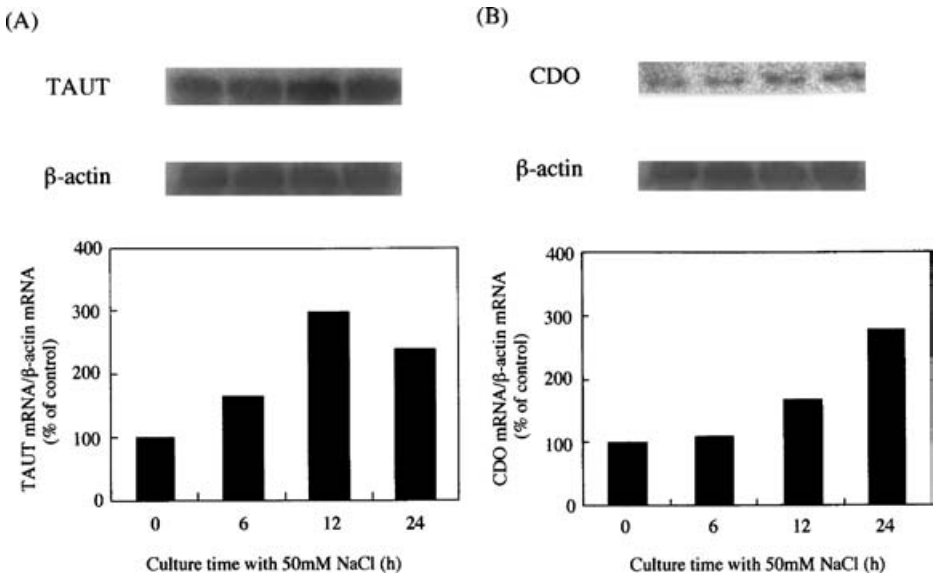

Figure 7 Expression level of TAUT and CDO mRNA under hypertonic conditions in HepG2 cells

RNA isolated from HepG2 cells cultured with 50 mM sodium chloride for different times (0, 6, 12 or 24 h) was subjected to a Northern-blot analysis. Polyadenylated RNA (10 μg) was used for the analysis of TAUT (**A**), and 20 μg of total RNA was used for the analysis of CDO (**B**) and β-actin.

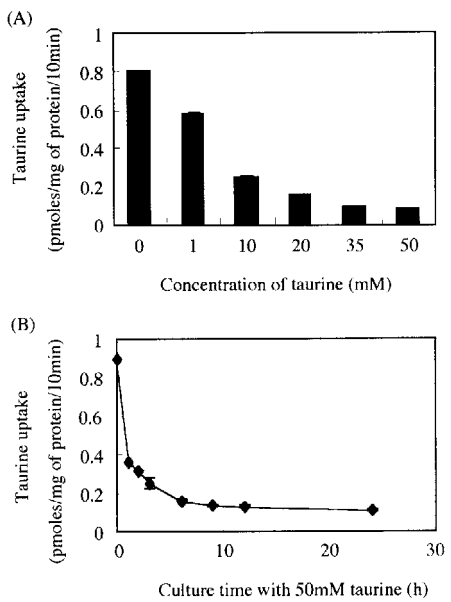

Figure 8 Taurine-induced down-regulation of the taurine uptake by HepG2 cells

(**A**) HepG2 cells were precultured for 24 h in a medium containing various concentrations of taurine. Uptake experiments were then performed as described in the Materials and methods section. (**B**) Cells were precultured in the medium containing 50 mM taurine for different times (0–24 h), uptake experiments then being performed as described in the Materials and methods section. Each value is the means ± S.E.M. ($n = 6$).

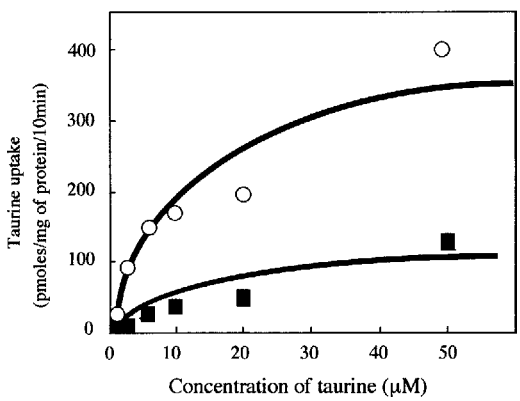

Figure 9 Kinetics of the taurine uptake by control and taurine-treated HepG2 cells

HepG2 cells were precultured in a medium with (■) or without (○) 50 mM taurine for 24 h. The taurine uptake was then measured over the concentration range 1–50 μM taurine. Eadie–Hofstee plots were constructed to calculate the V_{max} and K_m values for taurine uptake. The correlation coefficient $r = 0.993$ for Eadie–Hofstee plots.

a few minutes. (This phenomenon is called regulatory volume increase.) When the cells were exposed to hypertonicity, non-perturbing osmolytes are gradually accumulated in replacement of electrolytic ions in several hours. Thus the extracellular hypertonicity in mammalian cells is balanced by maintaining a high intracellular content of osmolytes, e.g. betaine, *myo*-inositol, sorbitol and several amino acids including taurine [11,28].

These osmolytes work in a tissue-specific manner, e.g. betaine, *myo*-inositol and taurine are all known to work in the kidney [29], whereas *myo*-inositol and taurine behave as osmolytes in the eye and brain [10,11]. There is only one report on osmoregulation in hepatic cells [30]. Warskulat et al. [30] have indicated, by using rat primary hepatocytes and H4IIE rat hepatoma cells, that taurine was the main osmolyte in H4IIE rat hepatoma cells. They have also demonstrated that TAUT was constitutively expressed and subjected to osmoregulation, whereas betaine and *myo*-inositol played little or no role in the osmolyte strategy for these cells. We measured the amino acid content of human hepatoma HepG2 cells cultured under hypertonic conditions and found that the taurine content was significantly increased (Figure 4), suggesting taurine to be an osmolyte in human hepatoma HepG2 cells.

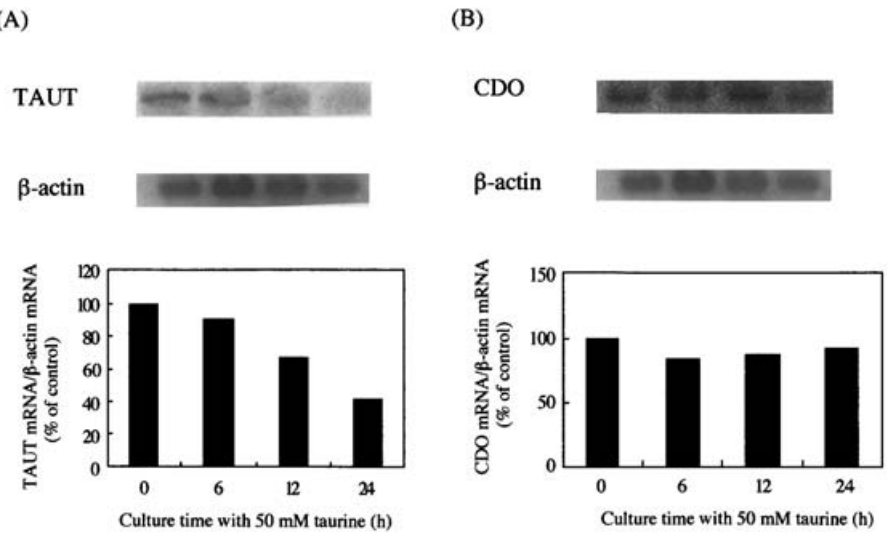

Figure 10 Expression levels of TAUT and CDO mRNA in HepG2 cells cultured with or without taurine

RNA isolated from HepG2 cells cultured with 50 mM taurine for different times (0, 6, 12 or 24 h) was subjected to a Northern-blot analysis. Polyadenylated RNA (10 μg) was used for the detection of TAUT (**A**), and 20 μg of total RNA was used for the detection of CDO (**B**) and β-actin.

In human hepatic HepG2 cells, the hypertonicity-induced response of TAUT appears to reach equilibrium at 12 h (Figures 5 and 7). On the other hand, the osmotic response of TAUT in human intestinal Caco-2 cells reached equilibrium at 72 h [9]. This difference in time to reach equilibrium for hypertonicity-induced response of TAUT between hepatic HepG2 cells and intestinal Caco-2 cells suggests that the human hepatic cells are more sensitive to osmotic stress than the human intestinal epithelial cells.

Osmosensitive genes are known to exist: these include the genes of such osmolyte transporters as the betaine transporter, *myo*-inositol transporter, TAUT and aldose reductase. Aldose reductase participates in the synthesis of sorbitol, which is also an osmolyte. The promoter regions of several osmosensitive genes have been extensively investigated, and the functional consensus TonE (tonicity-responsive enhancer) characterized [31]. Furthermore, Miyakawa et al. [32] have identified and characterized the TonE-binding protein. As shown in Figure 7 and reported previously for various tissues [6,9,10], the gene expression of TAUT is also osmoregulated. It is therefore plausible that the functional consensus tonicity-responsive element is also present in the promoter region of TAUT. We are now trying to identify and characterize TonE in the promoter region of the human TAUT.

For CDO, osmosensitivity is likely to be tissue-specific. An increased gene expression of CDO has been reported in the hyperosmotic renal papilla of anti-diuretic rats [20]. The results of our present study also show that the expression level of CDO mRNA was increased under hypertonic conditions in human hepatic HepG2 cells (Figure 7B). On the other hand, Bitoun and Tappaz [20] have reported that the expression of CDO mRNA was unexpectedly not up-regulated by hyperosmolarity in rat astrocyte primary cultures, although the expression of TAUT mRNA was up-regulated. It is presumed from this that there exists a repression mechanism which blocks the activation of some of the osmosensitive genes in tissues such as astrocytes.

The down-regulation of taurine uptake caused by culturing the cells with taurine is called 'adaptive regulation' and has also been reported in the cell lines of the kidney, placenta and small intestine [12–14]. We have found in the present study that such a regulation system also exists in the human hepatic HepG2 cell line. We also found that this down-regulation of taurine uptake occurred at least at the transcriptional level (Figure 10A). It has been assumed that the promoter region of TAUT has the TREE (taurine-responsive element): the taurine-induced transcriptional factor binds to TREE and represses the transcriptional activity. Han et al. [33] have reported that at least one TREE exists between -574 and -963 of the 5′-flanking region of the rat TAUT gene.

In contrast with the TAUT, the expression level of CDO was not influenced by the external taurine concentration (Figure 10B). It has been reported that the rat liver CDO activity was regulated by dietary factors such as additional protein and sulphur-containing amino acids [34–36]. However, there is little other information available on the regulation of human CDO. Tsuboyama-Kasaoka et al. [24] have isolated the human CDO gene and revealed that the CDO mRNA level in human HepG2 cells was changed by PMA treatment.

In the present study, both TAUT and CDO were regulated by hypertonicity, whereas only TAUT and not CDO, was regulated by taurine-rich conditions. This difference may reflect the physiological significance of taurine in liver cells. Extensive changes in osmolarity occur in the liver by nutrient absorption and hormones. It is also known that liver cells are exposed to extensive hyperosmolarity, especially from diseases which involve an unusual change in the serum osmolarity such as diabetes, hepatocirrhosis and dehydration [37]. Therefore it is presumed that the intracellular taurine content would be increased not only by up-regulating TAUT, but also by up-regulating the taurine biosynthetic pathway to protect and maintain the cellular function of liver cells against lethal hyperosmolarity.

On the other hand, under taurine-rich conditions, only the activity and expression level of TAUT were regulated, whereas the expression level of CDO mRNA was not affected. Since taurine itself has no toxicity [1,2], exposure to a high concentration of taurine had no adverse effect on the cell viability or cell functions in several cultured cell lines including HepG2 (results not shown). A change in the extracellular concentration of taurine is therefore not a lethal factor, and down-regulation of TAUT would be enough to maintain the intracellular taurine concentration at a suitable level.

In conclusion, human TAUT and CDO were co-operatively regulated in response to hypertonicity, but not so in response to taurine-rich conditions. This indicates that the TAUT and the taurine biosynthetic enzymes have an independent regulatory system. However, under certain conditions such as hypertonicity, they could be regulated in harmony with each other.

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