Osmoregulated taurine transport in H4IIE hepatoma cells and perfused rat liver

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The effects of aniso-osmotic exposure on taurine transport were studied in H4IIE rat hepatoma cells. Hyperosmotic (405 mosmol/l) exposure of H4IIE cells stimulated Na^+-dependent taurine uptake and led to an increase in taurine transporter (TAUT) mRNA levels, whereas hypo-osmotic (205 mosmol/l) exposure diminished both taurine uptake and TAUT mRNA levels when compared with normo-osmotic (305 mosmol/l) control incubations. Taurine uptake increased 30–40-fold upon raising the ambient osmolarity from 205 to 405 mosmol/l. When H4IIE cells and perfused livers were preloaded with taurine, hypo-osmotic cell swelling led to a rapid release of taurine from the cells. The taurine efflux, but not taurine uptake, was sensitive to 4,4′-di-isothiocyanatostilbene-2,2′-disulphonic acid (DIDS), suggestive of an involvement of DIDS-sensitive channels in mediating volume-regulatory taurine efflux. Whereas in both H4IIE rat hepatoma cells and primary hepatocytes TAUT mRNA levels were strongly dependent upon ambient osmolarity, mRNAs for other osmolyte transporters, i.e. the betaine transporter BGT-1 and the Na^+/*myo*-inositol transporter SMIT, were not detectable. In line with this, *myo*-inositol uptake by H4IIE hepatoma cells was low and was not stimulated by hyperosmolarity. However, despite the absence of BGT-1 mRNA, a slight osmosensitive uptake of betaine was observed, but the rate was less than 10% of that of taurine transport. This study identifies a constitutively expressed and osmosensitive TAUT in H4IIE cells and the use of taurine as a main osmolyte, whereas betaine and *myo*-inositol play little or no role in the osmolyte strategy in these cells. This is in contrast with rat liver macrophages, in which betaine has been shown to be a major osmolyte.

INTRODUCTION

Alterations in hepatocellular hydration, which reflect changes in liver cell volume on a short-term time scale, occur within minutes under the influence of hormones, cumulative substrate uptake or oxidative stress (for reviews, see [1,2]). Such changes in cell hydration were recognized as an independent signal which influences a variety of metabolic liver functions [3,4], such as protein turnover, carbohydrate metabolism, biliary excretion, viral replication and gene expression [5–13]. In view of this sensitive and potent osmoregulation of liver cell function, we investigated whether organic osmolytes participate in the cell volume homoeostasis of liver cells. Organic osmolytes are compounds that are specifically accumulated or released by cells in reponse to hyperosmotic cell shrinkage or hypo-osmotic cell swelling respectively, in order to maintain cell volume homoeostasis. Osmolytes need to be non-perturbing solutes that do not interfere with protein function, even when occurring at high concentrations (for reviews, see [14–16]).

In mammals, osmolytes have been identified in astrocytes, renal medulla cells, lens epithelia and macrophages [17–24]. Osmolytes such as betaine, taurine and *myo*-inositol are taken up via Na^+-dependent transporters, which are induced upon hyperosmotic exposure [18,20,21,23,25,26]. Organic osmolytes have not been identified with certainty in hepatocytes; however, recent studies identified betaine as an osmolyte in liver macrophages (Kupffer cells) and provided evidence that the osmolyte strategy used by these cells is a potential site in the regulation of macrophage function [23]. A volume-activated taurine channel is present in skate hepatocytes [27]. Hypotonic stress induces significant taurine release from trout hepatocytes [28]; however, only minor amounts of taurine are released from isolated perfused rat liver [29]. Taurine uptake by rat hepatocytes mainly consists of a saturable, sodium- and energy-dependent and carrier-mediated process [30], and hepatocytes possess a Na^+-coupled taurine transporter in the plasma membrane [31]. TAUT, a taurine transporter, has been cloned from MDCK (Madin–Darby canine kidney) cells [32], rat brain [33], mouse brain [34], a human thyroid cell line [35] and human placenta [36,37]. The present paper identifies taurine as an osmolyte in rat liver parenchymal cells.

MATERIALS AND METHODS

Materials

Guanidinium thiocyanate and SDS were from Fluka (Karlsruhe, Germany). First-strand cDNA synthesis kit was from Boehringer (Mannheim, Germany), and *Taq* polymerase was from Eurogentec (Seraing, Belgium). Oligonucleotide labelling kit was from Pharmacia (Freiburg, Germany). Cell-culture medium and fetal bovine serum were from Gibco BRL (Eggenstein, Germany). [α-^{32}P]dCTP (3000 Ci/mmol) was from ICN (Meckenheim, Germany), and [^{3}H]taurine (24 Ci/mmol), [^{14}C]betaine (48.1 mCi/mmol) and *myo*-[^{3}H]inositol (22.3 Ci/mmol) were from New England Nuclear DuPont (Bad Homburg, Germany). Hybond-N nylon membranes were from Amersham Buchler (Braunschweig, Germany). The salts required for preparation of the Krebs–Henseleit buffer were from Merck (Darmstadt, Germany). All other chemicals were from Sigma (Deisenhofen, Germany). Two plasmids containing full-length betaine trans-

Abbreviations used: BGT-1, betaine transporter; DIDS, 4,4′-di-isothiocyanatostilbene-2,2′-disulphonic acid; DME, Dulbecco's minimum essential medium; GABA, γ-aminobutyrate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MDCK cells, Madin–Darby canine kidney cells; MeAIB, methylaminoisobutyrate; SMIT, sodium/*myo*-inositol co-transporter; TAUT, taurine transporter.

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porter (BGT-1) [38] and Na^+/*myo*-inositol co-transporter (SMIT) [39] cDNAs were kindly provided by Dr. H. Moo Kwon (Division of Nephrology, The John Hopkins University School of Medicine, Baltimore, MD, U.S.A). The 1.0 kb cDNA fragment of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which was used for standardization, was from Clontech (Palo Alto, CA, U.S.A.).

Isolation and culture of rat hepatocytes and astrocytes

Isolated hepatocytes were prepared from livers of male Wistar rats, fed *ad libitum* on stock diet, by collagenase perfusion as described previously [40] and plated on collagen-coated 100 mm-diam. culture plates (Costar, Cambridge, MA, U.S.A.) at a density of about 1×10^6 cells/ml. Cell viability was more than 95 %, as assessed by Trypan Blue exclusion. Then, cells were cultivated in Dulbecco's minimum essential medium (DME)/F-12 medium (37 °C, 5 % CO_2, pH 7.4) containing glucose (5 mmol/l) and supplemented with 10 % fetal bovine serum, for 4 days. Medium was changed every day and 8 h before the start of the experiments. Osmolarity was altered by varying the NaCl concentration in the medium. Cells were maintained under various test conditions for 6 h.

Primary astrocytes were prepared from cerebral hemispheres of newborn Wistar rats as described [41] and cultured for 2–3 weeks in DME (37 °C, 5 % CO_2, pH 7.4) supplemented with 10 % fetal bovine serum in 75 cm^2 cell culture flasks (Costar). The purity of the cell culture, as determined by glial fibrillary acidic protein immunohistochemical staining, was > 95 %. Osmolarity was altered by varying the NaCl concentration in the medium. Cells were maintained under various test conditions for 8 h.

Culture of H4IIE hepatoma cells

H4IIE.C3 rat hepatoma cells (A.T.C.C. CRL 1600) were grown to near-confluency in DME/F12 medium (37 °C, 5 % CO_2, pH 7.4) plus glucose (5 mmol/l), supplemented with 10 % fetal bovine serum, in Cluster 6 dishes (Costar). The medium was changed 16 h before the cells were placed under various test conditions. Osmolarity was altered by varying the NaCl concentration in the medium. The volume of H4IIE cells was measured by flow resistance cytometry using a Casy 1 cell counter and analyser system (Schärfe Systeme, Reutlingen, Germany). In normo-osmotic medium, the average H4IIE cell volume was 1093 ± 34 fl (three different culture dishes). The protein content was 0.20 ± 0.03 mg/10^6 cells ($n = 3$). Assuming a water content of 80 % of the whole H4IIE cell volume, a mean intracellular water space of 4.4 μl/mg of protein is estimated.

Isolation and sequencing of a rat TAUT cDNA

To identify hepatocytes and H4IIE cells expressing TAUT mRNA, exact primers (24-mers) were designed, such that a 707 bp fragment of rat TAUT could be amplified from cDNA. The sense primer (5′ TCAGAGGGAGAAGTGGTCCAGCAA 3′) and antisense primer (5′ ATTTCATGCCTTCACCAGCA-CCTG 3′) correspond to nucleotide positions 243–266 and 929–952 of the rat brain TAUT [33]. Total RNA from H4IIE cells was reverse-transcribed using a first-strand cDNA synthesis kit (Boehringer Mannheim), and the resulting cDNA was subjected to PCR amplification (30 cycles of 94 °C for 2 min, 68 °C for 2 min and 72 °C for 3 min, with a 10 min extension time on cycle 30) with *Taq* polymerase using the primers described above. The PCR product was electrophoresed on a 1.5 % agarose gel, extracted and purified using a gel extraction kit (Qiagen, Hilden, Germany). Sequencing of the sense and antisense cDNA strands was by the dideoxy chain-termination method, using a dye terminator cycle sequencing kit (ABI-Perkin Elmer, Foster City, CA, U.S.A.) and analysis on an ABI 373A DNA Sequencer.

Northern-blot analysis

Total RNA from hepatocytes, astrocytes or near-confluent plates of H4IIE cells was isolated by using guanidinium thiocyanate solution as described in [42]. RNA samples (15 μg) were electrophoresed in 0.8 % agarose/3 % formaldehyde and then blotted on to Hybond-N nylon membranes with $20 \times$ SSC (3 mol/l NaCl, 0.3 mol/l sodium citrate). After brief rinsing with water and cross-linking (Hoefer UV-crosslinker 500; Hoefer, San Francisco, CA, U.S.A.), the membranes were observed under UV illumination to determine RNA integrity and the location of the 28 S and 18 S rRNA bands. Blots were then subjected to a 3 h prehybridisation at 43 °C in 50 % deionized formamide in sodium phosphate buffer (0.25 mol/l, pH 7.2) containing 0.25 mol/l NaCl, 1 mmol/l EDTA, 100 μg/ml salmon sperm DNA and 7 % SDS. Hybridization was carried out in the same solution with approx. 10^6 c.p.m./ml [α-^{32}P]dCTP-labelled random-primed TAUT, SMIT, BGT-1 or GAPDH cDNA probes. Membranes were washed three times in $2 \times$ SSC/0.1 % SDS for 15 min, twice in sodium phosphate buffer (25 mmol/l, pH 7.2)/EDTA (1 mmol/l)/0.1 % SDS for 10 min and twice in sodium phosphate buffer (25 mmol/l, pH 7.2)/EDTA (1 mmol/l)/1 % SDS for 10 min at 53 °C. Blots were then exposed to Kodak AR X-Omat film at −70 °C with intensifying screens. Suitably exposed autoradiograms were then analysed by densitometry scanning (PDI, New York, NY, U.S.A.) to determine the densities of the mRNAs. Relative mRNA levels were determined by standardization of the density of GAPDH mRNA.

Measurement of taurine transport

H4IIE rat hepatoma cells were maintained for 16 h in serum-free hypo-osmotic (205 mosmol/l), normo-osmotic (305 mosmol/l) or hyperosmotic (405 mosmol/l) DME/F-12 medium. The osmolarity was altered by varying the NaCl concentration in the medium. Then the cells were rinsed twice and incubated for 15 min at 37 °C with 1 ml of Krebs–Henseleit buffer with an osmolarity as used in the previous medium. This solution was replaced with an identical buffer containing [^{3}H]taurine, [^{14}C]betaine or *myo*-[^{3}H]inositol (100 μmol/l; 0.5 μCi/ml) and incubated for various time periods (0–240 min) at 37 °C. Thereafter, the cells were rinsed four times with 1 ml of ice-cold stop solution (10 mmol/l Tris/Hepes, pH 7.4, 300 mmol/l mannitol, 300 mmol/l NaCl) and harvested with 1 ml of 0.25 M NaOH/0.01 % SDS. ^{3}H or ^{14}C in the supernatant and in the cells was measured by scintillation counting, and the protein concentration was determined using a Bio-Rad protein assay (Bio-Rad, Hercules, CA, U.S.A.).

For studies on betaine efflux, H4IIE cells were maintained for 16 h in serum-free hyperosmotic (405 mosmol/l) DME/F12 medium and then the medium was replaced with an identical medium containing [^{3}H]taurine (100 μmol/l; 0.5 μCi/ml) in order to load the cells with radioisotope. After a loading period of 2 h at 37 °C in 5 % CO_2, the cells were rinsed three times with hyperosmotic (405 mosmol/l) Krebs–Henseleit buffer. The osmolarity was altered by varying the NaCl concentration in the medium. Then the cells were incubated for 0–30 min in taurine-free hyperosmotic (405 mosmol/l) or hypo-osmotic (205 mosmol/l) Krebs–Henseleit buffer. For inhibitor

studies, 0.1 mmol/l DIDS (4,4′-di-isothiocyanatostilbene-2,2′-disulphonic acid) was added during the last 15 min of the loading period and the entire efflux measurement period. Thereafter the medium was collected and the cells were harvested with 1 ml of 0.25 M NaOH/0.01 % SDS. [^{3}H]Taurine appearance in the supernatant was measured by scintillation counting and expressed as a percentage of total [^{3}H]taurine (contained in cells plus supernatant). As shown by HPLC analysis using an isocratic system with acetonitrile/ethanol/acetic acid/ammonium acetate (1 mol/l)/sodium phosphate (0.1 mol/l) (800:68:2:3:127:10, by vol.), at a flow rate of 1.5 ml/min, and a Perkin–Elmer Pecosphere-3CSi (5 μm; 4.6 mm × 83 mm) column, more than 95 % of ^{3}H radioactivity released into the supernatant co-eluted with [^{3}H]taurine standards.

Liver perfusion

Male Wistar rats (120–200 g body weight), fed *ad libitum* with standard chow, were raised in the local institute for laboratory animals and held according to local ethical guidelines. Livers were isolated and perfused as described previously [43] in a non-recirculating manner from the portal to the hepatic vein with bicarbonate-buffered Krebs–Henseleit saline containing lactate (2.1 mmol/l) and pyruvate (0.3 mmol/l). The perfusate was equilibrated with O_2/CO_2 (19:1); the perfusate flow rate was approx. 4 ml/min per g of liver tissue. The temperature was 37 °C. The osmolarity of normo-osmotic perfusion buffer was 305 mosmol/l. During hypo-osmotic perfusion, the NaCl concentration in the perfusion buffer was decreased by 40 mmol/l, yielding an osmolarity of 225 mosmol/l. The K^+ concentration in the effluent perfusate was monitored with a K^+-sensitive electrode (Radiometer, Willich, Germany). The portal pressure was monitored continuously with a pressure transducer. Livers were prelabelled with [^{3}H]taurine (25 μCi) for 20 min during normo-osmotic perfusion, and 30 min later the perfusion medium was replaced with hypo-osmotic medium as indicated. In inhibitor studies, DIDS (dissolved in DMSO) was added with a syringe and a precision micropump, yielding an influent concentration of 100 μmol/l. Samples of 1 ml of effluent perfusate were collected every 1 min and counted for radioactivity.

Statistics

Data are given as means ± S.E.M., where n is number of culture dishes or perfusion experiments. Statistical analysis was performed using Student's t test; $P < 0.05$ was considered to be statistically significant.

RESULTS

Molecular identity of the TAUT expressed in H4IIE rat hepatoma cells

An osmoregulated Cl^-- and Na^+-dependent TAUT has been identified from rat brain, the cDNA of which was cloned [33]. The H4IIE cell cDNAs obtained by reverse transcription of total RNA were amplified by PCR using specific primers for rat brain TAUT. The nucleotide sequence of the 0.7 kb PCR product was determined to confirm identity with the portion of the TAUT that is flanked by the PCR primers. This sequence was found to be exactly the same as nucleotide positions 243–952 of the rat brain TAUT cDNA (results not shown), and represents approx. 37 % of the coding region.

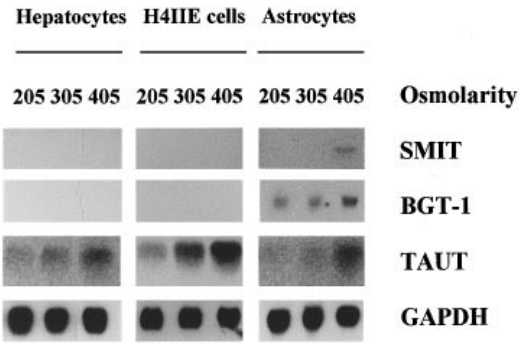

Figure 1 Organic osmolyte transporter gene expression in rat hepatocytes, H4IIE hepatoma cells and astrocytes

Hepatocytes or H4IIE cells were exposed for 6 h and astrocytes were exposed for 8 h to hypo-osmotic (205 mosmol/l), normo-osmotic (305 mosmol/l) or hyperosmotic (405 mosmol/l) medium. The osmolarity changes were obtained by appropriate changes in the NaCl concentration. Thereafter, the cells were harvested for RNA isolation and subjected to Northern-blot analysis for TAUT, SMIT, BGT-1 and GAPDH mRNAs (15 μg of total RNA per lane). This experiment is representative of three separate experiments.

Effects of aniso-osmolarity on TAUT mRNA levels in rat hepatocytes and H4IIE cells

Hyperosmotic (405 mosmol/l) exposure of isolated rat hepatocytes for 6 h due to the addition of 50 mmol/l NaCl to the cell culture medium led to an increase of 118 ± 10 %, whereas hypo-osmotic exposure (205 mosmol/l, by lowering the NaCl concentration by 50 mmol/l) led to a decrease of 51 ± 10 % in TAUT mRNA levels, compared with normo-osmotic (305 mosmol/l) control incubations (Figure 1). In normo-osmotic media, TAUT mRNA levels were 2–3-fold higher in H4IIE cells when compared with hepatocytes. GAPDH mRNA levels were chosen as standard, because this mRNA was shown to be uninfluenced by aniso-osmolarity [10]. Aniso-osmotic exposure of H4IIE cells led to changes in TAUT mRNA levels similar to those observed with the rat hepatocytes (Figure 1). In H4IIE cells, hypo-osmotic and hyperosmotic exposure resulted in a level of TAUT mRNA that was 59 ± 1 % lower and 222 ± 29 % higher respectively than that seen with normo-osmotic exposure. mRNAs for BGT-1 and SMIT were not detectable in hepatocytes or in H4IIE cells, and there was no induction by hyperosmolarity (Figure 1). As a control, mRNA levels for these transporters were studied in astrocytes. Here, SMIT mRNA was not detectable under normo-osmotic incubations, but was strongly increased during hyperosmotic incubation. BGT-1 and TAUT mRNAs observed in normo-osmotic incubations were expressed osmosensitively in astrocytes.

Cultured H4IIE cells were then used to characterize further the modulation of TAUT mRNA levels and taurine transport by aniso-osmolarity. The effect of osmolarity on TAUT mRNA levels was strongly osmolarity-dependent (Figure 2). Time courses of induction are given in Figure 3. Under normo-osmotic conditions, TAUT mRNA levels were lowered within 3 h after a change of medium, but rose within the next 6 h. When compared with the normo-osmotic conditions, significant changes in TAUT mRNA levels were detectable after 1 h of hyperosmotic exposure and after 6 h of hypo-osmotic exposure (Figure 3). After a 20 h incubation the hyperosmotic effect on TAUT mRNA levels was still present in H4IIE cells. The simultaneous addition of 1 mmol/l taurine diminished TAUT mRNA levels by more than

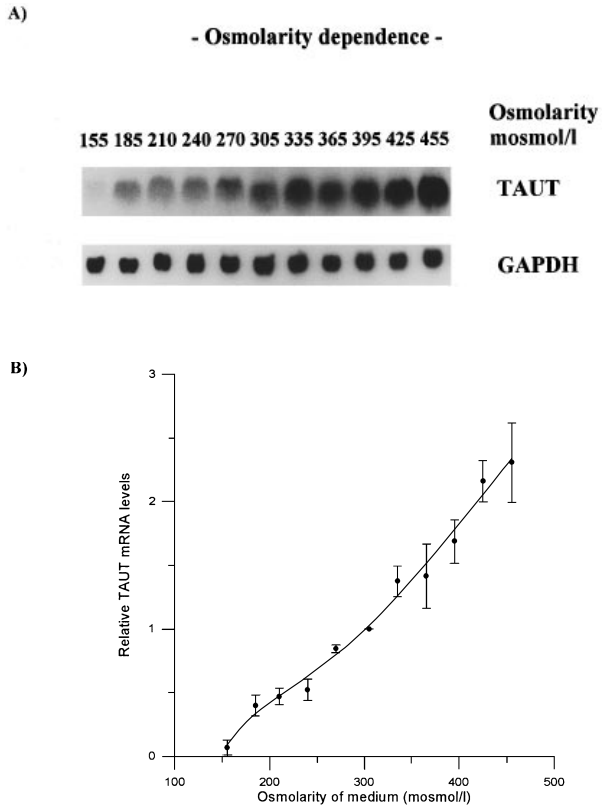

Figure 2 Effects of aniso-osmotic exposure on TAUT and GAPDH mRNA levels in H4IIE rat hepatoma cells

H4IIE cells were exposed for 6 h to media with osmolarities as indicated. The osmolarity changes were obtained by appropriate changes in the NaCl concentration. Thereafter, the cells were harvested for RNA isolation and subjected to Northern-blot analysis for TAUT and GAPDH. (**A**) Representative Northern-blot experiment (15 μg of total RNA per lane). In (**B**), TAUT mRNA levels were normalized to GAPDH mRNA levels, and the relative TAUT mRNA level found in normo-osmotic incubations (305 mosmol/l) was arbitrarily set to 1. Data are means $\pm$ S.E.M., and are from three different culture dishes for each condition.

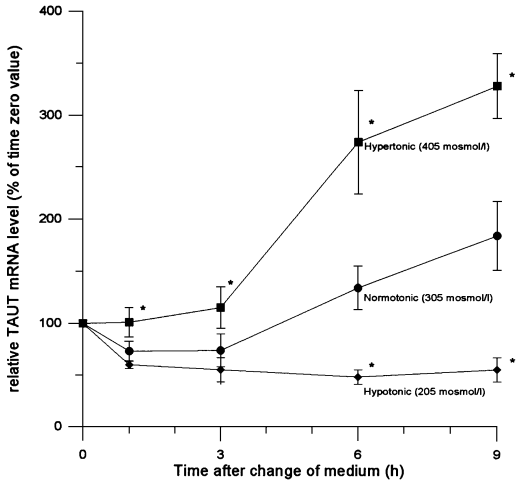

Figure 3 Time-dependent induction of TAUT mRNA levels in H4IIE cells during hyperosmolarity

H4IIE cells were incubated for the time periods indicated in normo-osmotic (305 mosmol/l), hypo-osmotic (205 mosml/l) or hyperosmotic (405 mosmol/l) medium. The osmolarity changes were obtained by increasing or lowering the NaCl concentration by 50 mmol/l. At the time points indicated, cells were harvested for RNA isolation and subjected to Northern-blot analysis for TAUT and GAPDH. TAUT mRNA levels were normalized to GAPDH mRNA levels. The time zero value was set to 100%, and the relative TAUT mRNA levels found at each time point were expressed as percentages thereof. Data are means $\pm$ S.E.M. and are from three different culture dishes for each condition. *Significantly different from the normo-osmotic (305 mosmol/l) incubations ($P < 0.05$).

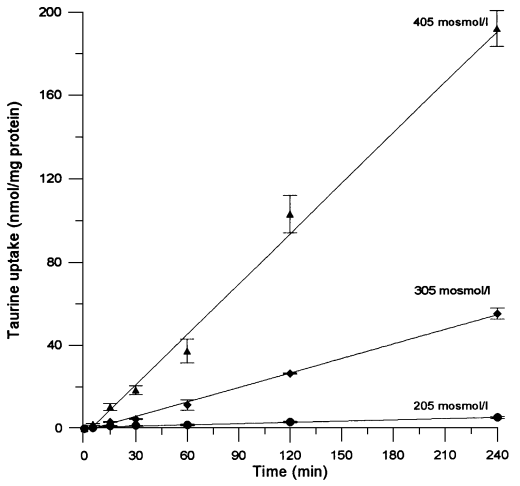

Figure 4 Osmosensitive taurine transport in H4IIE cells

H4IIE cells were preincubated for 16 h in hypo-osmotic (205 mosmol/l), normo-osmotic (305 mosmol/l) or hyperosmotic (405 mosmol/l) medium. Osmolarity changes were obtained by appropriate addition/removal of NaCl. The uptake of [^{3}H]taurine (100 μmol/l) was measured in medium with the same osmolarity. Data are means $\pm$ S.E.M. ($n = 3$).

50% in normo-osmotic (305 mosmol/l) and hyperosmotic (405 mosmol/l) incubations (U. Warskulat, M. Wettstein and D. Häussinger, unpublished work).

Effect of aniso-osmolarity on taurine uptake

Taurine was taken up by H4IIE rat hepatoma cells when incubated in normo-osmotic (305 mosmol/l) medium (Figure 4). Taurine uptake was linear over a 4 h period and was stimulated 4-fold when the cells were exposed to hyperosmotic (405 mosmol/l) medium for 16 h (Figure 4). On the other hand, exposure of H4IIE cells to hypo-osmotic (205 mosmol/l) medium for 16 h lowered taurine uptake by about 90% (Figure 4). At an extracellular concentration of 100 μmol/l, the intracellular taurine concentration increased under hyperosmotic incubations to about 100 nmol/mg of protein within a 2 h period of exposure to taurine. Given an intracellular water space of approx. 4.4 μl/mg of protein (see the Materials and methods section), an intracellular taurine concentration of about 23 mmol/l can be calculated. Thus, after hyperosmotic exposure, an intracellular/extracellular concentration gradient for taurine of about 230:1 is created. Irrespective of the medium osmolarity, taurine uptake in hyperosmotically pre-exposed cells was inhibited by more than 95% when Na^+ was replaced by either Li^+ or choline, indicating that taurine uptake occurred via an Na^+-dependent route. Substitution of Cl^- by gluconate also strongly inhibited taurine uptake (Table 1).

Figure 5 shows the concentration-dependence of the 2 h taurine uptake after a 16 h pre-exposure to hypo-osmotic (205 mosmol/l), normo-osmotic (305 mosmol/l), or hyperosmotic (405 mosmol/l) medium. The findings suggest that the hyperosmolarity-induced increase in taurine uptake involves an

Table 1 Na^+- and Cl^--dependence of hyperosmolarity-induced taurine transport

H4IIE cells were preincubated for 16 h in hyperosmotic medium (405 mosmol/l; prepared by addition of 50 mmol/l NaCl), in order to induce taurine transport activity. Then, the uptake of [^{3}H]taurine (100 μmol/l) was measured over 1 h in control hyperosmotic medium (405 mosmol/l), or in hyperosmotic media in which Na^+ was replaced by choline or Li^+ or in which gluconate was substituted for Cl^-. Data are means ± S.E.M. ($n = 3$).

	Taurine uptake (nmol/h per mg of protein)	Inhibition of taurine uptake (%)
Control	41.7 ± 1.4	
Sodium replacement by:		
Lithium	1.3 ± 0.8	97
Choline	< 0.1 ± < 0.1	> 99
Chloride replacement by:		
Gluconate	13.7 ± 0.5	67

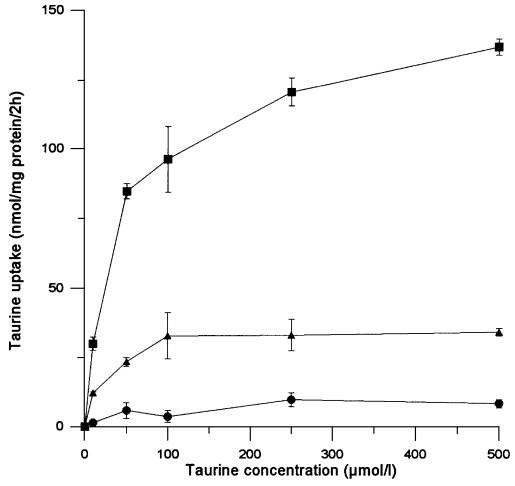

Figure 5 Concentration-dependence of taurine uptake in H4IIE cells

H4IIE cells were preincubated for 16 h in hyperosmotic (405 mosmol/l, ■), normo-osmotic (305 mosmol/l, ▲) or hypo-osmotic (205 mosmol/l, ●) medium. Osmolarity changes were obtained by appropriate addition/removal of NaCl. Then [^{3}H]taurine was added at the concentrations indicated, and taurine uptake was measured over a 2 h period. Data are means ± S.E.M. ($n = 3$–6).

increase in transport capacity. As shown in Table 2, taurine (100 μmol/l) uptake in hyperosmotically pre-exposed H4IIE cells was inhibited by γ-aminobutyrate (GABA; 5 mmol/l) by 63 %. This again indicates the involvement of the TAUT, which was shown to be sensitive to inhibition by GABA in rat brain [33]. Proline and L-alanine inhibited taurine uptake by 46 % and 36 % respectively. On the other hand, methylaminoisobutyrate (MeAIB), which is a model substrate for amino acid transport system A [44], did not inhibit taurine uptake significantly (Table 2), indicating that taurine transport occurred to only a negligible extent via system A. Betaine, *myo*-inositol and choline also did not affect taurine uptake (Table 2).

In order to determine whether H4IIE cells use osmolytes other than taurine, we tested the extent to which H4IIE cells accumulate betaine and *myo*-inositol. Recent studies identified betaine as an osmolyte in rat liver macrophages (Kupffer cells), and betaine transport is induced in response to increases in ambient osmolarity [23]. *myo*-Inositol plays a role in the osmoregulation

Table 2 Inhibition of hyperosmolarity-induced taurine uptake by various agents

H4IIE cells were exposed for 16 h to hyperosmotic medium (405 mosmol/l); thereafter [^{3}H]taurine (100 μmol/l) uptake was measured over 1 h in the absence (control) or presence of various agents, which were each added at a concentration of 5 mmol/l. Data are means ± S.E.M. ($n = 3$). Significant difference compared with control: $^*P < 0.05$.

	Taurine uptake (nmol/h per mg of protein)	Inhibition of taurine uptake (%)
Control	44.8 ± 3.3	–
GABA	16.4 ± 1.4*	63
Proline	24.2 ± 4.4*	46
L-Alanine	28.7 ± 0.9*	36
Betaine	38.6 ± 3.2	14
MeAIB	39.6 ± 1.9	12
Choline	46.5 ± 4.1	0
myo-Inositol	48.8 ± 2.3	0

of MDCK cells [21], rat brain glia cells [20] and bovine lens epithelial cells [19]. Thus H4IIE cells were preincubated for 16 h in hypo-osmotic (205 mosmol/l), normo-osmotic (305 mosmol/l) or hyperosmotic (405 mosmol/l) medium and then the uptake of 100 μmol/l [^{3}H]taurine, [^{14}C]betaine or *myo*-[^{3}H]inositol was measured for 2 h in medium with the same osmolarity. In contrast with taurine transport, betaine and *myo*-inositol uptake was low in H4IIE cells, and there was only slight stimulation of betaine, but not *myo*-inositol, uptake by hyperosmolarity (Table 3).

Osmoregulation of taurine efflux

To examine whether taurine efflux from the cells is osmosensitive, H4IIE cells were incubated in hyperosmotic (405 mosmol/l) medium for 16 h and then preloaded with [^{3}H]taurine (100 μmol/l) for another 2h in the same medium. After washing the cells, they were exposed to taurine-free medium and the appearance of radioactivity in the supernatant was monitored. As shown in Figure 6, following removal of extracellular taurine while maintaining the osmolarity, less than 5 % of the cellular taurine was released within 30 min. When, however, the osmolarity was decreased to 205 mosmol/l, taurine efflux was significantly stimulated and occurred within the first 5 min of the hypo-osmotic stress. This taurine efflux was largely inhibited by the anion-exchanger inhibitor DIDS. On the other hand, DIDS had no effect on cumulative taurine uptake in hyperosmotic

Table 3 Organic osmolyte transport in H4IIE cells

H4IIE cells were preincubated for 16 h in hypo-osmotic (205 mosmol/l), normo-osmotic (305 mosmol/l) or hyperosmotic (405 mosmol/l) medium. Osmolarity changes were obtained by appropriate addition/removal of NaCl. Then, the uptake of 100 μmol/l [^{3}H]taurine, [^{14}C]betaine or *myo*-[^{3}H]inositol was measured for 2 h in medium with the same osmolarity. Data are means ± S.E.M. ($n = 3$).

	Uptake (nmol/2 h per mg of protein)		
	205 mosmol/l	305 mosmol/l	405 mosmol/l
Taurine	2.9 ± 0.3	26.3 ± 0.3	102.8 ± 4.4
Betaine	0.7 ± 0.1	1.7 ± 0.1	8.2 ± 0.2
myo-Inositol	5.4 ± 0.7	2.4 ± 0.2	1.9 ± 0.4

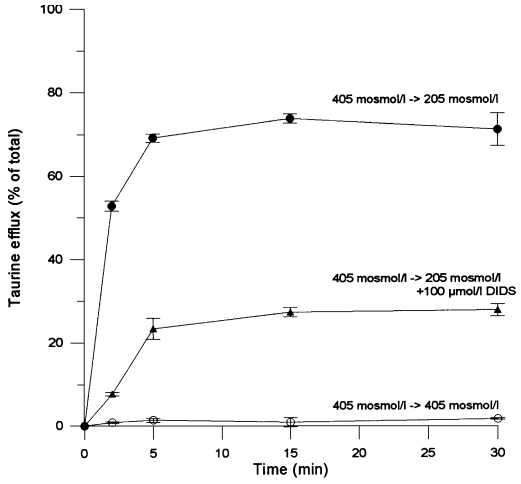

Figure 6 Hypo-osmolarity-stimulated taurine efflux from H4IIE cells

H4IIE cells were preincubated for 16 h in hyperosmotic (405 mosmol/l) medium. Then cells were allowed to accumulate [^{3}H]taurine (added at a concentration of 100 μmol/l) for 2 h and washed three times. Thereafter, cells were exposed to taurine-free hypo-osmotic (205 mosmol/l) (●) or hyperosmotic (405 mosmol/l) (○) medium. In inhibitor studies (▲), 0.1 mmol/l DIDS was added during the last 15 min of the loading period and throughout the efflux measurement period. [^{3}H]Taurine appearance in the supernatant was measured and expressed as a percentage of total [^{3}H]taurine (contained in cells plus supernatant). Data are means ± S.E.M. ($n = 3$).

Table 4 Taurine uptake in the presence of DIDS

H4IIE cells were exposed for 16 h to hypo-osmotic (205 mosmol/l), normo-osmotic (305 mosmol/l) or hyperosmotic (405 mosmol/l) medium. Osmolarity changes were obtained by appropriate addition/removal of NaCl. Then, [^{3}H]taurine (100 μmol/l) uptake was measured over 1 h in medium with the same osmolarity in the absence (control) or presence of 0.1 mmol/l DIDS. Data are means ± S.E.M. ($n = 3$). Values in parentheses are taurine uptake in the presence of DIDS expressed as a percentage of that in the absence of DIDS. Significant difference compared with control: $^*P < 0.05$.

	Taurine uptake (nmol/2 h per mg of protein)	
Osmolarity (mosmol/l)	Control	+ DIDS
205	2.9 ± 0.2	4.6 ± 0.7* (159%)
305	16.0 ± 0.2	18.3 ± 0.3* (114%)
405	44.5 ± 3.8	42.7 ± 2.7 (96%)

medium (Table 4). When the 1 h taurine uptake was studied under normo- and hypo-osmotic incubations, the presence of DIDS increased cellular taurine by 14% and 59% respectively (Table 4). These findings indicate that the taurine efflux pathway is barely, if at all, active under normo-osmotic or hyperosmotic incubations, but is activated by hypo-osmolarity.

Rapid hypo-osmolarity-induced taurine efflux was also observed in the isolated perfused rat liver after preloading with [^{3}H]taurine (Figure 7). During normo-osmotic perfusion (305 mosmol/l), there was a basal release of radioactivity into the effluent perfusate. Changing to a hypo-osmotic perfusion medium led to a marked stimulation of radioactivity release, reaching a maximum 3–4 min after the onset of hypo-osmotic stress and with a time course closely resembling that of volume-regulatory K^+ efflux [8,9]. On HPLC separation, this radioactivity coeluted with [^{3}H]taurine standards. After the initial peak,

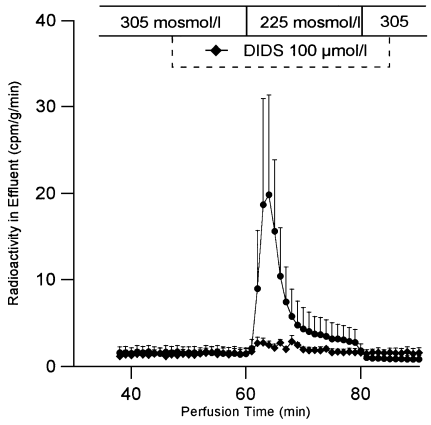

Figure 7 Hypo-osmolarity-induced release of [^{3}H]taurine in the perfused rat liver

Livers were prelabelled with [^{3}H]taurine (25 μCi) for 20 min during normo-osmotic perfusion at the beginning of each experiment. Data are means ± S.E.M. ($n = 3$).

radioactivity in the effluent perfusate remained elevated by 2–3-fold compared with the basal radioactivity release during normo-osmotic perfusion. Administration of DIDS did not influence basal radioactivity release, but inhibited the hypo-osmolarity-induced radioactivity release by more than 90% (Figure 7).

DISCUSSION

The present study shows that taurine transport is osmoregulated in rat hepatocytes and H4IIE hepatoma cells, indicating a role for taurine as an osmolyte in these cells. This conclusion is based on the following findings: (i) a strong osmosensitivity of taurine transport and its induction by hyperosmotic stress, (ii) an increase or decrease in mRNA levels for TAUT in response to hyper-osmolarity and hypo-osmolarity respectively when compared with normo-osmotic control incubations, and (iii) a rapid release of taurine from the cells in response to hypo-osmotic stress. It is likely that the osmoregulated taurine-transporting activity is similar to or identical with the osmosensitive TAUT system in rat brain. Several lines of evidence indicate that osmosensitive taurine uptake in H4IIE rat hepatoma cells is mediated by the TAUT, i.e. the osmosensitivity of TAUT mRNA levels, the Na^+- and Cl^--dependence of taurine transport (Table 1), its inhibition by GABA (Table 2), and the lack of effect of MeAIB, which is a model substrate for transport via system A. Thus H4IIE cells represent another cell type that uses an osmolyte strategy in order to counteract osmotic stress and to maintain cell volume homoeostasis. Apart from regulation of cell volume, the functional relevance of an osmolyte strategy in hepatocytes and the hepatoma cell line, however, remains to be established. The rationale for an osmolyte strategy in these cells is less obvious than in cells from kidney or brain, i.e. organs either facing extreme osmotic stresses or being encapsulated by rigid bone. The taurine concentrations used in the present study are well within the range of the physiological plasma concentration, reported to be 29–99 μmol/l [45,46]. Further, the taurine concentration in the portal vein of the rat is 249 μmol/l, whereas the intrahepatic taurine concentration is 5.5 μmol/g of liver [47].

Taurine occurs at relatively high concentrations (65 mmol/l) in skate hepatocytes, and a cell/plasma gradient for taurine of

500:1 was established [48]. From the data in the present paper, an intracellular taurine concentration of about 23 mmol/l and an intracellular/extracellular concentration gradient for taurine of about 230:1 can be calculated for H4IIE cells when the ambient taurine concentration is 100 μmol/l. These data indicate that taurine may also be a quantitatively important osmolyte in H4IIE cells. Considering the concentration-dependence of taurine uptake (Figure 5), it is likely that, apart from ambient osmolarity, physiological fluctuations of the plasma taurine concentration will be an important determinant of the hepatocellular taurine content.

Whereas taurine appears to be a quantitatively important organic osmolyte in H4IIE cells, the roles of betaine and *myo*-inositol are negligible. This is shown by the undetectable mRNA levels for the osmolyte transporters SMIT and BGT-1 (Figure 1), the low extent and lack of osmosensitivity of *myo*-inositol uptake and the small, but osmosensitive, rate of betaine uptake by H4IIE cells (Table 3). The low rate of osmosensitive betaine uptake in the absence of detectable BGT-1 mRNA may be explained by the contribution of amino acid transport system A. Osmosensitive betaine uptake via system A was shown to occur in SV-3T3 cells, in which BGT-1 mRNA is also undetectable [49], and evidence for an MeAIB-sensitive component in addition to BGT-1-mediated betaine uptake has recently been provided in macrophages [23,24].

Under normo-osmotic conditions, TAUT mRNA levels are apparently greater in H4IIE rat hepatoma cells than in normal hepatocytes. Previous studies have shown that cell swelling is one prerequisite for cell proliferation [50], and *ras* oncogene expression in NIH-3T3 fibroblasts is accompanied by a 30% increase in cell volume [51]. Greater taurine uptake may be also associated with a higher proliferation rate in some cultured cell lines [35]. Thus one might speculate that the high constitutive expression of TAUT in the H4IIE cell line, the activity of which tends to swell the cells, is related to the malignant phenotype of these tumour cells.

In line with findings on betaine transport in Kupffer cells [23], taurine efflux from H4IIE cells in response to hypo-osmolarity was much faster than taurine uptake during hyperosmolarity. In the intact perfused rat liver, taurine is released in response to hypo-osmotic exposure. There is general agreement that osmolyte efflux occurs via routes distinct from those mediating uptake. In C6 glioma cells and MDCK cells, volume-sensitive anion channels mediate the passive loss of certain organic osmolytes from cells [52,53], and it is likely that such channels are also involved in H4IIE cells. In line with this, taurine efflux from H4IIE cells and perfused liver was sensitive to inhibition by DIDS, as was reported previously for taurine efflux from flounder erythrocytes and skate hepatocytes [27,54]. DIDS, however, had no effect on taurine release from perfused rat liver under normo-osmotic conditions (Figure 7). Taurine uptake was shown to be uninfluenced by 0.5 mmol/l DIDS under normo-osmotic conditions in skate hepatocytes [48]. Addition of 0.1 mmol/l DIDS affected taurine uptake only slightly or not at all in normo-osmotically and hyperosmotically exposed H4IIE cells (Table 4). When the 1 h taurine uptake was studied in hypo-osmotic incubations, the presence of DIDS increased cellular taurine levels by 59% (Table 4). Because taurine uptake by H4IIE cells is the net result of unidirectional influx (presumably via TAUT) and simultaneous unidirectional efflux (via a presumed volume-regulated anion channel), these findings suggest that the taurine efflux pathway is barely, if at all, active under normo- and hyperosmotic conditions, but is activated in response to hypo-osmolarity. This points to the existence of a swelling-activated taurine efflux route in rat hepatocytes.

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