Osmoregulatory Alterations in Taurine Uptake by Cultured Human and Bovine Lens Epithelial Cells

Patrick R. Cammarata, Grant Schafer, Shiuh-Wei Chen, Zhen Guo, and Rustin E. Reeves

Purpose. Comparative assessment of cultured human lens epithelial cells (HLECs) and bovine lens epithelial cells (BLECs) established the nature of the relationship between taurine-concentrating capability and intracellular polyol accumulation or extracellular hypertonicity.

Methods. The kinetic characteristics of active taurine accumulation based on the measurement of in vitro [^{3}H]-taurine uptake were resolved by side-to-side review of cultured HLECs and BLECs pre-exposed to either galactose-supplemented medium or extracellular hypertonicity. Competitive RT-PCR was used to appraise variation in taurine transporter (TauT) mRNA abundance from cells maintained in hyperosmotic medium over a 72-hour exposure period.

Results. The capacity to accumulate [^{3}H]-taurine was significantly lowered after prolonged (20-hour) incubation of cultured BLECs in 40 mM galactose in contrast to HLECs, the latter cells' velocity curve being indistinguishable from control cells in physiological medium. Inhibition of the intracellular taurine transport site appeared to be noncompetitive, in that there was a marked reduction in the V_{max} without significant alteration in the K_m to a high-affinity transport site. Galactitol content in BLECs exceeded five times that found in HLECs. The coadministration of the aldose reductase inhibitor, sorbinil, with 40 mM galactose completely prevented the inhibitory effect of galactose on [^{3}H]-taurine uptake. Acute exposure (3 hours) of HLECs and BLECs to a range of 10 to 40 mM galactitol or 10 to 40 mM galactose plus sorbinil-supplemented medium suggested by Dixon plot that neither galactitol nor galactose interacted with the extracellular taurine transport site. In contrast, [^{3}H]-taurine accumulation was markedly elevated in both HLECs and BLECs after prolonged exposure to galactose-free medium made hyperosmotic by supplementation with sodium chloride. The enhanced taurine uptake capacity involved increase in peak velocity (V_{max}) without significant change in Michaelis-Menten constant (K_m). Cultured HLECs and BLECs responded to hypertonicity with an inducible but transitory upregulation of TauT mRNA.

Conclusions. These results demonstrate that lens epithelial cells express a high-affinity TauT protein capable of active uptake, but predisposed to inhibition by intracellular galactitol when the sugar alcohol is present in sufficiently high concentration to interfere with cell metabolism. Furthermore, lens epithelial cells respond to hypertonic stress by raising taurine transport activity. The increase in taurine uptake is due to an increase in the number of high-affinity TauTs expressed as a result of an increase in the manifestation of taurine mRNA stemming from exposure to hypertonic medium. (*Invest Ophthalmol Vis Sci.* 2002;43:425–433)

The lens can counterbalance water stress, either by accumulating osmotically active, nonperturbing organic osmolytes (regulatory volume increase) or by release from cell to medium of organic osmolytes (regulatory volume decrease).[1,2] To date, three compatible organic osmolytes have been identified in cultured lens epithelial cells, sorbitol, *myo*-inositol, and taurine, the latter being an amino acid derivative and the focus of this study.

As a putative lens osmolyte, taurine may participate in volume regulation when the lens epithelial cell experiences osmotic perturbation. Active uptake of taurine into the cell (influx) occurs through an Na^+- and Cl^--dependent taurine transporter (TauT).[3,4] The mechanism of passive movement of taurine out of the cell (efflux) is controversial. To date, whether the taurine efflux pathway occurs through a volume-sensitive organic osmolyte anionic (chloride) channel or volume-activated taurine efflux occurs through a pathway independent of volume-sensitive anion channels remains an open question.[5–9]

To identify the cellular mechanisms by which experimental diabetes, coincident with polyol accumulation and taurine depletion, impairs normal lens function, a reliable set of in vitro cellular parameters should be identified. We have reported that galactose inhibits the ouabain-sensitive uptake of *myo*-inositol.[10] The coadministration of the aldose reductase (AR) inhibitor, sorbinil (Pfizer, Groton, CT), to galactose-supplemented medium corrects the attenuated *myo*-inositol uptake, indicating that galactitol interferes with the *myo*-inositol transport system. In addition, we have provided evidence that an increase in *myo*-inositol uptake could be accounted for by an increase in the number of *myo*-inositol transporters, due to upregulation of *myo*-inositol transporter mRNA, resulting from cell incubation in hypertonic medium.[10]

The studies reported herein were designed to investigate the effects of high extracellular galactose on [^{3}H]-taurine uptake, using a range of taurine concentration from 1.5 to 400 μM, as well as the influence on taurine uptake by exposure to hypertonic medium. Experiments such as these, using a concentration range of taurine and hyperosmotic insult, are necessary to uncover potential relationships between elevated intracellular polyol content, hyperosmolarity, and taurine accumulation.

Materials and Methods

Cell Culture

Bovine eyes obtained from a local abattoir were transported on ice to the laboratory, where the lenses were removed aseptically. Bovine lens

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epithelial cells (BLECs) were isolated and cultured in 10% bovine calf serum–supplemented Eagle's minimal essential medium (MEM, 257 ± 2 mOsm), as previously described.[11] The osmolarity of the culture medium was determined with a vapor pressure osmometer (model 5500; Wescor, Salt Lake City, UT). Human lens epithelial cells (HLE-B3, hereafter referred to as HLECs) were obtained from Usha Andley (Washington University School of Medicine, Department of Ophthalmology, St. Louis, MO) and cultured in 20% fetal bovine serum–supplemented MEM (287 ± 8 mOsm). All studies with BLECs were performed on cells of passage 2, whereas experiments with the immortalized HLECs were started at passage 11 and did not exceed passage 19.

Taurine Accumulation

The effect on [^{3}H]-taurine accumulation of prolonged exposure (hereafter operationally defined as a 20-hour incubation period in serum-supplemented medium) of cells to 40 mM D-galactose was performed as follows: The cultured cells were divided into groups in 25-cm^2 flasks and the medium replaced with physiological medium containing 5.5 mM glucose or 5.5 mM glucose supplemented with an additional 40 mM galactose (Sigma Chemical Co., St. Louis, MO) in the presence and absence of 0.1 mM sorbinil for 20 hours. Thereafter, the cells were rapidly rinsed by and then transferred to a simpler uptake medium (medium A), containing: 5.5 mM glucose, 135 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$ and 10 mM HEPES (pH 7.4). Studies with BLECs used serum-free medium A. For studies with HLECs, medium A contained 0.5% fetal bovine serum (because the cells would not remain anchored to the tissue culture flasks for the 3-hour taurine uptake period in serum-free medium). Cells maintained in physiological medium or 40 mM galactose were rapidly rinsed in galactose-free medium A (containing 5.5 mM glucose). Cells previously maintained in 40 mM galactose plus 0.1 mM sorbinil were rinsed in galactose-free medium A (containing 5.5 mM glucose) plus 0.1 mM sorbinil. Cell cultures were then switched to fresh medium A containing a trace of [^{3}H]-taurine (1.0 μCi/mL; Amersham, Arlington Heights, IL) over a concentration range of 1.5 to 400 μM taurine for a 3-hour uptake period at 37°C. After isotope incubation, the medium was removed and the cell monolayers prepared for liquid scintillation counting, as previously described.[12] Replicate 1.0-mL aliquots were used for liquid scintillation counting (Packard Instrument Co., Meriden, CT). Triplicate 25-μL aliquots were used for protein determination, according to the method of Bradford[13] with bovine serum albumin (Sigma Chemical Co.) as the standard.

Experiments for Dixon plot analysis were performed as follows: Cells previously maintained in physiological medium were rapidly rinsed with medium A before being divided into four groups of glucose-free medium A containing 10, 20, 30, and 40 mM galactose. The experiment was performed at a fixed osmolarity of medium A as a fructose supplementation was reduced accordingly as the galactose concentration was raised from 10 to 40 mM. All experimental sets contained 0.1 mM sorbinil. Taurine accumulation was followed by the addition of [^{3}H]-taurine (1.0 μCi/mL) at concentrations of 1.57, 6.25, and 25 μM taurine for a 3-hour uptake period at 37°C. Essentially the same experiment was performed with 10, 20, 30, and 40 mM galactitol with the exception that, in this situation, the medium A also contained 5.5 mM glucose. This experiment was also performed at a fixed osmolarity by fructose supplementation, and sorbinil was omitted during the uptake period.

The effect on [^{3}H]-taurine uptake of prolonged exposure of cells to hypertonic medium was conducted as follows: Cells were maintained in physiological medium (257 ± 2 mOsm) or physiological medium made hyperosmotic by the addition of 116 mM NaCl. HLECs were cultured in hyperosmotic MEM with 20% fetal bovine serum (513 ± 11 mOsm), and BLECs were cultured in hypertonic MEM with 10% calf serum (473 ± 6 mOsm) for 20 hours. The cells were then switched to medium A or hypertonic medium A with a trace of [^{3}H]-taurine (1.0 μCi/mL) over a concentration range of 1.5 to 400 μM taurine for a 3-hour uptake period at 37°C. A 3-hour [^{3}H]-taurine uptake period was chosen for the velocity studies subsequent to determination of a linear time course of taurine uptake. For the determination of linear taurine uptake, the experiment was performed essentially the same, with the exception that medium A or hypertonic medium A contained a fixed concentration of 100 μM taurine, and triplicate flasks were collected at 1, 2, 3, 4, 6, and 8 hours.

The effect of prolonged exposure of HLECs to hypertonicity and galactose exposure was performed with the following modifications to the described experimental protocol. The cultured cells were divided into groups in 25-cm^2 flasks, the medium replaced with physiological medium containing 20% fetal bovine serum and supplemented with an additional 116 mM NaCl and 40 mM galactose with (566 ± 17 mOsm) and without (560 ± 11 mOsm) 0.1 mM sorbinil, and the cells maintained under these conditions for 20 hours. Thereafter, the cells were transferred, without rinsing, to fresh hypertonic medium in the absence of 40 mM galactose, with and without sorbinil and a trace of [^{3}H]-taurine (0.5 μCi/mL) over a concentration range of 6.25 to 200 μM for a 3-hour uptake period at 37°C. Note that the taurine uptake portion of this experiment was performed in hyperosmotic MEM in the continuous presence of 20% fetal bovine serum (instead of the more simplified hypertonic medium A).

For the determination of taurine efflux, HLECs grown to near confluence in 20% fetal bovine serum–supplemented MEM (287 ± 8 mOsm) were transferred to sodium hypertonic medium (MEM + 116 mM additional NaCl;513 ± 11 mOsm) for 24 hours at 37°C in a humidified 5% CO_2-95% air atmosphere. The media also contained 0.5 μCi/mL [^{3}H]-taurine. After 24 hours, the cells were rinsed three times (5 mL/rinse) with isotope-free high-salt medium to remove free [^{3}H]-taurine. One-half of the flasks of cultured cells were treated with high-salt medium containing 0.1 mM sorbinil for an equilibration period of 2 hours. Control cells were transferred to fresh hypertonic medium for the same 2-hour period. [^{3}H]-taurine efflux was monitored at the end of this initial 2-hour equilibration period and was negligible. Thereafter, the cells were transferred to 5 mL fresh 20% fetal bovine serum–supplemented isotonic MEM containing 5.5 mM glucose and an additional 40 mM galactose (341 ± 10 mOsm, hereafter defined as isotonic galactose medium; IGM) in the continued presence or absence of sorbinil. The rapid reduction in medium osmolarity from sodium hypertonic medium to IGM, coupled to the simultaneous exposure of HLECs to 40 mM galactose, permitted an evaluation of tonicity-activated and polyol-exacerbated [^{3}H]-taurine efflux.

The medium was collected for liquid scintillation counting at specified time intervals up to 4 hours, and triplicate sets of flasks were collected at each time point. The efflux medium was removed from each culture flask at the predetermined intervals, transferred to a 15-mL polystyrene centrifuge tube, and spun at 2500*g* at room temperature for 5 minutes. Aliquots of 1 mL were taken from each centrifuge tube for liquid scintillation counting. The culture flasks were prepared for protein determination, as previously described.[12]

Determination of Intracellular Galactitol

The concentration of intracellular galactitol was resolved by anion-exchange chromatography and pulsed-chase electrochemical detection, using a chromatography system (BioLC; Dionex, Sunnyvale, CA), as previously described.[12] HLECs and BLECs were grown to confluence in 150-cm^2 flasks in physiological medium and transferred to physiological medium or 40 mM galactose-containing medium for 20 hours before dispersion with trypsin in serum-free physiological medium (MEM) and centrifuged at 2500*g* at 4°C for 8 minutes. The cells were suspended in 0.9 mL of 0.3 N zinc sulfate (Sigma Chemical Co.) and were disrupted by rapid freezing in liquid nitrogen and thawing at 37°C for three repetitions. The samples were transferred to a 5-mL homogenizer (Dounce; Bellco Glass Co., Vineland, NJ) and subjected to five strokes. The homogenate was centrifuged at 18,000*g* at 4°C for 20 minutes, and the cell pellet was saved for protein determination. The supernatant was adjusted to 1 mL with 0.3 N zinc sulfate and precipitated with 1.0 mL of 0.3 N barium hydroxide sulfate (Sigma

Chemical Co.). The precipitate was centrifuged at 2500*g* at 4°C for 8 minutes, and the supernatant was removed and stored without further modification at −20°C for subsequent galactitol analysis.

Measurement of TauT mRNA by Competitive PCR

Northern blot analysis makes it difficult to detect and judge small changes in mRNA amount. In the present study, we have put to use competitive PCR, which is 1,000 to 10,000 times more sensitive than Northern blot analysis in monitoring the transcriptional activity of a gene.[14] In competitive PCR, the target cDNA and an internal standard DNA fragment (the mimic), having the same primer annealing sequences as the endogenous target cDNA, are put in the same PCR reaction, and compete for the endogenous primers. To differentiate between the PCR products generated from the target and the mimic, the mimic is designed to be larger or smaller than the target.[15,16]

Reverse Transcription–Polymerase Chain Reaction

Total RNA from HLECs was extracted using extraction reagent (RNAzol; Tel-Test, Friendswood, TX) according to the supplier's protocol. The RNA pellet was dried in vacuo for 15 minutes, the dried pellet dissolved in 20 μL deionized water at 65°C for 15 minutes, and the absorbance of RNA measured at 260 nm and 280 nm. Reverse transcription was performed on 2.5 μg total RNA, using 62.5 U Moloney murine leukemia virus reverse transcriptase (Perkin-Elmer, Norwalk, CT) in a 25-μL total volume containing 1× PCR buffer; 1 mM each dATP, dGTP, dCTP, and dTTP; 25 U RNasin (Perkin-Elmer); and 62.5 pmol oligo(dT)$_{12-18}$ primers (Perkin-Elmer). For competitive PCR, 2 μL of the above cDNA was amplified in a 50-μL total volume containing the mimic DNA; 0.2 μM target gene primers (S1 and AS1); 0.75 mM $MgCl_2$; 0.2 mM each of dATP, dGTP, dCTP, and dTTP; 1.25 U *Taq* polymerase (Perkin-Elmer); and 2 μCi [^{32}P]-dCTP (3000 Ci/mmol) in 1× PCR buffer. (The amount of the mimic used in the competitive PCR reaction was predetermined for each target gene by amplification of a constant amount of cDNA from the RT reaction of total RNA isolated from cells maintained at physiologic condition and 10-fold serial dilutions of the mimic with 0.2 μM of the target gene primers [S1 and AS1].) A ratio of approximately 1:1 target gene PCR product to mimic PCR product, as determined by image quantitation of a scanned x-ray film, was used to select the working concentration of the mimic). The PCR protocol consisted of 26 cycles for amyloid precursor protein (APP) and the Na^+/*myo*-inositol cotransporter (SMIT) or 28 cycles for AR and TauT of denaturation, annealing, and extension for 1 minute, 1 minute, and 2 minutes, at 94°C, 57°C, and 72°C, respectively. The PCR product was separated by electrophoresis on a 6% sequencing gel (Gibco-BRL, Grand Island, NY) and exposed to x-ray film (Eastman Kodak, Rochester, NY) for approximately 20 hours at −70°C with an intensifying screen. For image quantification, the x-ray film was scanned on a densitometer and the signal analyzed, using the associated analysis software (PDI, Huntington Station, NY). The data are plotted as the relative density of (target gene/mimic)/(APP/mimic). To verify that the amplified PCR products were from mRNA and not genomic DNA contamination, reverse transcriptase was omitted from the RT reaction. APP was used as the housekeeping gene, because its gene expression was found to be stable over the entire time course of hyperosmotic exposure from 1 to 72 hours.

Primer Construction

The mimic shares the same antisense primer (AS1) used for target gene amplification but uses a different sense primer (S2). The S2, S1, and AS1 primers were designed for the SMIT, AR, and TauT genes as follows: SMIT, S2 primer: 5′-GTGGAATGGCTGGCTTTGTTGCCTACCGTGCCCCAGAATG-3′, S1 primer: 5′-GTGGAATGGCTGGCTTTGTT-3′, and AS1 primer: 5′-CCGTTGGGAATGATGTGGTT-3′[17]; AR, S2 primer: 5′-ATCGCAGCCAAGCACAATAAGTCTGTGACACCAGAACG-3′, S1 primer: 5′-ATCGCAGCCAAGCACAATAA-3′, and AS1 primer: 5′-CCAGCAGGGTAGAAAGAAGG-3′[18]; and TauT, S2 primer: 5′-CCTCGCTCTCTGCCTTCTTTTCTACTTCACAGCCACTTTT-3′, S1 primer: 5′-CCTCGCTCTCTGCCTTCTTT-3′, and AS1 primer: 5′-ATGTCCACCCCTTGCTCTTG-3′.[19] The mimic for APP was prepared using a kit (PCR Mimic Construction Kit; Clontech, Palo Alto, CA) according to the supplier's protocol. APP composite primers, S2 primer: 5′-TAGCCGTTCTGCTGCATCTTGGTTGAGTCCATGGGGAGCTTT-3′ and AS1* primer: 5′-CCGTGGAGCTCCTCCCCGTGCGCAAGTGAAATCTCCTCCG-3′; APP target primers, S1 primer: 5′-CCGTGGAGCTCCTCCCCGTG-3′ and AS1 primer: 5′-TAGCCGTTCTGCATCTTGG-3′.[20]

Preparation of the Mimic

The mimic was amplified in a final volume of 50 μL containing 2 μL cDNA from the RT reaction, 1.25 mM $MgCl_2$, each deoxynucleoside triphosphate at 200 μM, 1× PCR buffer, 1.25 U *Taq* polymerase, and a selected set of primers S2 and AS1 (AS1* for APP) from one of the target genes, SMIT, AR, TauT or APP, at 0.2 μM. The DNA was denatured at 94°C for 5 minutes and then subjected to 35 amplification cycles (94°C for 1 minute, 57°C for 2 minutes, and 72°C for 2 minutes) and elongated at 72°C for 7 minutes. Thereafter, 2 μL of this first PCR reaction was removed and diluted to 200 μL in H_2O, and 2 μL of this dilution was amplified a second time in a final volume of 50 μL containing all the above components, except for substitution of S1 (for S2) and AS1 for each target gene at 0.2 μM. The number of amplification cycles and the cycle protocol were the same. Primers and reaction components were removed from the PCR product by passing 50 μL of the secondary PCR reaction through each of two prespun columns (Chrome Spin+ TE-100 columns; Clontech). The quality of the mimic was examined by electrophoresis and quantified by spectrophotometric analysis.

Calculations and Statistical Analysis

Calculations to determine the Michaelis-Menten constant (K_m) and peak velocity (V_{max}) were performed on computer (TableCurve; Jandel Scientific, now SPSS, Chicago, IL). Appropriate statistical analyses were applied to each group of data, as indicated.

Results

Long-Term Effect of Exposure of 40 mM Galactose and AR Inhibition on Taurine Uptake

Cultured HLECs and BLECs responded with a rapid accumulation of taurine for up to 8 hours in a fixed concentration of 100 μM taurine containing a trace of [^{3}H]-taurine (Fig. 1). Once an appropriate time for uptake studies was determined from a linear region of Figure 1, the long-term effect of exposure of extracellular galactose on taurine uptake was investigated. Cultured HLECs were exposed to 40 mM galactose in the presence and absence of 0.1 mM sorbinil for 20 hours. After being switched to galactose-free medium A, with and without sorbinil, [^{3}H]-taurine uptake was recorded for a 3-hour period over a concentration range of 1.5 to 400 μM taurine. Correspondent control cells were maintained in physiological medium and [^{3}H]-taurine uptake was similarly recorded. The accumulation curve for [^{3}H]-taurine for cultured HLECs was indistinguishable, irrespective of whether the cells were preincubated in physiological medium, 40 mM galactose, or 40 mM galactose plus sorbinil (Fig. 2A). Repeating this experiment with cells preincubated in physiological medium made hypertonic by the addition of NaCl and 40 mM galactose in the presence and absence of sorbinil similarly resulted in indistinguishable curves (data not shown). For the latter experiment, preincubation of the HLECs in hypertonic medium with supplemented galactose achieved two goals: AR was upregulated during the 20-hour exposure to hypertonic medium, and the cell exposure to excess galactose favored conversion to galactitol. On the contrary, the uptake of [^{3}H]-taurine was reduced after

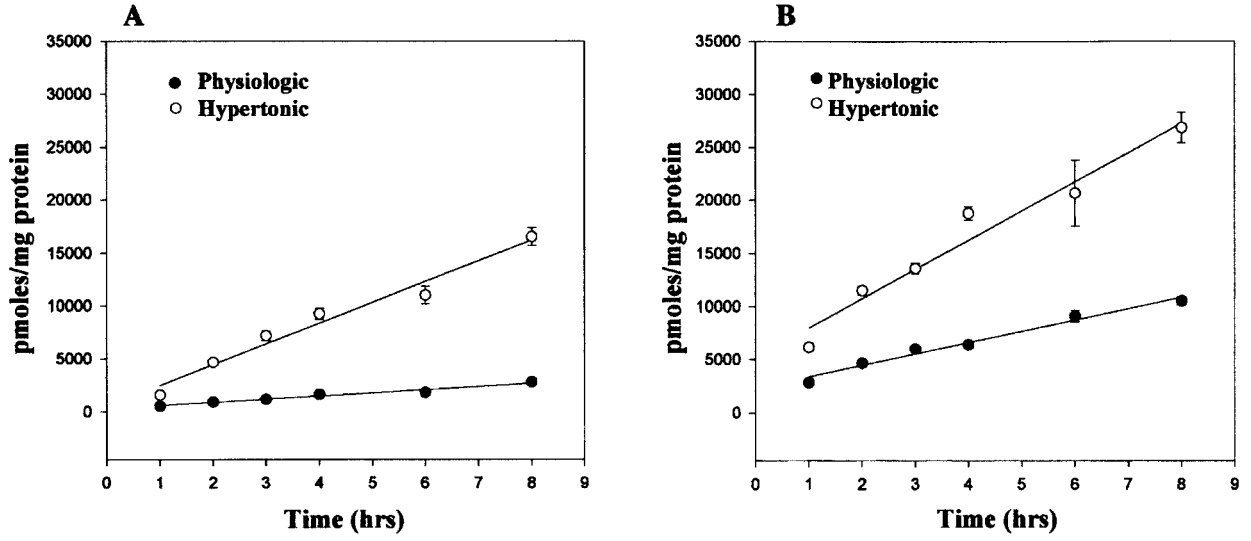

FIGURE 1. Time dependent [^{3}H]-taurine uptake. HLECs were incubated in 0.5% fetal bovine serum with medium A, and BLECs were incubated in serum-free medium A. Both uptake media contained approximately 100 μM [^{3}H]-taurine. Taurine uptake was determined by collecting triplicate flasks for each of the designated times. The data are the mean ± SE. In all figures, some small error bars are obscured by the symbols. Data points were plotted by linear regression. For HLECs (**A**) the correlation coefficients for physiologic and hypertonic conditions were 0.95 and 0.91, respectively. For BLECs (**B**) the correlation coefficients for physiologic and hypertonic conditions were 0.88 and 0.95, respectively.

prolonged exposure of cultured BLECs in 40 mM galactose (Fig. 2B). The coadministration of sorbinil with 40 mM galactose completely prevented the inhibitory effect of galactose on [^{3}H]-taurine uptake. Neither human nor bovine lens epithelial cells showed a statistically significant change in K_m for taurine transport under any experimental condition (Table 1), but a significant change of V_{max} was observed for the BLECs exposed to extracellular galactose.

Intracellular galactitol levels were determined by anion-exchange chromatography for HLECs and BLECs maintained in physiologic medium (257 ± 2 mOsm) or high extracellular galactose medium (Phys + 40 mM galactose, 285 ± 4 mOsm) for 24 hours. As shown in Table 2, intracellular galactitol was dramatically increased in BLECs maintained in galactose, the polyol level being greater than 325 nmol/mg protein after a 24-hour incubation period. By contrast, intracellular galactitol content in HLECs was more than five times lower, not exceeding 60 nmol/mg protein, under identical culture conditions. The difference in accumulated polyol content probably reflects the relative levels of AR activity between the two species of cultured cells.

Tonicity-Activated [^{3}H]-Taurine Efflux in the Presence of Exogenous Galactose

When extracellular osmolarity was reduced from 513 ± 11 mOsm (NaCl hypertonic medium) to 341 ± 10 mOsm isotonic

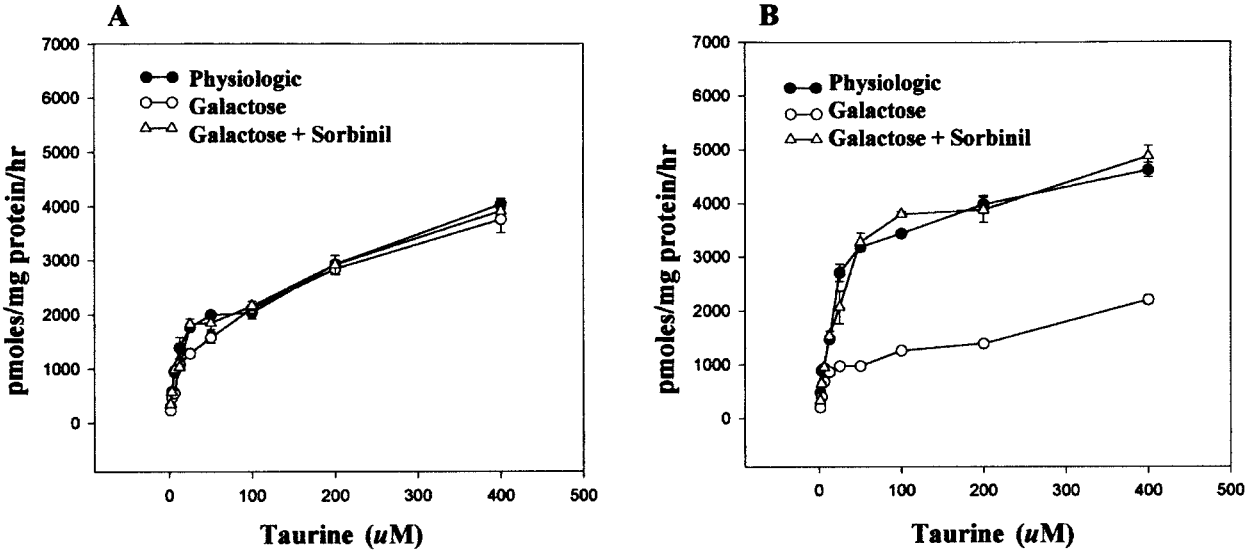

FIGURE 2. Effect of prolonged galactose exposure and sorbinil on [^{3}H]-taurine uptake. HLECs (**A**) and BLECs (**B**) were preincubated in 40 mM galactose, 40 mM galactose plus 0.1 mM sorbinil, or physiological medium (5.5 mM glucose) for 20 hours before the experiment. Galactose was not present during the 3-hour [^{3}H]-taurine uptake period, during which concentration ranged from 1.5 to 400 μM. Data represent triplicate determinations from individual flasks. Data points are means ± SEM. *Significantly different from physiological medium using reciprocal data transformation and an independent *t*-test comparison of the slope in a linear regression ($P < 0.001$).

TABLE 1. Kinetic Parameters of the Taurine Transporter in HLECs and BLECs in Physiological and Galactose-Supplemented Media

Conditions	K_m		V_{max}	
	HLEC	BLEC	HLEC	BLEC
Physiological (5.5 mM glucose)	7.2 ± 1.8	21.4 ± 1.7	1891 ± 204	4546 ± 98
Galactose (40 mM)	7.7 ± 3.1	17.0 ± 2.9	1510 ± 297	1737 ± 73*
Galactose + sorbinil (0.1 mM)	7.1 ± 2.0	28.5 ± 3.2	1926 ± 174	4884 ± 153

Data are the mean ± SE of three experiments. The units for K_m and V_{max} are micromolar and picomoles per milligram protein per hour, respectively.

* Significant difference, $P < 0.05$.

galactose medium (IGM) approximately 80% to 90% [^{3}H]-taurine was released into the medium in the first 60 minutes, and flux continued from cell to medium at a much slower but sustained rate during the next 3 hours. The loss of [^{3}H]-taurine from human lens epithelial cell to medium was virtually identical, irrespective of whether galactitol formation was favored or inhibited by the inclusion of an AR inhibitor (data not shown).

Acute Effect of Exposure of Galactose and AR Inhibition on Taurine Transport Activity

HLECs and BLECs were briefly exposed to 10 to 40 mM galactose containing the inhibitor of galactitol biosynthesis, sorbinil. By applying this experimental approach, the acute (3-hour exposure) effect of galactose on taurine transport could be analyzed without interference from accumulated intracellular galactitol. Figure 3A is a Dixon plot resulting from the acute exposure of HLECs with D-galactose and sorbinil using three random doses of taurine at 1.57, 6.25, and 25 μM and a trace of [^{3}H]-taurine. The failure of the Dixon plot to intersect with all values of substrate verifies that D-galactose does not interact with the taurine transport system in HLECs. The data shown in Figure 3B were similarly generated using BLECs. These data, as with the HLECs, demonstrate that D-galactose had no inhibitory effect on taurine uptake in BLECs.

Acute Effect of Exposure of Galactitol on Taurine Transport Activity

The effect of high extracellular galactitol on taurine transport was also examined by Dixon plot analysis. Figure 4A is a Dixon plot of [^{3}H]-taurine uptake resulting from an acute (3-hour) incubation of HLECs with galactitol. The concentrations of galactitol were 10, 20, 30 and 40 mM, and the taurine concentrations were 1.57, 6.25, and 25 μM, with a trace of [^{3}H]-taurine included in the uptake media. The Dixon plot analysis of the data showed no intersection of lines for the HLECs (Fig. 4A). Therefore, as with galactose, exogenous galactitol had no acute inhibitory effect on taurine transport. The same conclusion was reached using BLECs (Fig. 4B) in place of HLECs.

TABLE 2. Intracellular Galactitol Content in HLECs and BLECs

Conditions	HLEC	BLEC
Physiological (5.5 mM glucose)	ND	ND
Galactose (40 mM)	60 ± 1.6	334 ± 7.0

Data were acquired by anion-exchange chromatography and are the mean ± SE nanomoles per milligram protein. Data points represent duplicate determinations from triplicate flasks. ND: nondetectable.

Hypertonicity-Induced Enhancement of Taurine Uptake

HLECs and BLECs were pre-exposed to an NaCl-supplemented hyperosmotic environment for 20 hours. The addition of sodium chloride promoted enhanced taurine accumulation in cultured HLECs and BLECs, irrespective of whether a fixed concentration of taurine was examined (Fig. 1) or whether a 3-hour uptake period over a dose range of 1.5 to 400 μM taurine was considered (Fig. 5). The kinetic character of [^{3}H]-taurine uptake was to increase the V_{max} without significant alteration to the K_m (Table 3).

Hypertonicity-Induced Pattern of TauT mRNA

Using Northern blot analysis, we previously reported that enhanced *myo*-inositol transport and accumulation, characteristic of upregulation of SMIT mRNA, was an adaptive osmoregulatory response to hypertonicity in cultured BLECs.[10] In the present study, we considered the response of HLECs subjected to hypertonic insult for 72 hours and observed the abundance of TauT mRNA. The levels of SMIT and AR mRNA were simultaneously monitored. The effect of hypertonic medium on TauT, SMIT, and AR mRNA levels in HLECs is shown in Figure 6. HLECs responded to hyperosmolarity with an inducible transitory upregulation of TauT mRNA. The induction profile of TauT mRNA exhibited a temporal pattern of expression identical with SMIT mRNA, but differed in amplitude. The TauT mRNA was maximally expressed by 10 hours. Thereafter, TauT mRNA, as with SMIT mRNA, steadily declined to near physiological levels for the duration of the 72-hour exposure to sodium hypertonic medium. Unlike the early-onset pattern of induction of TauT and SMIT mRNA, AR mRNA achieved maximal expression by 24 hours of cell exposure to hypertonicity and remained elevated for the duration of the 72-hour exposure in sodium hypertonic medium.

DISCUSSION

The nature of the taurine transport system has been reported in monolayer cultures of several human cell lines, including colon carcinoma,[21] retinal pigment epithelium,[22] and glioma.[23] The transport process is typified by a single saturable high-affinity site with a K_m ranging from 2 to 11 μM. Studies with cloned TauT from rat kidney cortex[24] or a mouse retinal library[25] expressed in *Xenopus laevis* oocytes has confirmed a high-affinity transport system with a K_m of 22.5 μM and 13.2 μM, respectively. To date, data describing the characteristics of active taurine accumulation in lens epithelial cells are not available, nor has the impact of aldose sugars or their sugar alcohol counterparts on the taurine transport system been previously considered.

[^{3}H]-taurine uptake in cultured HLECs was found to be

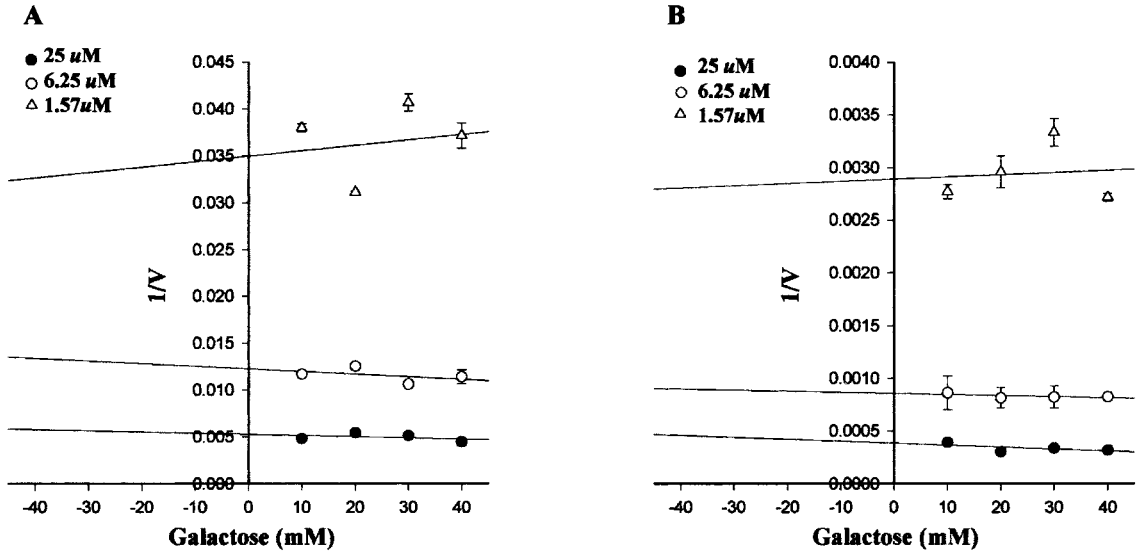

FIGURE 3. Dixon plot of acute D-galactose and sorbinil exposure on [^{3}H]-taurine uptake. HLECs (**A**) and BLECs (**B**) were incubated in medium A containing 1.57, 6.25, and 25 μM taurine and a trace of [^{3}H]-taurine. Data points were plotted by linear regression, and the correlation coefficients for the Dixon plot resulting from 1.57, 6.25, and 25 μM taurine were 0.60, 0.90, and 0.94 for (**A**) and 0.62, 0.95, and 0.94 for (**B**), respectively. The incubation mixtures for (**A**) and (**B**) also contained 10, 20, 30, or 40 mM galactose plus 0.1 mM sorbinil. Taurine uptake was determined after a 3-hour uptake period. Data points are the mean ± SE of triplicate determinations from individual flasks.

unaffected by preincubation in 40 mM galactose when compared with 40 mM galactose with the coadministration of sorbinil (Fig. 2A), both curves being equivalent to physiological control. A similar experiment was performed with cultured HLECs preincubated in hypertonic medium and 40 mM galactose in the presence and absence of sorbinil with like results (data not shown). On the contrary, the intracellular accumulation of galactitol equated with an observed reduction in [^{3}H]-taurine uptake resulting from pre-exposure of cultured BLECs to 40 mM galactose (Fig. 2B). The uptake curve for [^{3}H]-taurine in the presence of 40 mM galactose and sorbinil was indistinguishable from physiological control (Fig. 2B). Clearly, the level of AR activity in the HLEC is marginal when compared with that in the BLEC. The observed inhibition of [^{3}H]-taurine accumulation could not be attributed to galactose, because the uptake measurement was performed in galactose-free medium. Our kinetic measurement identified a high-affinity taurine transport site. Based on the velocity curve data, galactitol-induced inhibition of active taurine uptake for this high-affinity transport site in BLECs is noncompetitive, because there was a marked reduction in V_{max}, without significant alteration of K_m (Table 1).

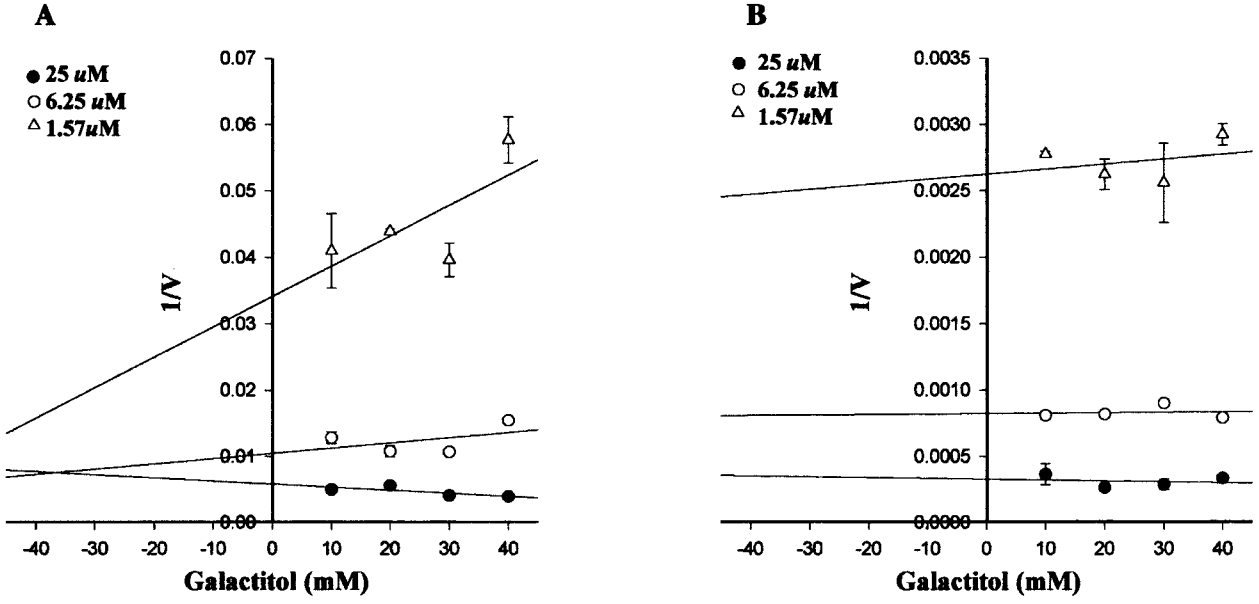

FIGURE 4. Dixon plots of acute galactitol exposure on [^{3}H]-taurine uptake. HLECs (**A**) and BLECs (**B**) were incubated in medium A containing 1.57, 6.25, and 25 μM taurine and a trace of [^{3}H]-taurine. Data points were plotted by linear regression, and the correlation coefficients for the Dixon plot resulting from 1.57, 6.25, and 25 μM taurine for (**A**) were 0.65, 0.89, and 0.94 and (**B**) 0.65, 0.93, and 0.94, respectively. The incubation medium of (**A**) and (**B**) also contained 10, 20, 30, or 40 mM galactitol. Taurine uptake was determined after a 3-hr uptake period. Data points are the mean ± SE of triplicate determinations from individual flasks.

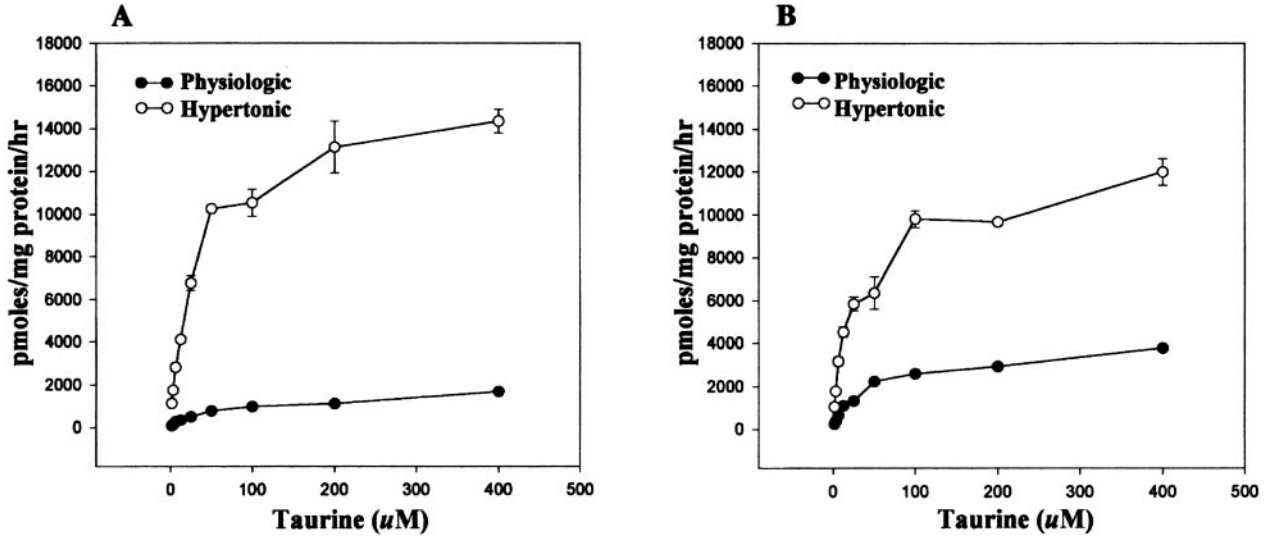

FIGURE 5. Effect of hypertonicity on [^{3}H]-taurine uptake. HLECs (**A**) and BLECs (**B**) maintained in physiological medium or physiological medium adjusted with 116 mM sodium chloride for 20 hours. Data points are the mean ± SE of triplicate determinations from individual flasks. The hypertonicity curves differed significantly from the physiological curves, using reciprocal data transformation and an independent *t*-test comparison of the slope in a linear regression ($P < 0.001$).

Dixon plot analysis further confirmed that galactose does not interact with the high-affinity taurine transport site in either HLECs or BLECs (Fig. 3). These experiments were performed in the presence of extracellular galactose and sorbinil (thereby eliminating the possibility of galactitol formation and intracellular accumulation), so that they were designed to analyze the direct course of action of galactose on the taurine transport system. Similar experiments were also performed with extracellular galactitol. Exogenous galactitol also did not interact with the taurine transport system as corroborated by Dixon plot analysis for either HLECs (Fig. 4A) or BLECs (Fig. 4B). These results were incompatible with the velocity curve data in Figure 2B. It should be noted that experiments in which exogenous galactitol is used probably do not reveal the mechanism by which intracellular galactitol might influence taurine uptake. The duration of incubation of exogenous galactitol did not exceed 3 hours, and galactitol does not readily cross cell membranes. Therefore, it is unlikely that exogenous galactitol could have accumulated inside the cell to a degree high enough to adversely affect the cytoplasmic side of the taurine transport system.

Collectively, these studies uncovered an incongruity between the galactitol insensitivity of the human taurine transport system (Fig. 2A) and the galactitol sensitivity of the bovine taurine transport system (Fig. 2B). That apparent discrepancy is probably explained by the higher rate of polyol formation and accumulation in the BLECs than in the HLECs (Table 2). The data are supportive of the fact that intracellular galactitol in excess of 325 nmol/mg protein probably has adverse effects on taurine transport. Whether this inhibition of taurine transport is the result of direct interaction with the taurine transport site or is due to some secondary effect on cellular metabolism could not be determined from these data alone, and further studies therefore seem to be warranted.

Cultured lens epithelial cells gain osmolytes through distinct intracellular enzymes or membrane transport pathways and lose osmolytes through discrete channel pathways. Exposure to hypertonicity immediately shrinks cultured cells, concentrating internal solutes through transient uptake of inorganic ions. This is promptly followed by the active accumulation, from medium to cell, of small organic molecules or conversion of aldose sugars to their respective sugar alcohols collectively identified as organic osmolytes. To date, these have been characterized, in this laboratory, to include *myo*-inositol, taurine, and sorbitol. Conversely, hypotonicity causes rapid cell swelling and the accompanying loss from cell to medium (i.e., efflux) of organic osmolytes and inorganic ions. This perfunctory gain or loss of osmolytes in response to cell volume fluctuation constitutes a fundamental constituent of the volume regulatory mechanisms associated with cellular regulatory increase (RVI) and decrease (RVD). It follows then that the process of organic osmolyte accumulation is in dynamic equilibrium with the release from cell to medium of organic osmolytes, depending on the state of osmotic homeostasis of the cell in any given period. It should be mentioned that should the lens epithelial cell be exposed to a high level of intracellular galactitol, it is likely that an increased taurine efflux would lead to a net reduction of taurine retained

TABLE 3. Kinetic Parameters of the Taurine Transporter in HLECs and BLECs in Hypertonic Medium

	K_m		V_{max}	
Conditions	**HLEC**	**BLEC**	**HLEC**	**BLEC**
Physiological (5.5 mM glucose)	19.1 ± 3.4	21.4 ± 3.5	1891 ± 204	2757 ± 424
Hypertonic (232 mM NaCl)	25.5 ± 7.7	17.0 ± 2.9	14633 ± 908*	9999 ± 461*

Data are the mean ± SE. The units for K_m and V_{max} are micromolar and picomoles per milligram protein per hour, respectively.

* Significant difference, $P < 0.05$.

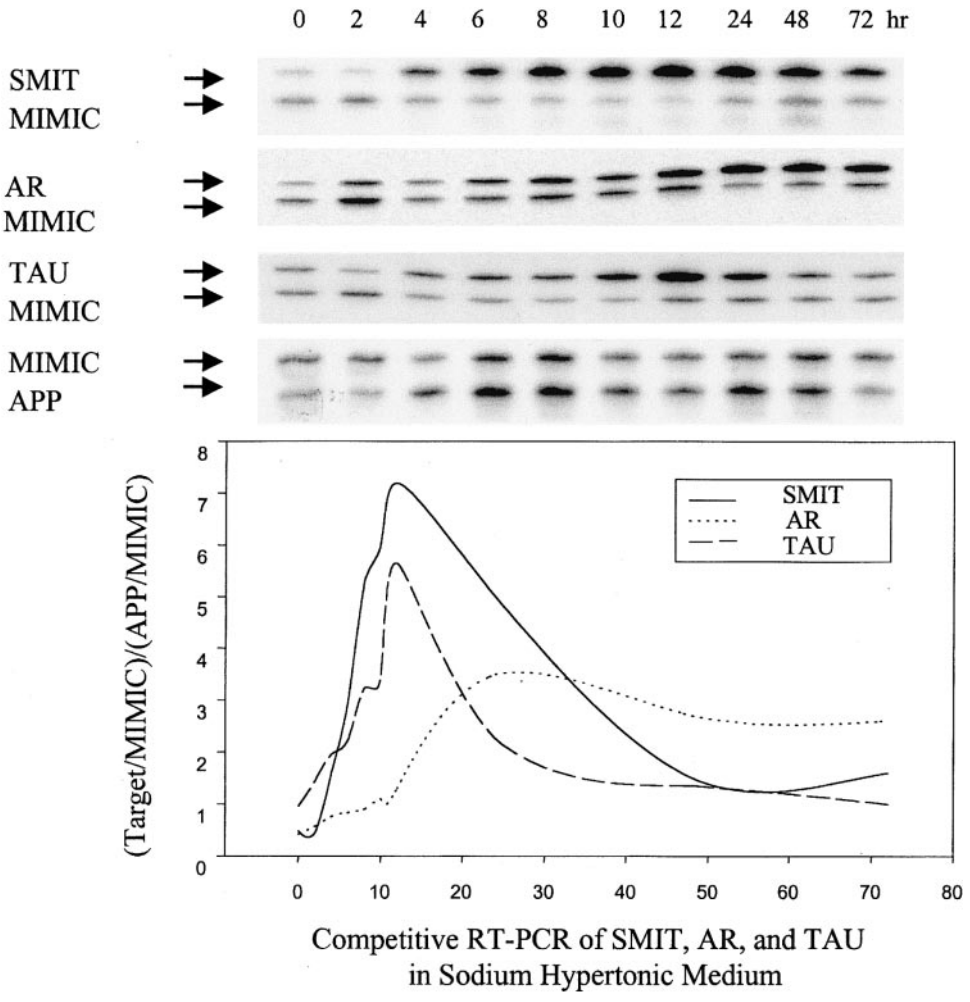

FIGURE 6. Competitive RT-PCR quantification of TauT (TAU), SMIT, and AR mRNA in HLECs (HLE-B3). HLE-B3 cells were exposed to sodium hypertonic medium for 72 hours. TauT, SMIT, and AR mRNA were quantified using APP mRNA as a reference. The data were plotted as the ratio of the relative densitometric density of (target gene/mimic)/(APP/mimic) versus the duration of exposure in hyperosmotic medium. The data shown represent one of three individual experiments with like results.

in the cell. We previously demonstrated, using cultured BLECs, that tonicity-activated movement of *myo*-inositol from cell to medium and *myo*-inositol efflux, as induced by intracellular polyol accumulation, appear to be interactively associated with chloride movement and moderated by a common anionic (chloride) channel.[26] We attempted to repeat this effect with HLECs that were hypertonically adapted and switched to isotonic galactose medium (IGM), with and without sorbinil. The pattern of release of [^{3}H]-taurine was indistinguishable over the 4-hour efflux period. This was certainly because an insufficient level of galactitol was synthesized, so that a considerable difference in intracellular polyol osmotic stress between the AR-inhibited and AR-uninhibited cells had not been achieved. This result is consistent with the apparent low level of AR activity in the human lens epithelium observed with the taurine uptake studies.

Several laboratories have reported that Na^+- and Cl^--dependent TauT is regulated by hypertonicity. That the increase in TauT activity is due to an increase in the abundance of TauT mRNA has previously been shown with several cell culture systems, including Madin-Darby canine kidney cells,[3,27] rat hepatoma cells,[28] human intestinal cells,[29] and bovine aortic endothelial cells.[30] Few studies are available in which the absolute pattern (amplitude, temporal display, regional incident) of upregulation of osmosensitive genes has been monitored.[10,31,32] Morimura et al.[31]reported no change in the eyes of acute hypernatremic rats for TauT mRNA but chronic hypernatremia markedly increased TauT mRNA in the retina. Data presented herein show that TauT mRNA was modulated in cultured lens epithelial cells in response to hyperosmotic stress in a temporal pattern that is similar, if not identical with, SMIT. That is, both osmosensitive genes showed an adaptive osmoregulatory response to hypertonicity that appears to be an early-onset protective mechanism against the effects of acute water stress. Upregulation of the AR gene appears to be a late-onset defensive mechanism against the effects of prolonged water stress.

In conclusion, taurine transport was found to be unaffected by exposure to galactose. The taurine transport system appears to be hindered by intracellular galactitol only if a suitably high level of galactitol is amassed. The mechanism of interference of intracellular galactitol on taurine uptake was not clear from these studies. We further showed that, just as with the osmotically responsive SMIT and AR genes, an increase in taurine uptake in cultured lens epithelial cells was dependent on an increase in the number of TauTs resulting from upregulation of TauT mRNA. The time course of upregulation of the TauT is identical with that of SMIT, but differs markedly from that of AR, indicating that there are factors in the lens epithelium that can selectively modify temporal upregulation of osmosensitive genes.

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