

Molecular characterization of taurine transport in bovine aortic endothelial cells

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Abstract

Cultured bovine aortic endothelial (BAE) cells expressed a Na^+/Cl^- -dependent taurine uptake activity that saturated with an apparent $K_{0.5}$ of $\sim 4.9 \mu\text{M}$ for taurine and was inhibited by β -alanine, guanidinoethane sulfonate, and homotaurine. We isolated a taurine transporter clone from a BAE cell cDNA library that revealed $>91\%$ sequence identity at the amino acid level to the previously cloned high-affinity mammalian taurine transporters. The biochemical and pharmacological properties of the bovine taurine transporter cDNA expressed in *Xenopus* oocyte was similar to those of the high-affinity taurine transporter. Surprisingly, F^- blocked taurine uptake in BAE cells with an IC_{50} of $\sim 17.5 \text{ mM}$. The endogenous taurine uptake was also inhibited by the protein kinase C activator phorbol 12-myristate 13-acetate, but not by its inactive analog, 4 α -phorbol 12,13-didecanoate. The endogenous uptake was stimulated, however, by hypertonic stress and the increase was due to an increase in the V_{max} of taurine uptake. Our results provide the first description of a molecular mechanism that may be responsible for maintaining the intracellular taurine content in the endothelial cells. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Taurine transport; Endothelial cell; Na^+ dependent; Cl^- dependent; Bovine taurine transporter; Cloning; Molecular properties

1. Introduction

Taurine, a sulfur containing β -amino acid, is one of the most abundantly found free amino acids in the central nervous system and muscles (for a review, see [1]). The de novo synthesis of taurine occurs primarily in the liver, from where the circulating blood delivers it to the target tissues. Most cells acquire taurine from the circulating plasma. A high-affinity taurine transporter catalyzes the transport of taurine

from the circulating blood into the cells. Moreover, the taurine transporter helps in maintaining the intracellular taurine concentration. While a large body of evidence suggests that taurine functions as an osmolyte in a variety of tissues, taurine is also known to attenuate the deleterious effects of radiation and oxidative damage in a number of tissues/cells [1].

The endothelial cell layer lines the blood vessels forming a blood-solute barrier. These cells are constantly exposed to the shear and stress of blood flow and to the changes in blood osmolarity. Little is known about the mechanism(s) by which the endothelial cells protect themselves from the long term deleterious effects of shear, stress, and osmotic changes.

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Although taurine transport has been demonstrated in capillary preparations from rat brains [2], in primary cultures of bovine brain capillary endothelial cells [3,4], and in intact brains [5], the nature and the properties of the taurine transport system in the endothelial cells in general remain mostly unknown. Because of taurine's ability in preventing cell damage and its role in regulating cell volume, it is of great importance to know whether the endothelial cells possess a mechanism that will concentrate taurine in the cells. Thus, the purpose of this study is to examine whether the endothelial cells endogenously express a taurine transport system and to determine its molecular properties. Our results demonstrate for the first time that a high-affinity, Na^+ - and Cl^- -dependent, and hypertonic stress-sensitive taurine transporter is expressed in cultured bovine aortic endothelial cells.

2. Materials and methods

2.1. Materials

All chemicals used were ultra-pure or ACS pure grade and were purchased from one of the general chemical suppliers such as Sigma Chemical Co. (St. Louis, MO), Fisher Scientific Co. (Pittsburgh, PA), Life Technologies (Gaithersburg, MD), and United States Biochemical (Cleveland, OH).

2.2. Cell culture

Bovine aortic endothelial (BAE) cells were generous gift from Dr Diana L. Kunze (Rammelkamp Center for Education and Research, MetroHealth Systems, Cleveland, OH). These cells were maintained in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin at 37°C under 95% O_2 /5% CO_2 . Passages between 8 and 21 were used for the studies described here.

2.3. RNA isolation and reverse transcriptase–polymerase chain reaction (RT–PCR) analysis

Total poly(A) RNA or total RNA was prepared from several batches of BAE cells. Total RNA was purified using the RNA purification kit from BioTex

(Houston, TX) or Ambion (Austin, TX). Total poly(A) RNA was purified from the total RNA using oligo(dT) spin columns (Invitrogen, Carlsbad, CA). For RT–PCR, typically $\sim 1 \mu\text{g}$ of total poly(A) RNA or $\sim 10 \mu\text{g}$ of total RNA was reversed transcribed into oligo(dT)/random primer-primed first strand cDNA using the PCR Cycling kit (Invitrogen). An aliquot of the first-strand cDNA was used for PCR amplification utilizing *Taq* DNA polymerase (Perkin–Elmer, Foster City, CA) and using various combinations of forward and reverse PCR primers. These PCR primers were synthesized previously using sequences based on the cDNA sequence of the mTauT mouse retinal taurine transporter [6]. DNA amplification reactions were carried out using the following PCR cycling conditions: denature at 94°C for 1 min, anneal at 50°C for 1 min, and extend at 72°C for 2 min. At the end of 40 cycles, the reaction mixture was incubated for 10 min at 72°C. DNA fragments of expected sizes were purified by agarose gel electrophoresis and were cloned subsequently into the pCR2-TA cloning vector (Invitrogen). Plasmid DNA was prepared from at least five independent clones containing DNA inserts and each clone was sequenced using the Sequenase Version 2.0 DNA Sequencing kit (United States Biochemicals, OH).

2.4. Rapid amplification of 5'-end

The 5'-end of the taurine transporter cDNA was isolated from BAE cell total poly(A) RNA (10 μg) using a 5'-RACE Amplification kit (Life Technologies) following the manufacturer's protocol and the insert specific reverse primer: 5'-CCAAGAAAAA-CACAGGCAG-3'. The amplified DNA was gel purified and cloned into the pCR2.1-TA cloning vector (Invitrogen). Several insert-containing clones were sequenced using Sequenase Version 2.0 DNA sequencing kit.

2.5. Assembly of full-length cDNA for functional expression in oocytes

To functionally express the bTauT cDNA in *Xenopus* oocytes, we first created an expression vector pBV β g by introducing a *Hind*III and *Sma*I digested DNA fragment of the plasmid pCrT.3 [7] to the *Hin*-

dIII and *EcoRV* digested pBV vector [8]. The *HindIII* and *SmaI* digested DNA fragment contained the 5'-untranslated region of the β -globin gene fused at a *NcoI* site upstream to a small portion of the CRT-1 cDNA coding region. The full-length coding region of the bTauT cDNA was assembled subsequently from two PCR-amplified overlapping DNA fragments and cloned into the pBV β g vector to create the bTauT expression plasmid (pBV β g.bTauT) as follows.

A cDNA fragment encoding the N-terminal 400 amino acids of the bTauT was amplified using the following primers: forward primer, 5'-GCCATGGC-CACGAAGGAGAAGCTGCA-3' (contains a *NcoI* restriction site, which is in italics; the first codon, within the *NcoI* site, is underlined), and reverse primer, 5'-CAGTCCAAGCAAGAGGAGCA-3'. Similarly, a cDNA fragment encoding a C-terminal portion of the bTauT (corresponding to amino acids 330–621) was amplified using the following two PCR primers: forward primer, 5'-ATGCCTGAA-CAGTGGTACCAG-3', and reverse primer, 5'-TCACATCATGGTCTCCACAATGA-3'. The amplified fragments were cloned into the pCR2-TA vector (Invitrogen). A recombinant plasmid containing the N-terminal coding cDNA fragment was digested with the restriction enzymes *NcoI* and *SacI*. Similarly, a recombinant plasmid containing the C-terminal coding cDNA fragment was digested with the restriction enzymes *SacI* and *EcoRI*. The appropriate DNA fragments were purified by agarose gel electrophoresis and cloned subsequently into the *NcoI* and *EcoRI* digested pBV β g expression vector. The resultant recombinant plasmid, which contained the entire coding region of the BAE cell taurine transporter, was termed pBV β g.bTauT.

2.6. Expression in *Xenopus* oocytes

Oocytes were isolated from *Xenopus laevis* and were used for microinjection as described [6,9]. Oocytes were microinjected individually with synthetic RNA (cRNA; ~ 5 –10 ng/oocyte) prepared from the pBV β g.bTauT recombinant plasmid. Seventy-two hours after injection, taurine uptake assays were performed to measure the functional expression of bTauT in the microinjected oocytes.

2.7. Taurine uptake in BAE cells

The BAE cells were seeded at a density of $\sim 1.9 \times 10^5$ cells/well in 6-well culture dishes and were incubated in the 37°C incubator for 2–3 days before they were used for uptake studies. Unless otherwise stated, taurine uptake experiments in cells were performed in sodium chloride buffer (NaCl-1 buffer, mM: 135 NaCl, 1 CaCl₂, 2 KCl, 5 MgCl₂, 5 Hepes; pH to 7.5 with Tris base). Sodium-free buffers were prepared by substituting the NaCl in the NaCl-1 buffer with equimolar amount of lithium chloride, potassium chloride, or choline chloride. Uptake buffers containing various concentrations of Na⁺ were prepared by substituting NaCl with LiCl or choline chloride and keeping the other ingredient concentrations the same. Uptake buffers containing various concentrations of Cl[−] were prepared by replacing only a given portion of the NaCl with an equimolar amount of sodium acetate or sodium fluoride, while acetate salts were used for the rest of the ingredients. In all cases, the total osmolarity of the cell uptake buffer was ~ 305 mosmol.

For uptake studies, cells were first washed with choline chloride buffer, and taurine uptake was initiated by adding 1 ml of a given uptake buffer containing [³H]taurine. After incubation at 37°C for a given period of time, the uptake was terminated by aspiration, and the cells were quickly washed three times (1 ml each time) with ice-cold choline chloride buffer. Subsequently, the cells were solubilized in 1 ml of 1% SDS by shaking for 5–10 min and then repeatedly passed through a pipette-tip to reduce the viscosity of the cell extract by shearing the chromosomal DNA. A fraction (900 μ l) of the solubilized cell extract from each well was transferred to a scintillation vial, mixed with 10 ml of Ultima Gold LSC-cocktail (Packard Instruments, Meriden, CT), and the amount of radioactivity in each fraction was measured using a Beckman LS 3800 liquid scintillation counter. Protein content in each extract was measured using the Pierce Protein Assay kit (Pierce, Rockford, IL).

To determine the effects of hypertonicity on taurine uptake, BAE cells were seeded in 6-well culture dishes as described above. Two to three days later, the growth medium was replaced with a hypertonic

(hyperosmotic) medium consisting of normal growth medium supplemented with either 100 mM NaCl or 200 mM raffinose to raise the osmolarity. Control cells were maintained in normal growth medium (iso-osmotic) in parallel cultures. Uptake studies were performed as described above 24 h after the growth medium was replaced.

2.8. Taurine uptake in oocytes

Taurine uptake experiments in oocytes were performed in the oocyte sodium chloride buffer (NaCl-2 buffer, mM: 100 NaCl, 1 CaCl₂, 2 KCl, 5 MgCl₂, 5 Hepes; pH to 7.5 with Tris base). Compositions of sodium-free (choline chloride, potassium chloride, and lithium chloride) and chloride-free (sodium acetate and sodium gluconate) uptake buffers, and uptake buffers with various concentrations of Na⁺ and Cl⁻ were previously described [6,7]. Taurine uptake assays using microinjected oocytes and uninjected oocytes (control) were performed as described earlier [6].

2.9. Additional uptake experiments

Taurine uptake was measured (30 min uptake, 37°C) as a function of various external concentrations of taurine (1–60 µM), Na⁺ (0–135 mM for cells and 0–100 mM for oocytes), and Cl⁻ (0–135 mM for cells and 0–100 mM for oocytes) as described [6] to determine their effects on the rate of uptake. Results were analyzed as described [6,7] by non-linear curve-fitting of the experimental data points to the modified Michaelis–Menten equation: $V = V_{\max} [S]^n / \{ (K_{0.5})^n + [S]^n \}$, where $K_{0.5}$ is the equilibrium constant and n is the Hill coefficient.

Taurine uptake was measured in the presence of various concentrations of known taurine transport inhibitors, β-alanine, guanidinoethane sulfonate (GES), and homotaurine [6]. The IC₅₀ value for each inhibitor was determined subsequently from its dose–response plot [6,7]. To determine the effect of A23187 on taurine uptake, 5 µM of A23187 was added directly to the uptake buffer. To determine the effects of phorbol esters on taurine uptake, cells were pre-treated with either 100 nM of phorbol 12-myristate 13-acetate (PMA), 100 nM of 4α-phorbol 12,13-didecanoate (4α-PDD) or vehicle solvent (DMSO)

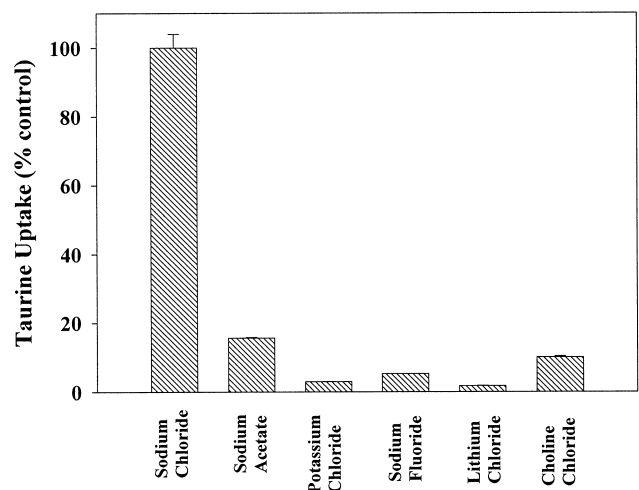


Fig. 1. Taurine uptake in cultured BAE cells is dependent on both Na⁺ and Cl⁻. Taurine uptake was initiated by adding 2 µM of [³H]taurine (100 mCi/mmol) to the cells in a given buffer, and the uptake was measured as described in Section 2. Results (average of duplicate measurements) are expressed as percentage of uptake in NaCl-1 buffer (control uptake). Control taurine uptake (100%) represents 19.45 pmol of taurine/mg protein per 30 min.

for 1 h at 37°C. Subsequently, treated cells were used for a 60-min uptake assay.

3. Results

3.1. Taurine uptake in bovine aortic endothelial cells

Fig. 1 shows taurine uptake by cultured monolayer BAE cells. As shown, removal of either the external Na⁺ (with choline, K⁺, or Li⁺) or the Cl⁻ (with gluconate) significantly inhibited the taurine uptake, suggesting that the endogenous taurine uptake system in BAE cells is dependent on both Na⁺ and Cl⁻. The taurine uptake increased linearly with time until saturation and was linear almost up to 110 min (results not shown). A number of drugs known to inhibit taurine transport in other cells [6], also inhibited the endogenously expressed taurine uptake activity in the BAE cells in a dose dependent manner. Table 1 lists the IC₅₀ values of these inhibitors. For comparison, the respective IC₅₀ values of these drugs for the mouse taurine transporter (mTauT) [6] are also included.

mTauT	1	MATKEKLQCLKDFHKDILKPSPGKSPGTRPEDEADGKPPQREKWSKIDFVLSVAGGFVG
rTauT	1	MATKEKLQCLKDFHKDILKPSPGKSPGTRPEDEADGKPPQREKWSKIDFVLSVAGGFVG
hTauT	1	MATKEKLQCLKDFHKDILKPSPGKSPGTRPEDEADGKPPQREKWSKIDFVLSVAGGFVG
dTauT	1	MATKEKLQCLKDFHKDILKPSPGKSPGTRPEDEADGKPPQREKWSKIDFVLSVAGGFVG
bTauT	1	MATKEKLQCLKDFHKDILKPSPGKSPGTRPEDEADGKPPQREKWSKIDFVLSVAGGFVG
mTauT	61	LGNVWRFPYLCYKNGGGAFLIPYFIFLFGSGLPVFFLEVIIGQYTSEGGITCWEKICPLF
rTauT	61	LGNVWRFPYLCYKNGGGAFLIPYFIFLFGSGLPVFFLEVIIGQYTSEGGITCWEKICPLF
hTauT	61	LGNVWRFPYLCYKNGGGAFLIPYFIFLFGSGLPVFFLEVIIGQYTSEGGITCWEKICPLF
dTauT	61	LGNVWRFPYLCYKNGGGAFLIPYFIFLFGSGLPVFFLEVIIGQYTSEGGITCWEKICPLF
bTauT	61	LGNVWRFPYLCYKNGGGAFLIPYFIFLFGSGLPVFFLEVIIGQYTSEGGITCWEKICPLF
mTauT	121	SGIGYASIVIVSLLNVYYIVILAWATYYLFHSFQKDLPPWAHCNHSWNTPQCMEDTLRRNE
rTauT	121	SGIGYASIVIVSLLNVYYIVILAWATYYLFQSFQKDLPPWAHCNHSWNTPQCMEDTLRRNE
hTauT	121	SGIGYASIVIVSLLNVYYIVILAWATYYLFQSFQKDLPPWAHCNHSWNTPQCMEDTLRRNE
dTauT	121	SGIGYASIVIVSLLNVYYIVILAWATYYLFQSFQKDLPPWAHCNHSWNTPQCMEDTLRRNE
bTauT	121	SGIGYASIVIVSLLNVYYIVILAWATYYLFQSFQKDLPPWAHCNHSWNTPQCMEDTLRRNE
mTauT	181	SHWVSLSTANFTSPVIEFWERNVLSLSSGIDNPGSLKWDALCLLLVWLVCFFCIWKGVK
rTauT	181	SHWVSLSTANFTSPVIEFWERNVLSLSSGIDNPGSLKWDALCLLLVWLVCFFCIWKGVK
hTauT	181	SHWVSLSTANFTSPVIEFWERNVLSLSSGIDNPGSLKWDALCLLLVWLVCFFCIWKGVK
dTauT	181	SHWVSLSTANFTSPVIEFWERNVLSLSSGIDNPGSLKWDALCLLLVWLVCFFCIWKGVK
bTauT	181	SHWVSLSTANFTSPVIEFWERNVLSLSSGIDNPGSLKWDALCLLLVWLVCFFCIWKGVK
mTauT	241	STGKVVFYFTATFPFAMLLVLLVRLTLPAGAGEGIKFYLYPDISRLCDPQVWIDAGTQIFF
rTauT	241	STGKVVFYFTATFPFAMLLVLLVRLTLPAGAGEGIKFYLYPDISRLCDPQVWIDAGTQIFF
hTauT	241	STGKVVFYFTATFPFAMLLVLLVRLTLPAGAGEGIKFYLYPDISRLCDPQVWIDAGTQIFF
dTauT	241	STGKVVFYFTATFPFAMLLVLLVRLTLPAGAGEGIKFYLYPDISRLCDPQVWIDAGTQIFF
bTauT	241	STGKVVFYFTATFPFAMLLVLLVRLTLPAGAGEGIKFYLYPDISRLCDPQVWIDAGTQIFF
mTauT	301	SYAICLGAMTSLGSYNKYKNSYRDCMLLGCLNSGTSFVSGFAIFSILGFMAQEQGVDA
rTauT	301	SYAICLGAMTSLGSYNKYKNSYRDCMLLGCLNSGTSFVSGFAIFSILGFMAQEQGVDA
hTauT	301	SYAICLGAMTSLGSYNKYKNSYRDCMLLGCLNSGTSFVSGFAIFSILGFMAQEQGVDA
dTauT	301	SYAICLGAMTSLGSYNKYKNSYRDCMLLGCLNSGTSFVSGFAIFSILGFMAQEQGVDA
bTauT	301	SYAICLGAMTSLGSYNKYKNSYRDCMLLGCLNSGTSFVSGFAIFSILGFMAQEQGVDA
mTauT	361	DVAESGPGGLAFIAYPKAVTMMPLPTFWSILFFIMLLLLGLDSQFVEVEGQITSLVDLYPS
rTauT	361	DVAESGPGGLAFIAYPKAVTMMPLPTFWSILFFIMLLLLGLDSQFVEVEGQITSLVDLYPS
hTauT	361	DVAESGPGGLAFIAYPKAVTMMPLPTFWSILFFIMLLLLGLDSQFVEVEGQITSLVDLYPS
dTauT	361	DVAESGPGGLAFIAYPKAVTMMPLPTFWSILFFIMLLLLGLDSQFVEVEGQITSLVDLYPS
bTauT	361	DVAESGPGGLAFIAYPKAVTMMPLPTFWSILFFIMLLLLGLDSQFVEVEGQITSLVDLYPS
mTauT	421	FLRKGYRREIFIAIICSIISYLLGLTMVTEGGMYVFQLFDDYAAAGVCLLWVAFFECFVIA
rTauT	421	FLRKGYRREIFIAIICSIISYLLGLTMVTEGGMYVFQLFDDYAAAGVCLLWVAFFECFVIA
hTauT	421	FLRKGYRREIFIAIICSIISYLLGLTMVTEGGMYVFQLFDDYAAAGVCLLWVAFFECFVIA
dTauT	421	FLRKGYRREIFIAIICSIISYLLGLTMVTEGGMYVFQLFDDYAAAGVCLLWVAFFECFVIA
bTauT	421	FLRKGYRREIFIAIICSIISYLLGLTMVTEGGMYVFQLFDDYAAAGVCLLWVAFFECFVIA
mTauT	481	WIYGGDNLYDGIEDMIGYRPGPWWKYSWAVITPCLCVGCFIFSLVKYVPLTYNKVYRYPD
rTauT	481	WIYGGDNLYDGIEDMIGYRPGPWWKYSWAVITPCLCVGCFIFSLVKYVPLTYNKVYRYPD
hTauT	481	WIYGGDNLYDGIEDMIGYRPGPWWKYSWAVITPCLCVGCFIFSLVKYVPLTYNKVYRYPD
dTauT	481	WIYGGDNLYDGIEDMIGYRPGPWWKYSWAVITPCLCVGCFIFSLVKYVPLTYNKVYRYPD
bTauT	481	WIYGGDNLYDGIEDMIGYRPGPWWKYSWAVITPCLCVGCFIFSLVKYVPLTYNKVYRYPD
mTauT	541	WAIGLGWGLALSSMCVPLVIVIRLCRTEGFLVRKYLTPREPNRWAVEREGATPYS
rTauT	541	WAIGLGWGLALSSMCVPLVIVIRLCRTEGFLVRKYLTPREPNRWAVEREGATPYS
hTauT	541	WAIGLGWGLALSSMCVPLVIVIRLCRTEGFLVRKYLTPREPNRWAVEREGATPYS
dTauT	541	WAIGLGWGLALSSMCVPLVIVIRLCRTEGFLVRKYLTPREPNRWAVEREGATPYS
bTauT	541	WAIGLGWGLALSSMCVPLVIVIRLCRTEGFLVRKYLTPREPNRWAVEREGATPYS
mTauT	601	RVTMNGALMKPSSHIVETMM
rTauT	601	RVTMNGALMKPSSHIVETMM
hTauT	601	RVTMNGALMKPSSHIVETMM
dTauT	601	RVTMNGALMKPSSHIVETMM
bTauT	601	RVTMNGALMKPSSHIVETMM

Fig. 2. Sequence alignment of the taurine transporters. Amino acid sequences of the taurine transporters encoded by the mouse retina (mTauT; [6]), rat brain (rTauT; [11]), human placenta (hTauT; [10]), MDCK cells (dTauT; [12]), and BAE cells (bTauT; this work; GenBank accession no. AF260239) cDNA clones were aligned using the CLUSTALW 1.8 program, and the aligned sequences were shaded using the BOXSHADE 3.21 program (accessed via the BCM Search Launcher, Baylor College of Medicine, Houston, TX). Identical residues are shown as white letters in black background.

3.2. Identification and isolation of the taurine transporter cDNA from the BAE cells

To identify the taurine transporter expressed in BAE cells, we initially used a RT–PCR based approach. Total RNA from the BAE cells were analyzed by RT–PCR using several mTauT-specific oligonucleotide primers. Initial results indicated that BAE cells expressed a taurine transporter cDNA which shared a high degree of sequence homology with that of the mTauT cDNA (results not shown). Subsequently, the entire sequence of the cDNA was assembled from three overlapping PCR-amplified fragments. We also used 5'-RACE method to amplify independently the 5'-end of the cDNA. Out of ten independent recombinant plasmids bearing the amplified insert, only three clones revealed DNA sequences comprising the anticipated 5'-end of the taurine transporter coding region and part of the 5'-untranslated region; the rest of the clones contained unrelated DNA sequences (results not shown). Sequencing of each taurine transporter 5'-RACE clone revealed differences in length of the 5'-untranslated region and also verified the sequence integrity of the N-terminal coding region. A cDNA clone containing the entire coding region of the bovine taurine transporter (bTauT) was assembled from two overlapping PCR amplified fragments as described in Section 2.

Fig. 2 depicts the amino acid sequence alignment of the bTauT taurine transporter with those of the human placental [10], mouse retinal [6], rat brain [11], and canine kidney derived MDCK cell [12] taurine transporters. As shown, the bTauT shared

Table 1
Inhibition of taurine uptake

Inhibitor	IC ₅₀ (μM)	
	BAE cells	mTauT ^a
β-Alanine	66.1	52.5
GES	120.2	89.1
Homotaurine	691.8	631.0

Taurine uptake (60 min uptake at 37°C using 5.0 μM of [³H]taurine) was measured as a function of various concentrations of a given inhibitor. IC₅₀ for each inhibitor was determined from the respective dose–response plot.

^aResults for the mTauT are taken from Vinnakota et al. [6] for comparison.

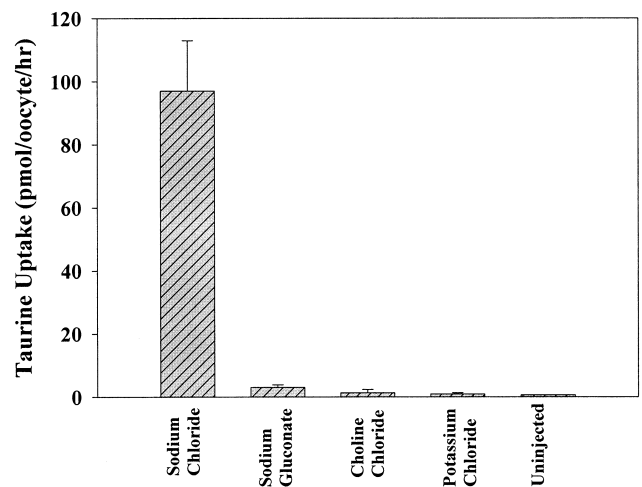


Fig. 3. Taurine uptake in *Xenopus* oocytes expressing the bTauT taurine transporter cDNA clone. Taurine uptake in oocytes individually microinjected with the bTauT cRNA was measured for 1 h using 5 μM [³H]taurine as described in Section 2. Results are expressed as average mean uptake ($n=8$ oocytes; \pm S.E.). Control taurine uptake (100%) in oocytes using NaCl buffer was 97.0 pmol/oocyte per h. Uninjected oocytes were used as a negative control.

~92.9% sequence identity at the amino acid level to the human, mouse, and rat taurine transporters, and ~96.8% sequence identity to the MDCK cell taurine transporter.

Fig. 3 shows the induced taurine uptake in *Xenopus* oocytes expressing the bTauT cRNA. The induced taurine uptake activity in each oocyte was robust (97.0 pmol/oocyte per h) and was significantly higher than that in the uninjected oocyte (0.6 pmol/oocyte per h). Furthermore, the uptake was dependent on both external Na⁺ and Cl[−]. Thus, the oocytes were able to sustain taurine uptake in NaCl buffer; however, the uptake was significantly inhibited in choline chloride, potassium chloride, and sodium acetate buffers.

3.3. Effects of external taurine, Na⁺, and Cl[−] on taurine uptake

The rate of taurine uptake both in cultured BAE cells (endogenous) as well as in oocytes (expressing the recombinant bTauT) increased when the external concentration of taurine, Na⁺ or the Cl[−] was increased independently (results not shown). The rate of uptake increased hyperbolically with the increase in the external taurine concentration (1–60 μM). The

rate of uptake also increased hyperbolically as the external Cl^- concentration was increased (0–135 mM for cells and 0–100 mM for oocytes) using acetate as the replacement anion for Cl^- . In contrast, the rate of uptake increased sigmoidally as the external Na^+ concentration was increased (0–135 mM for cells and 0–100 mM for oocytes). Similar results were obtained irrespective of whether the Na^+ concentration was varied by replacing Na^+ with either choline or Li^+ (results not shown).

The experimental results of taurine uptake as a function of various external concentrations of taurine, Na^+ , and Cl^- were subjected to non-linear curve-fit analyses as described [6] to determine the apparent kinetic parameters (i.e., equilibrium constant, V_{\max} , Hill coefficient), which are listed in Table 2. The taurine uptake in BAE cells followed Michaelis–Menten kinetics that saturated with a $K_{0.5}$ of 4.9 μM for taurine. Similar results were obtained with oocytes expressing the bTauT cDNA. Based on the Hill coefficients summarized in Table 2, the bovine taurine transporter requires at least two Na^+ and one Cl^- to transport one taurine across the cell membrane, as has been found with the mouse, human, and rat high-affinity taurine transporters [6,10,11].

In contrast to the studies described above, in which the external Cl^- concentration in the uptake buffer was varied using acetate as the replacement ion, a markedly different result was obtained when these studies were performed using buffers in which the Cl^- was replaced by F^- . As shown in Fig. 4, when Cl^- was replaced by acetate, the taurine uptake gradually decreased from 100% to about 10% of its original value as the acetate concentration was in-

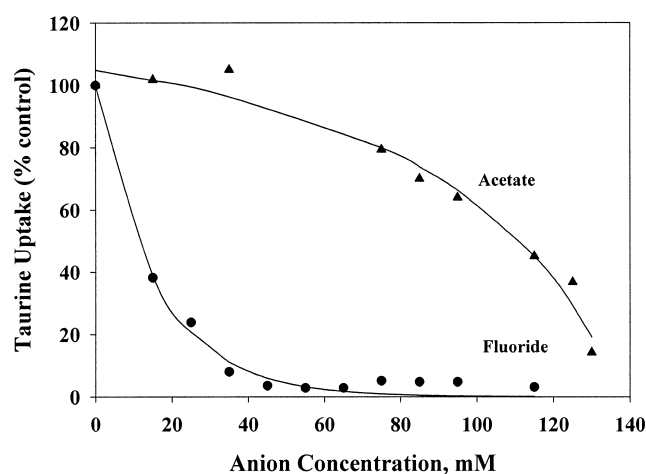


Fig. 4. Effect of replacing Cl^- with F^- on taurine uptake in BAE cells. Taurine uptake was measured using 5.0 μM of [^3H]taurine (60 min uptake, 37°C) in buffers containing various concentrations of Cl^- . The Cl^- concentration in the buffer was varied by replacing a given amount of NaCl in the NaCl-1 buffer with an equimolar amount of either NaF (●) or sodium acetate (▲) to keep the combined anion concentration at 135 mM. Results (average of duplicate measurements \pm deviation) are expressed as percentage of uptake in NaCl-1 buffer (control uptake) as a function of anion (acetate or F^-) concentration.

creased from 0 to 135 mM (i.e., the Cl^- concentration decreased from 135 to 0 mM). In contrast, when Cl^- was replaced by F^- , the uptake sharply decreased as the F^- concentration was increased from 0 to 30 mM. When the F^- concentration in the uptake buffer was 40 mM, there was virtually no residual taurine uptake even though the uptake buffer contained 95 mM of Cl^- . The estimated IC_{50} for F^- from this plot was ~ 17.5 mM. Similar results were also observed in oocytes expressing the bTauT

Table 2

Kinetic parameters of taurine transport

Substrate	Equilibrium constant ($K_{0.5}$)		V_{\max}		Hill coefficient (n)	
	BAE	bTauT	BAE ^a	bTauT ^b	BAE	bTauT
Taurine	4.9 μM	1.0 μM	17.0	77.9	1	1
Na^+	19.3 mM	41.9 mM	16.2	84.4	2.2	1.87
Cl^-	7.5 mM	28.3 mM	14.1	97.5	1	1

The external concentration of taurine, Na^+ , or Cl^- was varied independently and the taurine uptake was measured at each experimental condition as described in Section 2. Equilibrium constant, V_{\max} , and Hill coefficient values were determined by non-linear curve-fit analyses of experimental data points as described [6] using the Sigma Plot 4.0 program (Jandel Scientific, CA).

^a V_{\max} is expressed as pmol/mg protein per 30 min.

^b V_{\max} is expressed as pmol/oocyte per h.

(results not shown). A reasonable explanation of these results is that F^- is inhibiting the taurine uptake by interfering with the binding of the Cl^- to the transporter. Alternatively, the effect of F^- may be due to F^- -induced cytotoxicity [13]. In this regard, it is noteworthy that the Cl^- concentration-dependent taurine uptake in insulin secreting HIT-T15 cells decreased in a manner similar to the 'acetate' activity profile shown in Fig. 4 irrespective of whether Cl^- was replaced by acetate or F^- (Vinnakota, Edwards, Sarkar, in preparation). Even though we cannot completely rule out the possibility of F^- -induced cytotoxicity in BAE cells based solely on our results using the HIT-T15 cells, it is reasonable to suggest that the effect of F^- on taurine uptake is not a general phenomenon.

3.4. Modulation of taurine uptake in BAE cells

Taurine uptake is known to be modulated by various cellular factors [6,10,14]. So we examined the effect of calcium ionophore A23187 and phorbol esters on the endogenous taurine uptake in BAE cells. As shown in Fig. 5, direct addition of A23187 (5 μ M) to the uptake mixture stimulated the taurine uptake by about 28%. Pre-treatment of BAE cells

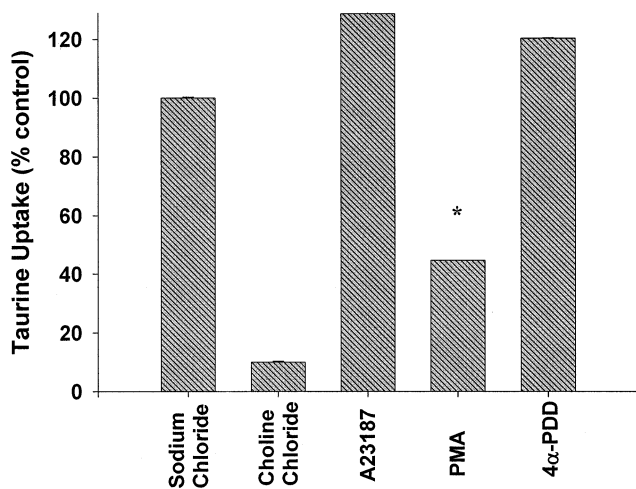


Fig. 5. Effects of A23187 and phorbol esters on taurine uptake. BAE cells were treated with A23187 and phorbol esters as described in Section 2. Taurine uptake was measured in NaCl-1 buffer using 2.0 μ M of [3 H]taurine (60 min uptake, 37°C). Results (average of duplicate measurements \pm deviation) are expressed as percentage of uptake in untreated cells (control uptake). * $P < 0.0001$.

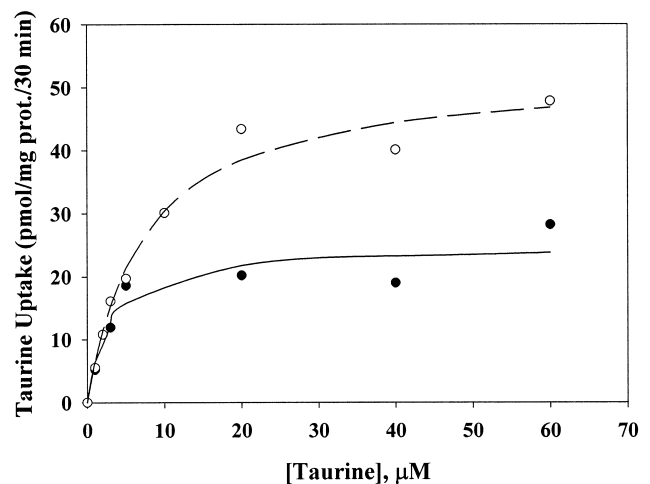


Fig. 6. Effect of hypertonic stress on taurine uptake in BAE cells. Cells were grown and exposed to iso-osmotic (control; ●) or hyperosmotic (○) condition as described in Section 2. Taurine uptake was measured (30 min uptake, 37°C) in control and experimental cells as a function of various external [3 H]taurine concentration (1–60 μ M). Smooth curve represents best fit of the experimental data points to Michaelis–Menten equation.

with 100 nM of the protein kinase C activator phorbol ester, phorbol 12-myristate 13-acetate (PMA), reduced the taurine uptake by $\sim 55\%$; however, the inactive phorbol ester analog, 4 α -phorbol 12,13-didecanoate (4 α -PDD), was unable to mimic the effect of PMA. These results suggest, but do not necessarily prove, that the taurine transporter in BAE cells might be regulated by intracellular factors, such as Ca^{2+} and protein phosphorylation.

3.5. Effect of hypertonic stress on taurine uptake

Hypertonic stress modulates taurine uptake in cells where taurine is known to play a role in cell volume regulation [15,16]. To determine whether taurine might play a similar role in BAE cells, we examined the effect of hypertonic stress on taurine uptake. The uptake was stimulated significantly in BAE cells grown in medium supplemented with either 100 mM NaCl or 200 mM raffinose over cells incubated in normal growth medium (results not shown). To determine whether the increase is due to an effect on the V_{max} or K_m of taurine uptake, we measured taurine concentration-dependent taurine uptake in cells exposed to hyperosmotic medium or iso-osmotic medium (Fig. 6). Analysis of the experimental results

shown in Fig. 6 using Michaelis–Menten equation revealed that exposure to hyperosmotic medium increased the V_{\max} for taurine uptake from 24.9 pmol/mg protein per 30 min for the control cells to 51.2 pmol/mg protein per 30 min for the hypertonically stressed experimental cells without significantly altering the K_m (3.0 and 6.9 μM for the control and experimental cells, respectively) for taurine. This observed increase in V_{\max} suggests that hypertonic stress may have stimulated taurine uptake by increasing the number of taurine transporters in BAE cells.

4. Discussion

Results described above for the first time demonstrate clearly that the cultured BAE cells possess a Na^+ - and Cl^- -dependent taurine transport system, which is biochemically and pharmacologically similar to the high-affinity taurine transport system found in a variety of mammalian tissues. Commensurate with this conclusion, the taurine transporter encoding cDNA isolated from the BAE cells (bTauT) showed a high degree of sequence homology at the amino acid level (>92%) with the taurine transporters previously cloned from the mammalian brain, kidney, retina, and placenta. Furthermore, the molecular properties of the recombinantly expressed bTauT in oocytes were also similar to those of the other cloned mammalian taurine transporters. Collectively, these results suggest that a high-affinity taurine transporter (bTauT) is expressed endogenously in the cultured BAE cells.

A Na^+ - and Cl^- -dependent taurine uptake activity was demonstrated previously in the primary cultures of bovine brain capillary endothelial cells [3,4]. Taurine uptake activity was found on both the apical and the basolateral surface of these endothelial cells. In both cases, the taurine uptake saturated with an apparent K_m of $\sim 12 \mu\text{M}$. Our results show that the endogenous taurine uptake in BAE cells and the bTauT-induced taurine uptake in oocytes saturated with a K_m between 1.0 and 5.0 μM for taurine, a value that is similar to the K_m value described for the brain capillary endothelial cells [3]. Based on these results, we suggest that the same taurine transporter is expressed in both the brain and the aortic endothelial cells. We do not know from our results,

however, whether bTauT is the only taurine transporter expressed in the BAE cells.

The physiological role of the taurine transporter in the endothelial cells is currently not known. The high-affinity taurine transporter is most likely a key player in transporting taurine into the endothelial cells and also in transporting taurine across the blood–brain barrier. The endothelial cells are exposed to the shear and stress of blood flow and to the changes in blood osmolarity. Information regarding the mechanism(s) protecting the endothelial cells from the long-term deleterious effects of shear, stress, and osmotic changes are lacking. Thus, these cells may possess potential mechanisms to counteract the rise and decrease in the extracellular osmolarity, including mechanisms involving modulation of intracellular concentration of the osmolyte taurine. Our observation that exposure to hypertonic stress resulted in an increase in the V_{\max} of taurine uptake in BAE cells supports the above contention. Even though our result does not prove that taurine functions as an osmolyte in the endothelial cells, a concomitant increase in intracellular taurine in response to hypertonicity is a prerequisite if taurine were to play the role of an osmolyte in endothelial cells. Recent demonstration that hypo-osmotic swelling activated taurine efflux in cultured calf pulmonary endothelial cells [17] also adds support to the idea that taurine might act as an osmolyte in endothelial cells.

Taurine was also suggested to play a cytoprotective role in human umbilical endothelial cells [18] and in human vascular endothelial cells [19] because of its ability to prevent stimuli-induced and high glucose-induced apoptosis, respectively. Although the exact mechanism by which taurine prevented cell damage in these studies remains to be elucidated, a critical factor required for the cytoprotective action of taurine may involve mechanisms that maintain the intracellular taurine content. Treatment with glucose may decrease the intracellular taurine content of the endothelial cells, possibly by downregulating the taurine transporter [20], and may consequently contribute to the endothelial cell apoptosis. Consistent with this idea, we have observed $\sim 40\%$ decrease in taurine uptake level in glucose-treated BAE cells (65 mM glucose for 3 days) compared to that in the untreated (control) BAE cells (Sarkar, unpublished observation). Pre-incubation with taurine would raise the

intracellular taurine content, which may explain why taurine prevented glucose-mediated apoptosis in endothelial cells [19]. Our observation that glucose treatment reduced relative taurine uptake level in BAE cells suggests that chronic exposure to high glucose concentrations may lead to a decrease in the intracellular taurine content, which may be a causal factor for the hyperglycemia-triggered vascular lesions found in human and experimental animals.

In summary, our studies provide the first molecular evidence that endothelial cells express a high-affinity taurine transporter, via which these cells can actively accumulate taurine from the extracellular medium (i.e., circulating plasma). It is noteworthy that taurine was shown to attenuate the effects of several cytotoxic agents in a variety of tissues and cells, where taurine transporter is also expressed [1,21]. Thus, we propose that the maintenance of the intracellular taurine content via the high-affinity taurine transporter is one of the mechanisms by which the endothelial cells counteract the deleterious effects of osmotic stress and a number of other cytotoxic agents.

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