Taurine synthesis and cysteine metabolism in cultured rat astrocytes: effects of hyperosmotic exposure

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Beetsch, Joel W., and James E. Olson. Taurine synthesis and cysteine metabolism in cultured rat astrocytes: effects of hyperosmotic exposure. Am. J. Physiol. 274 (Cell Physiol. 43): C866–C874, 1998.—We investigated mechanisms controlling taurine synthesis in cultured rat cerebral astrocytes. The mean ± SE rate of taurine synthesis from extracellular cysteine was 21.2 ± 2.0 pmol·mg protein⁻¹·min⁻¹, whereas taurine degradation was <1.3% of this rate. Eliminating cellular glutathione and inhibiting glutathione biosynthesis increased taurine synthesis from extracellular cysteine by 39%. In cell homogenates, cysteine dioxygenase (CDO) and cysteine-sulfinate decarboxylase activities were 2.4 ± 0.2 and 8.3 ± 2.8 nmol·mg protein⁻¹·min⁻¹, respectively. CDO activity was strongly dependent on cysteine concentration over physiological and pathophysiological ranges of intracellular cysteine concentration. Growth in hyperosmotic medium caused a greater increase in culture medium taurine content than that measured from cells in isosmotic growth medium. Hyperosmotic treatment transiently increased the rate of taurine accumulation and cellular cysteine and glutathione contents but had no effect on the synthesis rate of taurine from extracellular cysteine. Thus taurine is accumulated and then metabolized to taurine through CDO, whose activity depends on the intracellular cysteine concentration and appears to be rate limiting for taurine synthesis. Hyperosmotic exposure increases net taurine production yet has no effect on taurine synthesis from exogenously applied cysteine. Availability of substrate from intracellular pools must contribute to maintenance of high intracellular taurine during hyperosmotic exposure.


cysteine dioxygenase; cysteine-sulfinate decarboxylase; γ-glutamyltransferase; glutathione; volume regulation

TAURINE BIOSYNTHESIS in the central nervous system has been described previously (13), but mechanisms that regulate this pathway remain to be defined. Taurine can be synthesized from its amino acid precursor, cysteine, by a variety of mechanisms (13). In the central nervous system, however, taurine synthesis proceeds from cysteine to cysteine sulfinate, hypotaurine, and finally taurine, a sequence commonly called the cysteine-sulfinate decarboxylase (CSD; EC 4.1.1.29) route (42). Contributions to taurine synthesis via cysteamine or cysteine are very minor or insignificant (17). The taurine-synthesizing enzymes, cysteine dioxygenase (CDO; EC 1.13.11.20) and CSD, have been identified and characterized in the rat brain (24, 32). In addition, Kuriyama et al. (17) demonstrated conversion of cysteine to cysteine sulfinic acid, hypotaurine, and taurine in rat whole brain homogenates.

In hyperosmotic hypernatremia, contents of a number of organic osmolytes including polyamines, polyols, sugars, and amino acids are elevated in brain tissue (4, 12, 19, 44). In some animal models, accumulation of taurine accounts for as much as 50% of the additional osmolytes needed for brain volume regulation (44). Tissue culture studies of cerebral astrocytes have demonstrated increased cellular contents and rates of influx of taurine (6, 25, 36) and inositol (39) during hyperosmotic exposure. However, in situ, enhanced cellular accumulation of taurine only serves to sequester available taurine intracellularly, as unidirectional transport from blood to brain is not elevated by acute hyperosmotic treatment (40). Thus activation of de novo synthesis of taurine may play an important role in the brain’s adaptation to hyperosmotic conditions.

In addition to being a precursor for taurine synthesis, cysteine is a structural element of proteins and is one of three amino acids in glutathione (GSH, γ-glutamylcysteineglycine). Cysteine, which is in micromolar concentrations in the brain (37), is a limiting reagent for the production of GSH during changes in cellular oxidation states (8, 30, 34, 35). GSH, on the other hand, is present in millimolar concentrations in brain cells (22, 29, 35, 49). GSH serves as an intracellular reducing agent, a xenobiotic detoxifier, and a free radical scavenger (46). Studies have shown GSH to be present in higher concentrations in astrocytes than in neurons (22). Further evidence suggests that astrocytes provide the substrate for neuronal GSH synthesis by releasing cysteine into the extracellular space for accumulation by neurons (35). Release of GSH from cultured astrocytes also may supply neurons with GSH directly or perhaps may serve as a nonneuroactive precursor to glutamate (49).

Conversely, extracellular GSH may serve as a source of cellular cysteine. Hanigan and Ricketts (11) demonstrate that cysteine-dependent fibroblasts containing γ-glutamyltransferase (γ-GT; EC 2.3.2.2), a cell surface enzyme capable of hydrolyzing the γ-glutamyl bond of GSH or transferring the γ-glutamyl moiety to an acceptor amino acid, can use extracellular GSH both to replenish intracellular GSH stores and to provide a source of cysteine. This manner of GSH and cysteine cycling may be present in astrocytes, which also express γ-GT activity (22, 31). Thus alterations in GSH metabolism and cellular content may, in turn, affect taurine biosynthesis by regulating the availability of their common precursor, cysteine. Finally, activities of sodium-dependent and sodium-independent cysteine/cystine transporters (8, 34, 35) may regulate cellular pools of free cysteine in astrocytes and thus the biosynthesis pathways for GSH and taurine.
In this study, we examine rates of various transport and biochemical processes involved in the production of taurine from extracellular cysteine in cultured astrocytes from the rat cerebral cortex. Previous studies demonstrated that taurine synthesis is enhanced in astrocytes exposed to hyperosmotic conditions (5, 6). To further understand the regulation of taurine synthesis in vivo, we also explore mechanisms that contribute to this enhanced synthesis during hyperosmotic exposure.

**MATERIALS AND METHODS**

Cell culture. Primary astrocyte cultures were prepared from the cerebral cortices of 2- to 4-day-old Sprague-Dawley (Harlan Sprague-Dawley, Indianapolis, IN) rat pups by a modification of methods described previously (5, 26, 27). Cells were grown on 35- or 100-mm plastic culture dishes at an initial plating density of ~60,000 cells/cm². Astrocytes were plated in Eagle's minimum essential medium (MEM; GIBCO Life Technologies, Grand Island, NY) containing 20% (vol/vol) newborn calf serum (GIBCO Life Technologies), antibiotics (50 U/ml penicillin, 50 mg/ml streptomycin), four times the normal concentration of MEM vitamins, twice the normal concentration of MEM amino acids, and 1.6 mg/ml additional sodium bicarbonate. Plating medium was replaced 1–2 days later with an identical medium but containing only 10% (vol/vol) newborn calf serum. This formulation contained ~200 µM cysteine and no GSH or taurine from MEM; however, newborn calf serum may contain these constituents. Measured concentrations of taurine in fresh growth medium were 2–5 µM. Growth medium was changed twice weekly as astrocytes reached confluency over 2–3 wk. At the final change, some dishes received culture medium containing additional NaCl to raise the osmolality from the control value (300 mosmol/kg) to 350, 400, or 450 mosmol/kg. To avoid medium evaporation and elevations in medium osmolality, 35-mm dishes were placed into a covered 100-mm control value (300 mosmol/kg) to 350, 400, or 450 mosmol/kg.

To measure cell volume, astrocyte cell volumes were measured by determining the 3-O-[methyl-3H]-methyl-α-glucose ([3H]-OMG) space using a method modified from that described by Bender et al. (7). Cultures were incubated for 5 min at 37°C with glucose-free PBS containing 0.8 µCi/ml [3H]sucrose (400–700 mCi/mmol; NEN, Wilmington, DE), 0.8 µCi/ml 3-O-[methyl-3H]-methyl-α-glucose (60–90 Ci/mmol; NEN), 1 mM 3-OMG, and sufficient NaCl to match the osmolality of the culture medium. Cells were then rinsed free of culture medium using 37°C phosphate-buffered saline (PBS) containing (in mM) 2.7 KCl, 1.0 CaCl₂, 0.5 MgCl₂, 2.7 NaH₂PO₄, 0.5 KH₂PO₄, 5.5 glucose (pH 7.3), and sufficient NaCl to match the osmolality of the culture medium.

Cysteine derived from metabolism of endogenous GSH would artifically reduce the specific activity of taurine synthesized from extracellular [35S]cysteine. Therefore, in some experiments, cultured cells were treated with 1 mM butathionine-(S,R)-sulfoximine (BSO), a potent γ-glutamylcysteine synthetase (EC 6.3.2.2) inhibitor (10), for 24 h before experimentation. These cells also were exposed to 50 µM 1-chloro-2,4-dinitrobenzene (CDNB) for 10 min following BSO treatment to ensure complete depletion of intracellular GSH. Pilot studies showed that treatment with 1 mM BSO alone reduced GSH levels by 75% (data not shown). When these cells were also treated with CDNB, cellular GSH levels were undetectable (<1 nmol/mg protein).

Taurine degradation. Taurine degradation was determined for astrocyte cultures grown in isoosmotic conditions. Cells were exposed to 0.5 µCi/ml [3H]taurine in culture medium for 2 h before changing the medium to fresh isoosmotic culture medium of 300 mosmol/kg.

Taurine, cysteine, and GSH contents. Astrocyte taurine content was measured by high-performance liquid chromatography (HPLC) in deproteinized cell extracts of 0.6 M HClO₄ as previously described (6). Culture medium taurine concentrations were determined with a Waters model 420-AC fixed-wavelength (338-nm excitation, 425-nm cutoff emission; Waters, Milford, MA) fluorescence detector using mobile phase consisting of 28% methanol plus 72% (vol/vol) phosphate buffer (pH 5.5) flowing at 1.2 ml/min.

Cellular taurine content was determined by a modification of methods described previously by Bystroff et al. (7). Deproteinized cell extracts of 0.6 M HClO₄ were separated using a BioAnalytical Systems (BAS, West Lafayette, IN) chromatograph with a BAS Biosphere ODS (4.6 x 250 mm, 5 µm) column. Mobile phase flowed at 1.0 ml/min and contained 96% 100 mM monochloroacetic acid (pH 3), 4% methanol, and 2 mM heptane sulfonic acid (Alltech, Deerfield, IL) as an ion-pairing reagent. Cellular cysteine was detected with an Au/Hg electrochemical detector at +0.15 V vs. an Ag/AgCl reference electrode.

GSH content was determined by a modification of methods described by Jocelyn (15). GSH was extracted by scraping astrocytes into 3.6 mM EDTA and 3.4 M NaCl in 1.1% (wt/vol) glacial metaphosphoric acid. The suspension was centrifuged and protein was measured in the cellular pellet as described above. A 450-µl aliquot of supernatant was mixed with 225 µl of 0.5 M 5,5'-dithio-bis(2-nitrobenzoic acid) in 1% (wt/vol) sodium citrate and 1.8 ml of 300 mM Na₃HPO₄. Absorbance was read at 412 nm and compared with GSH standards prepared in the same manner.

Conversion of extracellular [35S]cysteine to cellular [35S]taurine. Each culture dish received 2 ml PBS containing 1 µCi/ml [35S]cysteine (~1,000 Ci/mmol; Amersham Life Science, Arlington Heights, IL), 1 mM unlabeled cysteine, and sufficient NaCl to match the osmolality of the culture medium. After 60 min at 37°C, the reaction was terminated by removing the PBS and rapidly rinsing three times with ice-cold 10 mM Tris (pH 7.3) plus sufficient sucrose to match the osmolality of the culture medium. The cells were then scraped from the dish into 1 ml of distilled water. The suspension was frozen at ~70°C and lyophilized, and the dried residue was resuspended in 100 µl of 0.6 M HClO₄. After centrifugation, amino acid contents were determined in this deproteinized extract by HPLC (6). The eluate was collected in fractions at 12-s intervals at times corresponding to the elution of the taurine peak. [35S]taurine was measured in the eluate fractions by liquid scintillation counting. Protein was determined in the pellet remaining after centrifugation (21).
medium. At 24 or 48 h later, the medium was removed and cells were rinsed in ice-cold 10 mM Tris (pH 7.3) plus sufficient sucrose to match the osmolality of the culture medium. Astrocytes were scraped into 1 ml of 0.6 M HClO₄ and centrifuged. Protein was determined in the pellet after solubilization in 1 M NaOH (21). Taurine content of the medium and cell supernatant was determined in triplicate by HPLC after derivitization to a fluorescent product as described previously (6). Fractions were collected for radioactivity counting during elution of the derivitized taurine reaction product. Integrated radioactivity in the samples corresponding to derivitized taurine was expressed relative to the specific activity of the carboxyl moiety was determined as described above for CDO; 750 µl of the remaining astrocyte-buffer homogenate were added to an equal amount of ice-cold incubation mixture, giving final concentrations of (in mM) 30 [¹³C]cysteine sulfinate (~0.0167 µCi/ml), 0.75 pyridoxal 5’-phosphate, 0.5 dithiothreitol, and 250 KH₂PO₄ (pH 7). The mixture was placed in a sealed 25-ml flask with a center well containing 300 µl of 2-aminoethanol-ethylene glycol (1:2 vol/vol) and folded filter paper. After 60 min at 37°C, the reaction was terminated by injecting 1 ml of 10% (wt/vol) trichloroacetic acid into the reaction mixture. The reaction flask was left for an additional 60 min to completely trap all evolved [¹⁴C]CO₂. Contents of the culture well were then removed for radioactive counting. Results from blank reactions, carried out using heat-inactivated cell homogenates, were subtracted from results from experimental cell homogenates.

A commercial kit (Sigma Diagnostics, St. Louis, MO) was used to determine γ-GT activity. Cells were scraped from the dish in 5 mM imidazole buffer (pH 7.35) and briefly sonicated. A sample of the homogenate was used for determination of protein content (21). A 500-µl aliquot of the remaining homogenate was added to 1 ml of a reaction mixture containing (in mM) 110 Tris buffer, 3.3 γ-glutamyl-3-carboxy-4-nitroanilide, and 110 glycylglycine plus 0.1% (wt/vol) sodium azide (osmolality of ~150 mosmol/kg). In some experiments, imidazole buffer and γ-GT reaction mixture osmolarities were raised by adding additional KCl. Absorbance was measured at 405 nm after 30 min. γ-GT activity was calculated using a millimolar absorptivity of 9.5 for 5-amino-2-nitro-benzoate, a product of the γ-GT reaction.

Lactate dehydrogenase (LDH) was measured in astrocyte culture medium using the method of Koh and Choi (16). LDH activity was calculated from the slope of the initial 45-s linear portion of the absorbance curve and expressed as the quantity of NADH oxidized per minute by astrocyte LDH released into the culture medium.

Data analysis. Data were analyzed by analysis of variance (Systat, Chicago, IL). Post hoc analysis using Dunnett’s test for multiple comparisons with a control population was used as appropriate. Differences between experimental groups were accepted as significant only when P < 0.05. Kinetic parameters were calculated using nonlinear (Quasi-Newton) curve-fitting procedures (Systat), assuming Michaelis-Menten kinetics.

RESULTS

Total culture taurine content. Total taurine content of the culture dish (medium plus cells) was significantly elevated 1, 8, 24, and 48 h after changing to a fresh culture medium (Table 1). As we previously described, at each time point the majority of total culture taurine was in the growth medium (6). The percentage of taurine contained in the medium varied from a maximum of 83% at 1 h to a minimum of 61% at 24 h. Taurine cellular content (per mg protein) and calculated intracellular concentration (in mM) decreased 1 h after the medium change but returned to the initial
Table 1. Astrocyte volume and metabolite contents following culture medium change

<table>
<thead>
<tr>
<th>Time After Culture Medium Change, h</th>
<th>0</th>
<th>1</th>
<th>8</th>
<th>24</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total culture taurine, nmol</td>
<td>26.6 ± 0.5</td>
<td>28.2 ± 0.2*</td>
<td>32.6 ± 0.3*</td>
<td>34.7 ± 0.5*</td>
<td>37.8 ± 0.5*</td>
</tr>
<tr>
<td>Total cellular protein, mg</td>
<td>0.189 ± 0.006</td>
<td>0.219 ± 0.007*</td>
<td>0.245 ± 0.009*</td>
<td>0.203 ± 0.003</td>
<td>0.232 ± 0.009*</td>
</tr>
<tr>
<td>Cellular taurine, nmol/mg protein</td>
<td>49.3 ± 2.7</td>
<td>21.2 ± 0.4*</td>
<td>29.9 ± 1.4*</td>
<td>67.4 ± 2.6*</td>
<td>55.6 ± 1.4*</td>
</tr>
<tr>
<td>Cell volume, µl/mg protein</td>
<td>5.74 ± 0.32</td>
<td>6.33 ± 0.47</td>
<td>6.24 ± 0.40</td>
<td>6.79 ± 0.22</td>
<td>5.57 ± 0.32</td>
</tr>
<tr>
<td>Taurine concentration, mM</td>
<td>8.6</td>
<td>3.3</td>
<td>4.8</td>
<td>9.9</td>
<td>10.0</td>
</tr>
<tr>
<td>Cellular cysteine, nmol/mg protein</td>
<td>4.2 ± 0.2</td>
<td>18.2 ± 1.1*</td>
<td>27.9 ± 1.7*</td>
<td>10.2 ± 0.3*</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>Cysteine concentration, mM</td>
<td>0.7</td>
<td>2.9</td>
<td>4.5</td>
<td>1.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Cellular glutathione, nmol/mg protein</td>
<td>33.1 ± 1.0</td>
<td>43.6 ± 1.1*</td>
<td>59.7 ± 2.4*</td>
<td>39.1 ± 1.0</td>
<td>32.6 ± 0.4</td>
</tr>
<tr>
<td>Glutathione concentration, mM</td>
<td>5.8</td>
<td>6.9</td>
<td>9.6</td>
<td>5.8</td>
<td>5.9</td>
</tr>
</tbody>
</table>

Values are means ± SE of 3–11 independent determinations. Total culture taurine equals the content of cells plus the content of culture medium. Total cellular protein is the protein content of the cells attached to the culture dish. Contents shown for 0 h were determined immediately after changing to fresh medium. Intracellular concentrations were determined by dividing mean cellular contents by the mean cell volume at the same time point. *Mean values are significantly different (P < 0.05) from value measured at 0 h.

value by the 48-h time point. Whereas total culture taurine content increased by 42%, total protein content of the culture dish increased by only 22% over the 48-h experimental period (Table 1).

Cultures grown in hyperosmotic culture medium had significantly greater taurine contents after 8 (450 mosmol/kg), 24 (350 mosmol/kg), and 48 h (400 mosmol/kg and 450 mosmol/kg) than those measured in control cultures at the same time point (Table 2). In contrast to the increase in total cell protein observed in control cultures over 48 h (Table 1), no change in total cellular protein was observed for cells in hyperosmotic medium (data not shown). Thus, similar to our previous data (6), cellular taurine concentration is significantly increased by hyperosmotic exposure. Cells exposed to 400 and 450 mosmol/kg medium for 1 h were 37 and 53% smaller than control cells measured at the same time point. Complete volume recovery was observed after 8 h in each hyperosmotic medium.

Taurine synthesis. The mean ± SE rate of conversion of extracellular [35S]cysteine to [35S]taurine in astrocytes was 21.2 ± 2.0 pmol·mg protein⁻¹·min⁻¹. Cells treated with BSO and CDNB to eliminate GSH synthesis and intracellular GSH content converted extracellular cysteine to taurine at a significantly (P < 0.02) faster rate of 28.9 ± 2.3 pmol·mg protein⁻¹·min⁻¹. Hyperosmotic treatment did not effect the rate of conversion of extracellular cysteine to taurine in untreated cells but significantly (P < 0.01) decreased this rate in cells treated with BSO and CDNB (24 h, 400 mosmol/kg, 13.9 ± 2.0 pmol·mg protein⁻¹·min⁻¹).

Taurine degradation. The fractional recovery of radioactivity eluted as derivitized [3H]taurine by HPLC was 0.262 ± 0.020 (mean ± SE) using a standard cocktail of amino acids. This fractional recovery is less than unity because of degradation of the fluorescent product during the time before elution from the column (14, 20). By comparison, 24 and 48 h after cells were loaded with radioactive taurine, 0.299 ± 0.012 and 0.302 ± 0.013 of the total radioactivities in the cell and medium supernatants, respectively, were recovered as radioactive derivitized taurine. These fractional recoveries are not significantly different from that measured in the standard cocktail. However, because of uncertainties in the actual mean values, we performed regression analysis using the fractional recovery from the standard cocktail as the baseline (0 h) value. This analysis yielded a mean (±SE) rate of change in taurine fractional recovery of 0.020 ± 0.011 per day. Using the calculated intercept at 0 h (0.268) and the lower bound of the 95% confidence interval for the rate of change in fractional recovery (−0.00349 per day), we computed a maximal taurine degradation rate of 1.3% per day.

Table 2. Total culture taurine content of astrocytes exposed to hyperosmotic conditions

<table>
<thead>
<tr>
<th>Culture Medium Osmolarity, mosmol/kg</th>
<th>0 h</th>
<th>1 h</th>
<th>8 h</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>26.6 ± 0.5</td>
<td>28.2 ± 0.2</td>
<td>32.6 ± 0.3</td>
<td>34.7 ± 0.5</td>
<td>37.8 ± 0.5</td>
</tr>
<tr>
<td>350</td>
<td>28.6 ± 0.3</td>
<td>32.0 ± 0.3</td>
<td>38.6 ± 0.9</td>
<td>37.2 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>28.6 ± 0.9</td>
<td>33.2 ± 0.4</td>
<td>37.9 ± 0.5*</td>
<td>43.5 ± 1.1*</td>
<td></td>
</tr>
<tr>
<td>450</td>
<td>28.5 ± 0.5</td>
<td>34.6 ± 0.5*</td>
<td>35.1 ± 0.2</td>
<td>40.7 ± 0.8*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE of 5 independent determinations. *Values are significantly different (P < 0.05) from value measured from cultures in 300 mosmol/kg culture medium at the same time point.
Biosynthetic enzyme activities. CDO activity measured directly from homogenates of isosmotic control cultures was 2.37 \( \pm \) 0.23 nmol \( \cdot \) mg protein \( ^{-1} \) \( \cdot \) min \( ^{-1} \) before medium change. This activity varied over the subsequent 48-h experimental period, reaching a maximal value of 3.55 \( \pm \) 0.34 nmol \( \cdot \) mg protein \( ^{-1} \) \( \cdot \) min \( ^{-1} \) 24 h after the medium change. CSD activity (mean \( \pm \) SE) in homogenates of cells grown in 300 mosmol/kg culture medium was 8.25 \( \pm \) 1.46 nmol \( \cdot \) mg protein \( ^{-1} \) \( \cdot \) min \( ^{-1} \). \( \gamma \)-GT activity was constant in control cultures throughout the experimental period, with a mean \( \pm \) SE value of 0.778 \( \pm \) 0.041 nmol \( \cdot \) mg protein \( ^{-1} \) \( \cdot \) min \( ^{-1} \).

CDO activity of control cells varied with cysteine concentrations, which were expected to occur intracellularly in these and hyperosmotically treated cells (Fig. 3). Kinetic parameters calculated from these data fit to an assumed Michaelis-Menten model gave an estimated (\pm asymptotic SE) maximal velocity of 1.17 \( \pm \) 0.27 nmol \( \cdot \) mg protein \( ^{-1} \) \( \cdot \) min \( ^{-1} \) and a \( K_m \) of 16.74 \( \pm \) 5.51 mM. Because the maximum concentration of cysteine used is lower than the calculated \( K_m \), these parameters give crude estimates of the enzyme kinetic parameters. This analysis is intended only to provide estimations of relative CDO activities over the physiological range of cysteine concentrations observed in this study (Table 1).

Hyperosmotic treatment had no effect on astrocyte CDO activity at any time after medium change compared with the activity in control cultures measured at the same time point. In addition, CDO activity after 48 h in control (300 mosmol/kg) or hyperosmotic (450 mosmol/kg) medium was not affected by altering the reaction mixture osmolality from 300 to 450 mosmol/kg with the addition of KCl (data not shown). Similar to CDO, CSD activity was not affected by 48 h of hyperosmotic (450 mosmol/kg) treatment compared with control cultures measured at the same time point. In contrast to the lack of effect of osmolality on taurine biosynthetic enzymes, elevating the osmolality of the \( \gamma \)-GT reaction mixture from 150 to 300 mosmol/kg significantly (\( P < 0.0001 \)) increased \( \gamma \)-GT activity from 0.778 \( \pm \) 0.041 to 1.086 \( \pm \) 0.021 nmol \( \cdot \) mg protein \( ^{-1} \) \( \cdot \) min \( ^{-1} \). However, when the reaction mixture osmolality was raised further to match that of the hyperosmotic...
astrocyte culture medium, the mean ± SE γ-GT activity was significantly (P < 0.02) lower at 400 mosmol/kg (48 h, 0.847 ± 0.031 nmol · mg protein⁻¹ · min⁻¹) and 450 mosmol/kg (8 h, 0.508 ± 0.032 nmol · mg protein⁻¹ · min⁻¹; and 24 h, 0.808 ± 0.033 nmol · mg protein⁻¹ · min⁻¹) compared with cultures grown in 300 mosmol/kg medium at the same time point.

LDH efflux. Release of LDH from cells into the culture medium provides a measure of cell viability (16). LDH activity of control cells scraped and sonicated in their culture medium was 21.4 nmol NADH/min, a value 6.2 times the activity measured in fresh culture medium. LDH activity of medium removed from cultures in each experimental condition (isosmotic, hyperosmotic, and following BSO and CDNB treatments) was slightly less than, or not different from, the activity measured in fresh culture medium (data not shown).

DISCUSSION

These studies examined several biochemical and physiological mechanisms that may regulate taurine synthesis and accumulation in astrocytes. From our data, we calculate a mean rate of taurine production of 19 pmol · mg protein⁻¹ · min⁻¹ by dividing the increase in total culture taurine over 48 h by the average protein content per dish (0.21 mg). This value is similar to that measured from the conversion of extracellular [³⁵S]cysteine to cellular taurine. This rate of taurine synthesis from extracellular cysteine would permit complete replacement of astrocyte taurine in 2 days. In contrast, degradation is <3% over a 48-h period. Our direct measurements of [³⁵S]taurine production from [³⁵S]cysteine and quantification of CDO and CSD activities represent the first demonstration that cultured astrocytes are capable of synthesizing taurine from extracellular cysteine. These results support the glial localization of CSD shown by Almarghini et al. (1) and suggest astrocytes are major contributors to organic osmolyte synthesis in the brain. Comparing relative rates of cysteine accumulation and CDO and CSD activities suggests CDO is rate limiting for taurine synthesis. Taurine synthesis from extracellular cysteine is enhanced if turnover of the GSH pathway is eliminated, suggesting catabolism of endogenous unlabeled GSH contributes to the pool of cysteine utilized by CDO. Finally, this rate of taurine synthesis from extracellular cysteine is supported by a robust rate of cysteine accumulation.

Similar to our previous data (6), total culture taurine (medium + cells) and cellular taurine concentration increase following exposure to hyperosmotic medium. The calculated rate of increase of total culture taurine over the 48-h experimental period for cells in 450 mosmol/kg medium is 26 pmol · mg protein⁻¹ · min⁻¹. This value is 37% higher than that calculated for control cells maintained in 300 mosmol/kg medium (18 pmol · mg protein⁻¹ · min⁻¹). By comparison, maximal taurine degradation in isosmotic conditions (1.3% per day) is 0.24 pmol · mg protein⁻¹ · min⁻¹. Thus inhibition of taurine degradation by hyperosmotic exposure cannot explain the increase in total culture taurine. These results strongly suggest hyperosmotic exposure leads to increased taurine synthesis. A similar increase in taurine synthesis of astrocytes in situ would contribute to the elevated taurine content observed in brains of animals exposed to systemic hyperosmolality (4, 45). Although intracellular taurine concentrations increase in our cell culture system, a significant portion of the total culture taurine appears in the extracellular fluid, a volume much larger than the intracellular space. Similar efflux of newly synthesized taurine in situ would rapidly elevate the extracellular taurine concentration, thus further limiting cellular taurine loss and increasing cellular reuptake.

Taurine is a major contributor to the increase in total brain amino acids in animal models of hypernatremia (4, 44), as well as in astroglial cultures exposed to hyperosmotic medium (25, 36). This increase in situ may be due to increased synthesis, increased influx, or decreased transport of taurine out of brain parenchyma. In the present studies, total culture taurine content was increased for each hyperosmotic condition, indicating taurine synthesis is accelerated throughout the range of osmolalities tested. We anticipate astroglial taurine synthesis is similarly increased in comparable models of hypernatremia, in which experimental serum osmolalities range from 335 to 400 mosmol/kg (4, 19, 44).

Taurine flux from blood to brain does not increase during acute hyperosmotic hypernatremia (40). Enhanced taurine uptake (6, 36), and decreased taurine efflux by astrocytes in situ (6), may cause intracellular taurine sequestration; however, these changes in cellu-
lar transport cannot directly increase brain taurine content. Increased glial accumulation of taurine may explain the decrease in extracellular taurine concentration described in hyperosmotic animals (18). Depending on the mechanism of brain taurine efflux, this decrease in extracellular taurine concentration may lead to decreased efflux. The contribution that changes in de novo synthesis make to the increase in total brain taurine must await quantitative data regarding the rate of taurine efflux across the blood-brain barrier of normal and hypernatremic animals.

In previous studies, we have shown that enhanced taurine uptake and inhibition of the taurine efflux pathway contribute to elevated intracellular taurine concentrations following prolonged (>24 h) hyperosmotic exposure (6). The results presented here suggest enhanced de novo taurine synthesis also occurs with hyperosmotic exposure. However, activities of key enzymes in the taurine biosynthetic pathway are not altered by hyperosmotic exposure. In addition, no change in CDO activity was measured when the reaction mixture osmolality was raised to match that which the cells experienced during the experimental treatment period, suggesting that enzyme activity is not directly altered by hyperosmotic conditions inside the cell. Although these data indicate that the quantity of enzyme is unaltered by hyperosmolarity, concentrations of substrates or enzymatic cofactors or enzyme phosphorylation states (41) not measured in these studies may be altered by the hyperosmotic treatment and thus may modify taurine biosynthesis in the cell. In particular, elevated intracellular cysteine concentrations caused by cell shrinkage and increased uptake would enhance, by kinetic means, the reaction velocity of CDO.

CDO activity is highly dependent on the concentration of cysteine in the reaction mixture. Thus taurine synthesis may be regulated by the substrate availability from the extracellular space or mobilization of intracellular cysteine pools. Cellular cysteine levels increased from 4.2 to 35.6 nmol/mg protein during hyperosmotic exposure (Fig. 1A), corresponding to intracellular cysteine concentrations ranging from <1 mM in isosmotic culture medium at 0 h to 5.6 mM in cultures treated in 450 mosmol/kg medium for 8 h. From Fig. 3, this elevation in cysteine concentration should increase CDO activity over sixfold. However, the production of intracellular taurine from extracellular cysteine is not increased in hyperosmotically treated cells. Conversion of extracellular [35S]cysteine to [35S]taurine is probably not limited by the cysteine influx rate, since, in all experimental conditions, cysteine uptake is rapid enough to equilibrate the radiolabel with the total intracellular cysteine content within the 60-min reaction period.

Cysteine utilized by CDO may be derived from extracellular or intracellular pools. We found an increased synthesis rate of taurine from extracellular cysteine when the γ-glutamyl cycle was inhibited by BSO and the remaining GSH was eliminated by CDNB, suggesting cysteine derived from GSH catabolism is shunted to taurine biosynthesis. Conversely, cysteine taken up from the extracellular space may be preferentially used by the γ-glutamyl cycle for GSH synthesis or another pathway. However, even with the GSH pathway eliminated, hyperosmotic treatment did not enhance taurine synthesis from extracellular cysteine.

Mechanisms surrounding the transient changes in cysteine and GSH contents in control (300 mosmol/kg) cultures cannot be explained by the results of this study. In previous experiments, we have shown significant decreases in intracellular taurine levels following a medium change (6). This decrease may be due to net efflux into fresh culture medium containing low concentrations of taurine or swelling-induced efflux caused by decreased osmolality of fresh growth medium. The reason for the increase in cysteine and GSH contents after 1 and 8 h is less apparent but may be a cellular response to the medium change. Nonetheless, taurine, cysteine, and GSH levels in control cells all returned to initial values by 24–48 h.

The transport system responsible for cysteine accumulation is not clear from our data, as both a sodium-independent, cystine-specific pathway and a faster sodium-dependent pathway for cysteine transport have been described in cultured astrocytes (8, 35). Although both cysteine and cystine may be present in the influx medium, Bannai (2) indicates that 90% or more of the substrate is present in the reduced form (cysteine) at the pH used during these experiments. Increased uptake in hyperosmotic conditions may be due to activation of one of these transporters, or, alternatively, increased concentrations of NaCl in the hyperosmotic experimental culture medium and influx incubation medium may enhance sodium-dependent cysteine uptake. Sodium dependence may contribute to the osmolality dependence of cysteine uptake at 1 and 8 h of hyperosmotic exposure but cannot account for decreases in accumulation that occur after 8 h. The transient increase in cysteine transport induced by hyperosmolarity is similar to that described for taurine accumulation in these and other cell types (6, 28, 39). Additional experiments are necessary to describe the sodium dependence of cysteine uptake and the mechanisms responsible for increased transport rates in hyperosmotically treated astrocytes.

Elevations in cellular cysteine match the time course of increases in cysteine uptake following exposure to hyperosmotic conditions, suggesting enhanced rates of cysteine uptake directly lead to elevated intracellular cysteine levels. Because accumulation was measured with a high extracellular cysteine concentration, changes in uptake rate represent alterations in the maximal velocity of the transporter. Similar increases in uptake would also be expected at the lower physiological concentrations of cysteine that are likely to occur in situ. However, with the high extracellular concentration of cysteine used in these uptake experiments, any change in uptake due to an alteration in the transporter $K_m$ would not be detected. Sagara et al. (34, 35) and Bannai and Tateishi (3) showed that astrocyte GSH levels depend on the concentrations of cysteine or...
cystine in the culture medium. At all time points, astrocyte GSH contents are at least two times larger than the cellular free cysteine pool. In addition, cystine uptake rates return to control values by 24 h while cellular cystine levels remain elevated. This difference may represent a lag period between the initial accumulation and subsequent metabolism of cysteine to other cellular compounds.

Total γ-GT activity is not altered by exposing astrocytes to hyperosmotic culture conditions, but the enzyme rate of reaction within the cell may be decreased directly by hyperosmolality. These results may explain increased measures in GSH contents and suggest amino acid transport mediated by γ-GT turnover (23) does not contribute to the observed accumulation of amino acids during exposure to hyperosmotic conditions (25). In addition, our data indicate that the increase in cellular cysteine in hyperosmotic astrocytes is not due to enhanced metabolism of GSH. Osmolarity dependence of enzymatic activity also has been described for pyruvate kinase (EC 2.7.1.40), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), and LDH (EC 1.1.1.27), as well as others, and may be related to changes in protein conformation due to altered ionic strength (38, 48). In addition, the synthesis of sorbitol, another organic osmolyte, has been shown to increase in renal cells subjected to increased osmolality due to an enhancement of aldose reductase (EC 1.1.1.21) activity (43).

In summary, the accumulation and maintenance of high levels of intracellular taurine in astrocytes occurs by several mechanisms. We previously reported that rapid taurine accumulation is enhanced by hyperosmotic exposure, whereas high intracellular taurine concentrations are maintained by decreases in taurine efflux (6). The present results suggest intracellular cysteine concentrations regulate taurine synthesis through the kinetic properties of CDO. During hyperosmotic exposure, increased levels of intracellular cysteine are metabolized to GSH and to taurine via CDO. Cellular GSH may be another accessible pool of cellular cysteine in astrocytes as it is in other cell types (11). This mechanism is supported, in part, by the increased rate of taurine synthesis from extracellular cysteine in GSH-depleted cells and the decline of cellular GSH during enhanced taurine synthesis in hyperosmotic cells.

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