Taurine Transport in Cultured Choroid Plexus

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Received December 20, 1996; accepted February 3, 1997

Purpose. Taurine, a β-amino acid, is a neuromodulator which interacts functionally with the glycinergic, GABAergic, cholinergic and adrenergic systems. Although a continuous cell culture model is not available for the choroid plexus epithelia, we recently described a primary cell culture of rabbit choroid plexus epithelia. The goal of the current study was to determine the suitability of this primary cell culture for the study of the Na^+-taurine transporter in the rabbit choroid plexus.
Methods. A primary cell culture of rabbit choroid plexus epithelial cells was grown on semi-permeable filters and kinetics of ^3H-taurine uptake were ascertained.
Results. Taurine transport in the cultured choroid plexus cells was Na^+-dependent and saturable (K_m = 156 μM). The β-amino acids, β-alanine and taurine, significantly inhibited Na^+-driven taurine transport whereas L-alanine partially inhibited taurine transport in the cultured cells. In addition, we observed that the activity of the Na^+-taurine transporter is affected by exposure to taurine in the media.
Conclusions. These results demonstrate that a Na^+-taurine transporter with characteristics similar to those in the intact tissue is expressed in cultured choroid plexus epithelial cells. The transporter may undergo adaptive regulation and play a role in taurine homeostasis in the central nervous system.

KEY WORDS: taurine; transport; choroid plexus; epithelium.

INTRODUCTION

Taurine, a β-amino acid, is critical in the normal development of the cerebellum and the visual cortex, affects the structure and function of the retina (1,2), and is a neuromodulator which has osmoregulatory properties in the brain (3) and the kidney (4).

Taurine is neither synthesized (5,6) nor metabolized to any significant degree in the central nervous system. At physiological pH, taurine is a zwitterion so that its passive diffusion across biological membranes is negligible. Recently, we described a Na^+-dependent taurine transporter in the choroid plexus, the blood-cerebrospinal fluid (CSF) barrier, which may serve as a portal for taurine between the blood and the brain (7). Subsequently, we demonstrated that the taurine transporter in the choroid plexus may be involved in the *in vivo* disposition of taurine in the CSF (8).

In the renal epithelium, a reciprocal relationship exists between the concentration of extracellular taurine and the activity of the Na^+-taurine transporter (9,10). The location of the choroid plexus within the brain suggests that an adaptive response in this tissue may provide an additional mechanism for taurine homeostasis in the central nervous system. The goal of this study was to determine whether a primary cell culture of choroid plexus, recently described in this laboratory (11), is suitable to study the choroid plexus taurine transporter. Our results demonstrate that cultured choroid plexus cells express a Na^+-taurine transporter with characteristics similar to those in intact tissue slices. Initial data suggest that the transporter, like that in the renal epithelium, may undergo adaptive regulation in response to extracellular taurine concentrations.

METHODS AND MATERIALS

Preparation of Choroid Plexus Cell Culture

Choroid plexus epithelial cells were cultured from adult New Zealand white rabbits by methods described previously in detail (11). For studies which examined the effect of taurine pre-exposure on the regulation of the Na^+-taurine transporter activity, the cultures were maintained in serum-free medium, supplemented with various taurine concentrations (0, 50 and 500 μM), from day 6 onwards; uptake was determined 48 hours later (day 8). Cultures enriched in fibroblasts were derived by maintaining cells on solid support for 15 passages. The protein concentration was determined with the Bio-Rad protein assay kit; serum albumin was used as the standard.

Uptake Studies in Choroid Plexus Cell Culture

The uptake of taurine by the primary cell culture of the choroid plexus epithelia was measured 8 days after seeding. Briefly, the monolayers were first washed twice with Na^+-containing buffer. Uptake (room temperature) was initiated by the addition of 200 μl of uptake medium, consisting of 25 μM [^3H]taurine in buffer (Na^+-containing or Na^+-free), to the apical compartment of each filter insert and terminated by aspiration of the medium. The monolayer was immediately rinsed 3 times with ice-cold Na^+-free buffer. The filters were cut out and placed in scintillation vials and the cells were solubilized overnight in 500 μl of Triton X-100 (0.5%). The total radioactivity was determined by liquid scintillation counting. For inhibition studies, various unlabeled compounds (2 mM) were tested for their potential to inhibit 25 μM [^3H]taurine when Na^+ was present. Uptake of taurine in 30 day-old cultures (on filters) and in fibroblastic cells (on solid support; Falcon 12-well cluster dishes) was performed in a manner similar to that described for 8 day-old cultures.

Data Analysis

To normalize the uptake of taurine per mg protein, the protein concentration from an aliquot of solubilized cells was determined with the Bio-Rad Protein Assay Kit, with bovine serum albumin as the standard. For Michaelis-Menten studies,

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the initial rate of taurine uptake is expressed as pmol/mg protein per 15 min and plotted against the concentration of taurine. The 15 min time point was selected because it was the earliest time in which statistically significant Na^+-dependent taurine transport was reproducibly obtained. In addition, the rate of Na^+-dependent taurine transport was not different at 25 min, but was lower at 45 min (data not shown). These data suggest that the uptake at 15 min was in the linear range. The data were fit to the Michaelis-Menten equation with a single, saturable term. The parameters (V_{max}, K_m) were estimated using a non-linear regression program on Minim 1.8 (obtained from Dr. R. D. Purves, University of Otago, New Zealand). Data are presented as mean ± SE; each point represents mean uptake from at least 4 wells. Means were compared using one-way ANOVA, followed by post-hoc Student-Newman-Keul's test; or Students t test, and a probability, $p < 0.05$ was considered significant.

Materials

[^{3}H]Taurine (specific activity: 21.9 Ci/mmol) was obtained from Du Pont-New England Nuclear (Boston, MA). All other chemicals were purchased from Sigma (St. Louis, MO). Cytoscint ES scintillation fluid was obtained from ICN (Irvine, CA). New Zealand White rabbits (2–3 kg) were purchased from Nitabell Rabbitry (Hayward, CA). Transwell-COL filter inserts were obtained from Costar (Cambridge, MA); 12-well cluster Falcon culture dishes were from Beckton Dickinson (Lincoln Park, NJ).

RESULTS AND DISCUSSION

Cultured rabbit choroid plexus cells, grown on filters, form confluent monolayers and express some, but not all, Na^+-coupled transporters found in plasma membrane vesicles and ATP-depleted choroid plexus tissue (11). Recently, we demonstrated the presence of a Na^+-taurine transporter in the choroid plexus (7), which may serve as a route for the passage of taurine between the blood and the brain. In this study, we tested the suitability of the choroid plexus cell culture as a model for taurine transport. Uptake of taurine in choroid plexus primary cell culture in the presence of Na^+ (174 ± 38 pmol/mg protein at 5 min) increased as a function of time until the last measured point (512 ± 27 pmol/ mg protein at 60 min) (Fig. 1). Uptake at 60 min was still rising suggesting that an equilibrium was not attained. Taurine uptake in the presence of Na^+ was consistently higher than that in its absence, consistent with a Na^+-dependent transport mechanism. In a separate experiment we found that the Na^+-dependent uptake of taurine at 15 min at 4° (232 ± 68 pmol/mg protein) was significantly different from the uptake at room temperature (355 ± 28 pmole/mg protein).

The activity of the transporter decreased with days in culture. Thirty-day-old cultures of the choroid plexus (on filters) do not express a Na^+-taurine transporter (data not shown). Choroid plexus cultures, enriched for fibroblasts by repeated subculturing (greater than 10 passages) on solid support, also do not express a Na^+-coupled taurine transporter (data not shown), suggesting that the transporter resides in the epithelial cells.

The rate of uptake of taurine (at 15 min), measured as a function of concentration in the presence of Na^+, increased with increasing substrate concentration and then achieved a plateau, in a manner consistent with saturable kinetics (Fig. 2). The rates of taurine uptake at 150, 200, and 250 μM are not different ($p = 0.193$, ANOVA), suggesting that a plateau rate had been achieved. The estimated V_{max} and K_m were 1827 ± 558 pmol/ mg protein per 15 min and 158 ± 83 μM, respectively. The affinity of the transporter for taurine in the cell culture is similar to that in ATP-depleted choroid plexus pieces (99 μM) (7). This is consistent with a physiological role for this transporter because taurine concentrations in the CSF and plasma are in the micromolar range.

The Na^+-dependent taurine transport in the choroid plexus occurs via a β-amino acid specific transport mechanism (7) and, in this respect, is similar to taurine transport in a number of other tissues, e.g. retina (12), jejunum (13), kidney (14),

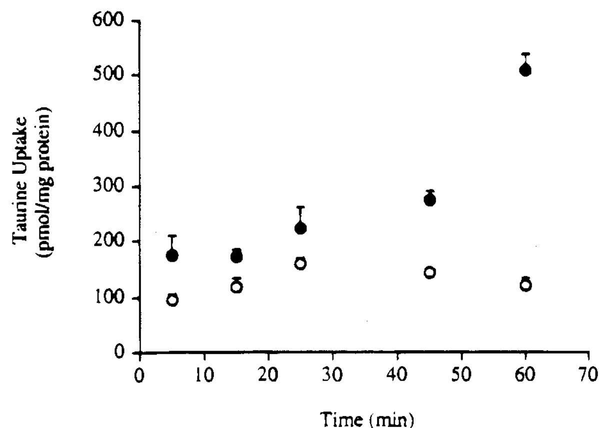

Fig. 1. Time course of [^{3}H]taurine uptake in choroid plexus cell culture in the presence (filled circles) or absence (open circles) of Na^+. Uptake medium containing 25 μM [^{3}H]taurine was added to the apical compartment and taurine uptake was determined. Uptake in the presence of Na^+ was significantly different from that in the absence of Na^+ at all times except 5 min. Data are mean ± SE, n = 4 wells.

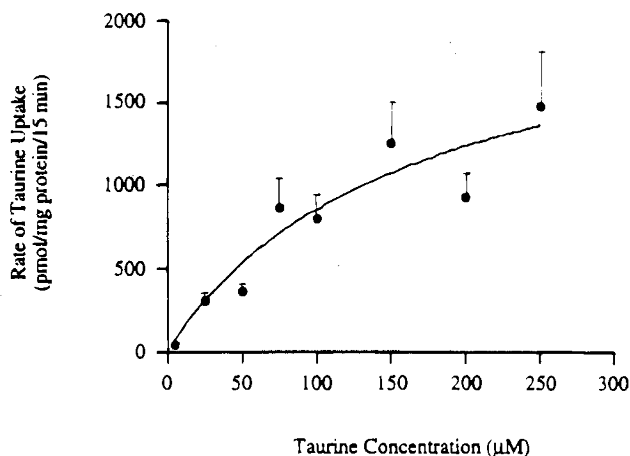

Fig. 2. Concentration dependence of Na^+-dependent taurine uptake in choroid plexus cell culture. Na^+-containing uptake medium (128 mM Na^+), containing different concentrations of [^{3}H]taurine, was added to the apical compartment and taurine uptake (37 C) was determined at 15 min. Data represent mean ± SE (n = 4–5). The estimated V_{max} was 1827 ± 558 pmol/mg protein per 15 min and the K_m was 158 ± 83 μM. The rates of taurine uptake at 150, 200, and 250 μM are not different.

placenta (15) and liver (16) (for a review, see (5)). In the cultured cells, the β-amino acids, β-alanine and taurine, significantly inhibited Na^+-driven taurine transport (28 and 29% of control uptake, respectively, $p < 0.001$) (Fig. 3), suggesting that the specificity of the transporter in the cultured choroid plexus is similar to that in the ATP-depleted choroid plexus tissue pieces (7). Interestingly, L-alanine, which does not inhibit Na^+-taurine transport in ATP-depleted choroid plexus pieces [7], partially inhibits taurine transport in the choroid plexus culture (66% of control uptake, $p < 0.05$) (Fig. 3). The reason is unclear; possibly, some specificity of transport may be lost after 8 days in culture. Alternatively, taurine uptake in cultured cells may differ from that in ATP-depleted tissue, a system in which transporters are driven by experimentally-imposed ion gradients. Regardless, the magnitude of the inhibition by L-alanine is significantly less than that by the β-amino acids (taurine and β-alanine). Collectively, our experiments suggest that an 8 day-old cell culture of the choroid plexus epithelium is an adequate model for the study of taurine transport in the choroid plexus.

In this study, we observed that the taurine level in the culture medium affects the activity of the Na^+-taurine transporter in an adaptive fashion (Fig. 4). Pre-exposure of the cells, for 48 hours, to culture medium containing 0 μM taurine resulted in an enhanced Na^+-dependent taurine uptake (15 min), compared to that following pre-exposure to 50 μM taurine (control, normal *in vivo* serum concentration): 443 ± 46 *vs* 249 ± 7.7 pmol/mg protein per 15 min, respectively ($p = 0.002$) (Fig. 4). On the other hand, pre-exposure of cells to 500 μM taurine resulted in a slight decrease in the mean Na^+-dependent taurine uptake compared to control; however, it did not reach statistical significance (191 ± 46 *vs* 249 ± 8 pmol/mg protein, respectively, $p = 0.06$). Na^+-independent uptake was not affected by pre-exposure of the cells to taurine in the culture medium ($p = 0.428$ by ANOVA) suggesting that the effects of taurine in the medium was on the Na^+-dependent transporter. In separate studies, no difference in taurine uptake was observed following a 1 min exposure of the cells to different taurine concentrations (data not shown). The lack of effect after 1 min exposure suggests that the effect depicted in Fig. 4 is a result of adaptive regulation, rather than direct inhibition of the Na^+-taurine transporter. Adaptive regulation of the Na^+-taurine transporter has been shown to occur *in vivo* in the renal epithelium of rats [17] and *in vitro* in renal cell culture [9,10]. The adaptive response of the choroid plexus Na^+-taurine transporter is similar to that in renal epithelium [9,10,17].

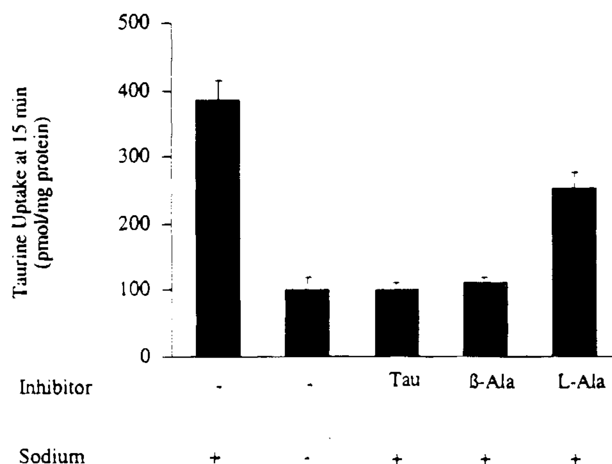

Fig. 3. Inhibition profile of taurine uptake in choroid plexus cell culture. Uptake of taurine (25 μM) was measured, at 15 min, with various unlabeled inhibitors (2 mM) and Na^+ (120 mM) present or absent from the uptake medium, as indicated in the legend. Where Na^+ was absent from the buffer, it was replaced with equimolar K^+. Key: −, absent; +, present; Tau, taurine; β-Ala, β-alanine; L-Ala, L-alanine. The data are the mean ± SE (n = 4).

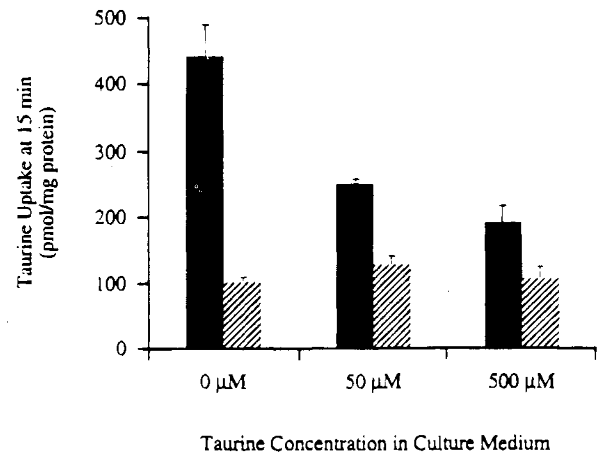

Fig. 4. Effect of taurine pre-exposure on [^{3}H]taurine uptake in choroid plexus cell culture. Cells were incubated in serum-free culture medium containing 0, 50 and 500 μM taurine on Day 6 after seeding. Forty eight hours later, [^{3}H]taurine (25 μM) uptake (15 min) by the culture was measured in the presence (filled bars) or absence (hatched bars) of Na^+. Data are mean ± SE, n = 6 wells.

Previous studies have determined that taurine levels in various regions of the brain are not altered following large, long-term (diet-induced) changes in plasma taurine concentrations (18). Chesney *et al.* (18) ascribed this constancy of brain levels of taurine (in the face of altered plasma concentrations) to the adaptive response of the Na^+-taurine transporter in the kidney. The proximity of the choroid plexus to the surrounding brain tissue, and its possible role in mediating blood-brain transport of taurine, suggest that the adaptive response identified in the choroid plexus cell culture may have physiological consequences. If adaptive regulation does occur *in vivo* in the choroid plexus, it may explain, at least in part, the relative constancy of taurine levels in the CSF and brain.

ACKNOWLEDGMENTS

This work was supported by NIH grant GM 26691.

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