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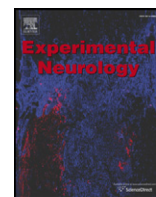

Regular Article

Na^+-dependent transport of taurine is found only on the abluminal membrane of the blood–brain barrier

Hector Rasgado-Flores *, Ashwini Mokashi, Richard A. Hawkins

Department of Physiology & Biophysics, The Chicago Medical School, Rosalind Franklin University of Medicine and Science, 3333 Green Bay Road, North Chicago, IL 60064-3095 USA

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ABSTRACT

Luminal and abluminal plasma membranes were isolated from bovine brain microvessels and used to identify and characterize Na^+-dependent and facilitative taurine transport. The calculated transmembrane potential was −59 mV at time 0; external Na^+ (or choline under putative zero-trans conditions) was 126 mM (T = 25 °C). The apparent affinity constants of the taurine transporters were determined over a range of taurine concentrations from 0.24 μM to 11.4 μM. Abluminal membranes had both Na^+-dependent taurine transport as well as facilitative transport while luminal membranes only had facilitative transport. The apparent K_m for facilitative and Na^+-dependent taurine transport were 0.06 ± 0.02 μM and 0.7 ± 0.1 μM, respectively. The Na^+-dependent transport of taurine was voltage dependent over the range of voltages studied (−25 to −101 mV). The transport was over 5 times greater at −101 mV compared to when V_m was −25 mV. The sensitivity to external osmolality of Na^+-dependent transport was studied over a range of osmolalities (229 to 398 mOsm/kg H_2O) using mannitol as the osmotic agent to adjust the osmolality. For these experiments the concentration of Na^+ was maintained constant at 50 mM, and the calculated transmembrane potential was −59 mV. The Na^+-dependent transport system was sensitive to osmolality with the greatest rate observed at 229 mOsm/kg H_2O.

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Introduction

Brain swelling can be caused by either intracellular ionic overload (e.g., hyperexcitability, hypoglycemia, status epilepticus, ischemia, acute ammonia toxicity, and anoxia) or by decreases in the extracellular ionic concentration (e.g., hyponatremia) (reviewed by Pasantes-Morales and Martin del Rio 1990; Verbalis, 2010). In response to such osmotic disturbances the concentrations of amino acids are altered, notably of taurine (Huxtable 1992). For instance, in hypoosmolar conditions, astrocytes and neurons release taurine to the extracellular fluid (Moran et al. 1992; Schousboe and Pasantes-Morales 1992; Franco et al. 2000; Pasantes-Morales and Tuz 2006) in an effort to decrease intracellular osmolarity and prevent further swelling.

Taurine (amino sulfonic acid) is a zwitterion with both positive and negatively charged groups and an isoelectric point of 5.16 (Huxtable 1992); it is therefore, almost completely negatively charged at pH 7.4. Because it is a zwitterion, taurine is hydrophilic and cannot pass cellular membranes without the assistance of transport pathways. The major metabolic pathway for taurine synthesis occurs in the liver via the cysteine sulfinic acid pathway. Taurine seems to be synthesized primarily by the liver, (Pasantes-Morales et al. 1980) entering and leaving the brain via transporters (Urquhart et al. 1974; Tamai et al. 1995; Kido et al. 2002). Taurine is non-toxic and its primary function seems to be involved in maintaining osmotic balance.

The brain capillaries of vertebrates are almost completely covered by cellular membranes, collectively known as the blood–brain barrier (BBB), through which metabolites must pass to enter or leave. Extensive networks of tight junctions separate the membranes of cerebral capillary endothelial cells into luminal (blood-facing) and abluminal (brain-facing) sides: hence they are polarized (Hawkins et al. 2006). Therefore, two membranes must be crossed to gain entrance or egress from the interstitial fluid (ICF) of the brain.

Hydrophilic nutrients, such as taurine and other amino acids, require the presence of carriers in both the luminal and abluminal membranes to traverse the BBB. We developed techniques to separate luminal and abluminal membranes, which allows the study of each side of the BBB independent of the other (Hawkins et al. 2002; Hawkins et al. 2006). The purpose of this study was to characterize the taurine transport systems in each membrane.

Materials and methods

Materials

[^{3}H]Taurine (20 Ci/mM) was from American Radiolabeled Chemicals, Inc. (St. Louis, MO). Collagenase Type IA and Protease Inhibitor cocktail were from Sigma (St. Louis, MO).

* Corresponding author. Fax: +1 847 775 6510.
E-mail address: Hector.Rasgado@rosalindfranklin.edu (H. Rasgado-Flores).

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Animals

Fresh bovine brains were bought from Strauss Veal & Lamb International Inc. (Franklin, WI). The cows were killed for food under USDA supervision (establishment number 2444) and the brains sold for human consumption.

Isolation and characterization of membrane vesicles

Membrane vesicles from the brain endothelial cells were prepared as previously described (Sánchez del Pino et al. 1992). Briefly, isolated microvessels from bovine cerebral cortices were obtained by the method of (Pardridge, et al. 1985). The microvessels were digested with collagenase Type IA to separate the basement membrane, pericytes, and glial fragments, and centrifuged at 5000 *g* for 10 min. Pericytes and other debris remained in suspension. The digested microvessels were suspended in buffer (10 mM Tris, 0.1 mM EDTA, 250 mM sucrose, 0.5 mM $MgCl_2$ and 0.1 ml/l Protease Inhibitor Cocktail) and homogenized with an IKA-Euroturrax® T-20 Standard (Wilmington, NC) for 45 s at setting 6. The homogenized capillaries were centrifuged at 5000 *g* for 15 min to remove unbroken cells and other large fragments. The supernatant was centrifuged at 20,000 *g* for 60 min to pellet the plasma membranes. Luminal and abluminal membranes were separated by centrifugation (175,000 *g* for 2.5 h) on a discontinuous Ficoll gradient (0 and 5%) (Sánchez del Pino et al. 1992). Luminal membranes accumulated at the interface while abluminal membranes formed pellets. Luminal and abluminal membranes were about 90% and 80% pure, respectively. The marker used for abluminal membranes was MeAIB (methylamino isobutyric acid) a marker of the system A amino acid transport system that is located exclusively on the abluminal side. The marker for luminal membranes was λ-glutamyl transpeptidase that is located only at the luminal membrane (Sánchez del Pino et al., 1995a, 1995b). The luminal membranes were passed over the Ficoll gradient for a second time resulting in a fraction that had no detectable contamination by abluminal membranes as judged by undetectable MeAIB uptake. The membranes were stored in aliquots at −70 °C.

Protein determination

Protein concentrations were measured using the Bio-Rad Protein Microassay (Hercules, CA), with bovine serum albumin as the standard (Bradford 1976).

Measurement of transport rates

Rates of taurine transport were measured by rapid filtration (Sánchez del Pino et al., 1992). Aliquots of membrane vesicles were thawed, centrifuged at 30,000 *g* for 30 min at 4 °C and suspended in 140 mM KCl, 20 mM HEPES, pH 7.4 containing valinomycin (12.5 μg/mg protein), a K^+-specific ionophore, that increases the vesicular membrane permeability to K^+ (Cammann 1985). The vesicles were allowed to equilibrate overnight at 4 °C. The final concentration of protein was between 5 and 10 μg protein/μl. The vesicle suspensions were divided into 10 μl aliquots that were pre-incubated at 25 °C for 1 min before initiation of transport measurements.

Except as indicated otherwise, the taurine transport measurements were initiated by the addition of 90 μl of reaction solution (140 mM NaCl and 20 mM HEPES, pH 7.4 at 25 °C) containing [^{3}H] taurine to 10 μl of vesicle suspension. The extravesicular NaCl at the start of the measurements was 126 mM and the internal concentration was nominally 0. A transmembrane potential was created by a 10:1 ratio of internal (140 mM) and external (14 mM) concentrations of K^+; this was calculated by the Nernst equation to be −59 mV (inside negative) at 25 °C (Hille 1992). Since in the presence of valinomycin the membrane potential and the reversal potential for K^+ were expected to be nearly identical, the transmembrane gradient for K^+ was expected to remain fairly constant. Facilitative transport was determined by substituting 140 mM choline$^+$ for Na^+ while maintaining the K^+ concentration gradient constant.

Reactions were stopped by adding 1 ml of stopping solution (145 mM NaCl and 10 mM HEPES, pH 7.4 at 4 °C) and filtered on a 0.45 μm Millipore membrane filter (Fisher, Pittsburg, PA) under vacuum. The filtered membranes were washed three times with 1 ml aliquots of stopping solution, after which the filters were counted by liquid scintillation spectroscopy. All dpm were corrected for non-specific transport, binding or trapping as measured at time 0.

Calculation of clearance

All measurement of taurine uptake were expressed as permeability-to-surface area products (clearance), in units of μl × mg protein^{-1} × min^{-1} according to the following equation

clearance = dpm per mg vesicle per min ÷ dpm per μl^{-1} of incubation solution. This is equivalent to the permeability × surface area ratio and reflects the μl of solution that would have been "cleared" of taurine (21).

Time course of taurine uptake

[^{3}H]Taurine uptake was measured in luminal and abluminal vesicles over time and the data fitted by non-linear regression analyses (Sigma Plot, SPSS, Chicago, IL) to the equation: uptake = a $(1-e^{-kt})$. The products of the constants a and k is considered the initial rate. Facilitative transport was measured by substituting choline mol-for-mol for NaCl.

Kinetic constants of taurine transport

The initial rate of [^{3}H]taurine (0.24 μM) uptake was measured in abluminal membranes. Non-radioactive taurine was used to create a range of concentrations up to 11.4 μM. The data were expressed as clearance in μl × mg protein^{-1} x min^{-1}. Non-linear regression analyses were used to fit the data to the equation $v/S = V_{max}/(S + K_m)$ to determine the kinetic constants (S = taurine concentration, V_{max} = maximal velocity and $K_m = V_{max}/2$).

Effect of osmolality on taurine transport

Solutions of varying osmolalities were used, each with 0.43 μM [^{3}H]taurine, 50 mM NaCl or 50 mM choline Cl and 10 mM HEPES buffer. The final pH of all solutions was 7.4. Osmolalities were measured with a vapor pressure osmometer (Westcor, Logan, Utah). Mannitol was added to adjust the following values 229, 297, 348 and 398 mOsm/kg H_2O. The vesicles were pre-loaded with 140 mM KCl and valinomycin as described above. The reaction was initiated by adding 90 μl of solution to 10 μl of vesicle suspension and stopping the reaction at 15 s. The calculated transmembrane potential was −59 mV at a temperature of 25 °C.

Transmembrane potential effect on transport

To create different initial transmembrane potentials, appropriate ratios of internal-to-external concentrations of K^+ were used to create calculated potentials from −28.2 mV to −101 mV. The reaction was initiated by the addition of an appropriate volume of solution to create the desired initial transmembrane potential as calculated by the Nernst equation.

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