Taurine Uptake in Apical Membrane Vesicles From the Bovine Retinal Pigment Epithelium

Yusei Miyamoto, Palaniappan Kulanthaivel, Frederick H. Leibach, and Vadivel Ganapathy

Characteristics of taurine uptake were investigated in apical membrane vesicles isolated from bovine retinal pigment epithelium. Uptake of taurine into these vesicles was stimulated markedly by the presence of an inwardly directed NaCl gradient across the membrane. The time course of the NaCl gradient-dependent uptake showed an overshoot, indicating a transient accumulation of taurine in the vesicles against a concentration gradient. Monovalent cations other than Na⁺ did not support taurine uptake. There was also an anion requirement for the uptake system, and Cl⁻ was the most potent among several monovalent anions tested. The NaCl-dependent taurine uptake was stimulated by inside-negative, K⁺, and H⁺ diffusion potentials, demonstrating the electrogenic nature of the system. Stoichiometric analysis revealed that two Na⁺ and one Cl⁻ ions were involved in the translocation of one taurine molecule. The NaCl-dependent taurine uptake was stimulated by excess amounts of unlabeled taurine, hypotaurine, β-alanine, and γ-aminobutyric acid. The relative potencies of the amino acids to inhibit taurine uptake were compared in three tissues, the bovine retinal pigment epithelium, the rat kidney, and the human placenta. In the apical membrane vesicles of the pigment epithelium, γ-aminobutyric acid was many times more potent than taurine itself in inhibiting radiolabeled taurine uptake. However, in the brush border membrane vesicles of the kidney and the placenta, taurine was many times more potent than γ-aminobutyric acid. It was concluded that the apical membrane of the retinal pigment epithelium has an uptake system for taurine and that this system is different from the uptake systems available for taurine in the placental and the renal brush border membranes.

The biologic importance of taurine in the retina has been recognized ever since the original observation that taurine deficiency in cats results in retinal degeneration and blindness.¹² Several roles for taurine in the retina were suggested, including antioxidation, membrane stabilization, and osmoregulation.³⁻⁴ Taurine is the most abundant free amino acid in the vertebrate retina; its intracellular concentration of 10⁻⁵⁰ mM was reported in retinal tissue.⁵⁻⁶ The retina is composed of several types of cells, and among them, taurine is most highly concentrated in the pigment epithelium and the photoreceptor cells.⁵⁻⁷⁻⁸ The high concentrations of taurine in the retinal cells are maintained by endogenous synthesis⁹⁻¹⁰ and active uptake from the choroidal blood and/or vitreous humor. The pigment epithelium, interposed between the choroid and the neural retina, plays a pivotal role in the maintenance of taurine levels in the retinal cells.

The retinal pigment epithelium is a polarized cell whose plasma membrane consists of two distinct regions, an apical membrane facing the neural retina and a basolateral membrane facing the choroid. It has been shown in vivo that radiolabeled taurine injected into blood first accumulates in the pigment epithelium and then is transferred to the neural retina.¹¹⁻¹³ A net transfer of taurine across the pigment epithelium in the neural retina-to-choroid direction also was reported.¹⁴⁻¹⁵ These studies suggest that active transport mechanisms for taurine should be present in both poles of the plasma membrane of the pigment epithelium. There have been numerous investigations on the characteristics of taurine uptake in the pigment epithelium, using tissue fragments and cultured cells.¹⁴⁻¹⁶⁻⁻¹⁹ These studies have limitations, however, owing to the polarized nature of the cell. It is not known at present whether the uptake mechanisms available for taurine in the apical and the basolateral membranes are similar or distinct. Because the uptake measurements made with isolated cells and tis-
sue fragments cannot distinguish the activity of the apical membrane uptake system from that of the basolateral membrane uptake system, it is difficult to assess the physiologic relevance of the taurine uptake characteristics observed in these studies. To obtain a clear understanding of the processes involved in the handling of taurine by the pigment epithelium under physiologic conditions, it is essential to study differentially the properties of taurine uptake occurring across the two poles of the plasma membrane. As an ideal means toward this goal, isolated plasma membrane vesicles consisting of predominantly either the apical or the basolateral membrane can be used to investigate taurine uptake. We describe isolation of apical membrane vesicles from the bovine retinal pigment epithelium and use these vesicles to investigate the characteristics of taurine uptake across this membrane.

Materials and Methods

We purchased 3H-taurine (specific radioactivity, 20.1 Ci/mmol) and γ-32P-adenosine triphosphate (ATP, radioactivity, 2 mCi/ml) from Du Pont-New England Nuclear (Boston, MA). Glycylproline p-nitroanilide was a gift from Professor A. Barth, Martin Luther University, Halle-Saale, Germany. Valinomycin and unlabeled amino acids were obtained from Sigma (St. Louis, MO), and carbonylcyanide p-trifluoromethoxyphenylhydrazone (FCCP) was from Aldrich (Milwaukee, WI). All other chemicals were of analytic grade.

Preparation of Apical Membrane Vesicles From the Retinal Pigment Epithelium

The apical membrane vesicles from the bovine retinal pigment epithelium were isolated as described previously, with some modifications. Two to three dozen bovine eyes, obtained fresh from a local slaughterhouse, were used for each preparation. The eyes were placed in ice-cold Krebs-Ringer’s solution and kept in the dark for 30 min to allow the retinal pigment epithelium to detach from the neural retina. All following steps were done at 4°C. The eyes were cut in half, and the pigment epithelium was collected. The collected tissue (0.43 ± 0.01 g/eye in wet weight, n = 13) was minced with scissors, and a 5% homogenate was made in 2.4 mM Tris/NaOH buffer (pH 7.1), containing 60 mM mannitol and 1 mM ethylene bis(oxyethylenenitrilo)tetraacetic acid (EGTA). Homogenization was done in a Waring blender (Waring Products Corp., New York, NY) for 1.5 min at high speed. A stock solution of MgCl2 (1 M) was added to the homogenate to give a final concentration of 30 mM. The mixture was stirred for 1 min and let stand for 15 min. After this it was centrifuged at 2500 g for 15 min. The supernatant was collected by filtering through 12 layers of cheesecloth and subjected again to centrifugation at 2500 g for 15 min. The resultant supernatant, containing the apical membranes, was centrifuged at 46,000 g for 45 min to pellet the membranes. The pellets were suspended in a desired preloading buffer using a 25-gauge needle. The preloading buffer in most experiments was 300 mM mannitol, buffered with 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)/Tris (pH 7.5). The membrane suspension was again centrifuged at 46,000 g for 45 min and the resulting pellets suspended in a small volume of the same preloading buffer. The protein concentration of the membrane suspension was adjusted to 5 mg/ml. The protein yield was 0.33 ± 0.02 mg/eye (n = 13). The membrane vesicles were stored in small aliquots in liquid nitrogen until use.

The major difference between our procedure and that of Zadunaisky et al was the selection of the divalent cation used to aggregate the nonapical membranes. The original procedure used Ca2+ for this purpose. In our method, Ca2+ was replaced by Mg2+ as the aggregating cation. This modification was prompted by a recent report that the membrane vesicles prepared by the Ca2+-aggregation method normally showed higher permeability for monovalent cations compared with those prepared by the Mg2+-EGTA-aggregation method and also by our own observation that the taurine transport activity measured in isolated membrane vesicles is reduced markedly by the exposure of the vesicles to Ca2+.

Preparation of Renal and Placental Brush Border Membrane Vesicles

The brush border membrane vesicles from the kidneys of F344 rats were prepared by a procedure similar to the one we previously described for the rabbit kidney. The method used Mg2+ as the aggregating cation in the presence of EGTA. The brush border membrane vesicles from normal-term human placentas were isolated as described previously except that EGTA (2 mM) was included in the membrane isolation buffer.

Assessment of the Membrane Purity

The purity of the apical membranes prepared from the bovine retinal pigment epithelium was assessed by the enrichment of Na, K-adenosine triphosphatase (Na, K-ATPase), a well-established marker for this membrane, and of alkaline phosphatase and 5'-nucleotidase, markers for plasma membrane. The activity of Na-K-ATPase was assayed with γ-32P-ATP as the substrate. Alkaline phosphatase was measured...
by the liberation of p-nitrophenol from p-nitrophenyl phosphate in 50 mM glycine/NaOH buffer (pH 9.2) in the presence of 5 mM MgCl₂. The activity of 5'-nucleotidase was measured by the release of P₁ from adenosine monophosphate in 50 mM Tris/HCl buffer (pH 8.5), containing 5 mM MgCl₂. In addition, we also tested the possibility of using dipeptidylpeptidase IV (DPP IV), an enzyme associated specifically with the apical membrane of intestinal and renal tubular cells, as a marker for the apical membrane of the retinal pigment epithelium. The activity of DPP IV was determined using glycylproline p-nitroanilide as a chromogenic substrate.

Uptake Measurements

Uptake of taurine in membrane vesicles was measured at room temperature (22–23°C) by a rapid filtration technique using Millipore filters (DAWP type, 0.65-μm pore size; Millipore Corp., Bedford, MA). Uptake was initiated by mixing 40 μl of membrane suspension (200 μg of protein) with 160 μl of uptake buffer containing 3H-taurine. The uptake buffer in most experiments was 10 mM HEPES/Tris (pH 7.5), containing 150 mM NaCl. Uptake was terminated by the addition of 3 ml ice-cold stop buffer (5 mM HEPES/Tris, 155 mM KCl, pH 7.5). The mixture was filtered and the filter washed with three changes of 5 ml each of the stop buffer. The filter then was transferred to a counting vial, and the radioactivity associated with the filter was determined by liquid scintillation spectrometry.

Statistics

Uptake measurements were done in duplicate or triplicate, and the variation among the replicate values was always less than ±10% of the mean value. Each experiment was done with two or more different membrane preparations. The results are expressed as means ± the standard error. Kinetic analyses were done using the computer package Statgraphics (STSC, Rockville, MD). Statistical analysis was done using the student t-test, and a P value < 0.05 was considered significant.

Results

Purity of the Apical Membrane Preparation From the Bovine Retinal Pigment Epithelium

The activities of marker enzymes, Na, K-ATPase, alkaline phosphatase, and 5'-nucleotidase, were measured in the homogenate and apical membranes prepared from the bovine retinal pigment epithelium. All three enzymes were enriched in the apical membrane preparation by about 12-fold compared with the homogenate (Table 1). These values were comparable to the corresponding values reported by Zadunaisky et al. for the apical membranes from the dog fish retinal pigment epithelium. The modifications introduced in our method by substituting Ca²⁺ with Mg²⁺ as the aggregating cation and by including EGTA in the homogenizing buffer to chelate endogenous Ca²⁺ did not compromise the purity of the apical membrane vesicles. In addition to these three enzymes, we also measured the activity of DPP IV in our preparations. This enzyme is associated with the plasma membrane in a number of nonpolarized cells, but in polarized cells such as intestinal and renal tubular cells, it is associated with the apical membrane specifically. The enrichment factor calculated for DPP IV in the apical membrane preparations from the bovine pigment epithelium was found to be comparable (about tenfold) to the values calculated for the other two marker enzymes (Table 1). Therefore, DPP IV appears to qualify as one of the marker enzymes for the apical membrane of the pigment epithelium.

Table 1. Enzyme activities in the original homogenate and the isolated apical membrane (the results represent means ± SE for three–five determinations)

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Homogenate</th>
<th>Apical membrane</th>
<th>Enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na-K-ATPase</td>
<td>0.10 ± 0.02</td>
<td>1.12 ± 0.16</td>
<td>11.2</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>1.08 ± 0.12</td>
<td>12.84 ± 1.50</td>
<td>11.9</td>
</tr>
<tr>
<td>5'-Nucleotidase</td>
<td>3.24 ± 0.42</td>
<td>41.22 ± 5.22</td>
<td>12.7</td>
</tr>
<tr>
<td>DPP IV</td>
<td>0.29 ± 0.01</td>
<td>3.01 ± 0.39</td>
<td>10.4</td>
</tr>
</tbody>
</table>

Time Course of Taurine Uptake

Figure 1 describes the time course of taurine uptake (0.1 mM) in the apical membrane vesicles isolated from the bovine retinal pigment epithelium. In this experiment, the vesicles were preloaded with 10 mM HEPES/Tris buffer (pH 7.5), containing 300 mM mannitol, and uptake was measured from an isosmolar medium containing 120 mM of either NaCl or choline chloride. Uptake of taurine from the NaCl-containing medium was rapid, and the time course of the uptake showed an overshoot (uptake at 5–10 min was many times greater than the uptake at 150 min), indicating a transient accumulation of taurine in the vesicles against a concentration gradient. However, uptake of taurine from the choline chloride-containing medium was slow, and there was no evidence for a concentrative uptake. Comparison of the initial uptake rates measured at 30 sec from both media re-
The results are given as means with bars representing SE for four determinations in two different membrane preparations. When not shown, the error bars lie within the symbol.

Table 2. Effects of inorganic monovalent cations and anions on taurine uptake*

<table>
<thead>
<tr>
<th>Inorganic salt</th>
<th>Taurine uptake (pmol/mg protein/30 sec)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>1.073 ± 0.227</td>
<td>100</td>
</tr>
<tr>
<td>LiCl</td>
<td>0.096 ± 0.009</td>
<td>9</td>
</tr>
<tr>
<td>KCl</td>
<td>0.090 ± 0.004</td>
<td>8</td>
</tr>
<tr>
<td>RbCl</td>
<td>0.075 ± 0.010</td>
<td>7</td>
</tr>
<tr>
<td>CsCl</td>
<td>0.085 ± 0.012</td>
<td>8</td>
</tr>
<tr>
<td>NaF</td>
<td>0.110 ± 0.018</td>
<td>10</td>
</tr>
<tr>
<td>Na gluconate</td>
<td>0.090 ± 0.008</td>
<td>8</td>
</tr>
<tr>
<td>NaSCN</td>
<td>0.734 ± 0.083</td>
<td>68</td>
</tr>
<tr>
<td>NaN3</td>
<td>0.317 ± 0.045</td>
<td>30</td>
</tr>
</tbody>
</table>

* Apical membrane vesicles were suspended in 300 mM mannitol, buffered with 10 mM HEPES/Tris, pH 7.5. The uptake buffer was 10 mM HEPES/Tris, pH 7.5, containing 150 mM of the respective inorganic salt. Taurine uptake from a 0.2 µM solution was measured for 30 sec. The results are given as means ± SE for four determinations.
Incubation time (min)

Fig. 2. Effect of an inside-negative, K+-diffusion potential on taurine uptake. The apical membrane vesicles were preloaded with 300 mM mannitol and 100 mM K-glucuronate, buffered with 10 mM HEPES/Tris, pH 7.5. The uptake buffer was 10 mM HEPES/Tris, pH 7.5, containing 150 mM NaCl and either 100 mM K-glucuronate (○) or 200 mM mannitol (●). Valinomycin was added to the uptake buffer to give a concentration of 5 nM during uptake measurements. The concentration of taurine was 0.1 μM. The results are given as means with bars representing SE for four determinations in two different membrane preparations.

brane potential in the presence of an outwardly directed K+ gradient. Figure 2 shows that the uptake of taurine was markedly (about twofold) stimulated by an inside-negative membrane potential.

We also studied the effect of an inside-negative H+ diffusion potential on taurine uptake. In this experiment, the membrane vesicles were preloaded with 25 mM 4-morpholineethanesulfonic acid/Tris buffer (pH 5.5), containing 300 mM mannitol. Uptake of 0.1 μM taurine was measured with a 1-min incubation from a pH 7.5 buffer (25 mM HEPES/Tris), containing 150 mM NaCl in the presence and absence of 10 μM FCCP. In the presence of an outwardly directed H+ gradient (pH$_{i}$ = 5.5; pH$_{o}$ = 7.5), FCCP, a H+ ionophore, is expected to generate an inside-negative H+ diffusion potential. Taurine uptake was found to be stimulated significantly by FCCP under these conditions (2.52 ± 0.01 pmol/mg of protein · min in the presence of FCCP versus 1.82 ± 0.02 pmol/mg of protein · min in the absence of FCCP, $P < 0.001$).

These results suggest that the uptake of taurine in the presence of an inwardly directed NaCl gradient is an electrogenic process and that the transport of taurine is associated with a net transfer of positive charge into the vesicles.

Stoichiometry

Taurine exists predominantly as a zwitterion with no net electrical charge at pH 7.5. That the NaCl-dependent taurine uptake is associated with a net flux of positive charge into the vesicles indicates that the ratio between the number of Na$^+$ and Cl$^-$ ions cotransported with one taurine molecule is greater than one. To determine the exact number of each ion associated with the transport of one taurine molecule, we studied the dependence of the initial rates of taurine uptake on the concentration of Na$^+$ and Cl$^-$. This approach to determine the stoichiometry of the cotransported ions is called the “activation method.” Figure 3 describes the influence of Na$^+$ concentration on the 15-sec uptake rate of taurine. In this experiment, the Na$^+$ concentration varied from 0–160 mM, but the Cl$^-$ concentration was maintained constant at 160 mM. When the Na$^+$-dependent uptake of taurine was plotted against the Na$^+$ concentration, a sigmoidal curve was obtained (inset), suggesting that more than one Na$^+$ ion was cotransported with one taurine molecule. A Hill-type equation was used to estimate the number of Na$^+$ ions

$$v = \frac{V_{\text{max}}[\text{Na}^+]^n}{K_n(\text{Na}^+) + [\text{Na}^+]^n}$$

involved in the process. In this equation, $v$ is the initial rate of taurine uptake and $n$ is the number of Na$^+$ ions. The plot of $v$ versus $v/[\text{Na}^+]^n$ was found to be a straight line ($r = -0.96$), only when $n$ was assigned a value of 2. The uptake rates at different concentrations of Na$^+$ with $n = 1, 2, 3$ were calculated theoretically from the equation using a value of 71.4 mM for the $K_n$ (Na$^+$) and a value of 2.02 pmol/mg of protein over 15 sec for the $V_{\text{max}}$. These uptake rates were used to construct theoretic curves describing the relationship between the uptake rate and the Na$^+$ concentration (inset). The experimentally determined uptake rates coincided with the theoretic values only when $n$ was equal to 2. These data indicate that two Na$^+$ ions are cotransported with each taurine molecule.

The number of Cl$^-$ ion associated with the transport of each taurine molecule also was determined using a similar approach, in which the initial rates of taurine uptake were measured at varying concentrations of Cl$^-$ (range, 0–160 mM) but at a constant Na$^+$ concentration (160 mM). The plot depicting the relationship between the uptake rate and the Cl$^-$ concen-
Fig. 3. Dependence of taurine uptake on Na⁺ concentration. The apical membrane vesicles were preloaded with 400 mM mannitol, buffered with 10 mM HEPES/Tris, pH 7.5. Uptake of 0.4 μM taurine was measured with a 15-sec incubation in 10 mM HEPES/Tris buffer, pH 7.5, containing varying concentrations of Na⁺ (0-160 mM). The concentration of Cl⁻ was maintained constant at 160 mM by substituting LiCl for NaCl. Only the Na⁺-dependent uptake rates (uptake rate in the presence of Na⁺ minus uptake rate in the absence of Na⁺) were used in the data analysis. The insert describes the dependence of taurine uptake on Na⁺ concentration. The insert also gives the three theoretical curves expected for a Hill coefficient (n) of 1, 2, or 3. (K_m(Na⁺) = 71.4 mM; V_max = 2.02 pmol/mg of protein × 15 sec). The Hill-type plot (v versus v/[Na⁺]n) was a straight line (r = -0.96) when n was assigned a value of 2. The results are given as means ± SE for four determinations in two membrane preparations. v, uptake rate in pmol/mg of protein × 15 sec.

Fig. 4. Dependence of taurine uptake on Cl⁻ concentration. The apical membrane vesicles were preloaded with 400 mM mannitol, buffered with 10 mM HEPES/Tris, pH 7.5. Uptake of 0.4 μM taurine was measured with a 15-sec incubation in 10 mM HEPES/Tris buffer, pH 7.5, containing varying concentrations of Cl⁻ (0-160 mM). The concentration of Na⁺ was maintained constant at 160 mM by substituting Na gluconate for NaCl. Only the Cl⁻-dependent uptake rates (uptake rate in the presence of Cl⁻ minus uptake rate in the absence of Cl⁻) were used in the data analysis. The insert describes the dependence of taurine uptake on Cl⁻ concentration. The Hill-type plot (v versus v/[Cl⁻]n) was a straight line (r = -0.99) when n was assigned a value of 1. Therefore, the number of Cl⁻ ion that is cotransported with one taurine molecule appears to be 1. From these data, it can be concluded that the stoichiometry of Na⁺:Cl⁻:taurine for the uptake system is 2:1:1.

Kinetics of Taurine Uptake

The dependence of the initial uptake rate on the concentration of taurine then was studied. The initial uptake rates were determined using a 15-sec incubation. Figure 5, inset, shows that a 15-sec incubation was suitable to measure the initial uptake rate. When the rates were determined at varying concentrations of taurine (range, 10-200 μM) in the presence of an inwardly directed NaCl gradient ([NaCl]₀ = 200 mM; [NaCl]₁ = 0), it was found that the plot of taurine concentration versus initial uptake rate was hyperbolic (not shown), indicating the saturability of the uptake system. An Eadie-Hofstee plot (initial uptake rate/taurine concentration versus initial uptake rate) of the data gave a straight line (r = -0.99), suggesting that a single system is involved in the uptake of taurine in these membrane vesicles. The apparent dissociation constant, K_d, for taurine was 96 ± 17 μM, and the maximal velocity, V_max, was 267 ± 24 pmol/mg of protein over 15 sec.
Substrate Specificity

The specificity of the uptake system was studied by investigating the ability of various unlabeled amino acids to compete with radiolabeled taurine for the uptake process. Uptake of 0.2 μM radiolabeled taurine was measured with a 30-sec incubation in the presence and absence of 100 μM of unlabeled amino acids. The uptake of radiolabeled taurine was inhibited markedly by taurine, β-alanine, hypotaurine (all three are β-amino acids), and γ-aminobutyric acid (Table 3). We also studied the effects of four α-amino acids on the uptake of radiolabeled taurine: α-aminoisobutyric acid (a specific substrate for the A system), α-alanine (a specific substrate for the ASC system), leucine (a specific substrate for the L system), and proline, an imino acid. The first three amino acids did not inhibit the uptake of taurine, indicating that the amino acid transport systems A, ASC, and L are not involved in the uptake of taurine in the apical membrane vesicles from the pigment epithelium. Proline caused a moderate inhibition of taurine uptake. Thus, the uptake system for taurine in these membrane vesicles appears to accept only the β- and γ-amino acids as primary substrates.

Comparison of the Substrate Affinities of the Taurine Uptake System in the Retinal Pigment Epithelium, the Kidney, and the Placenta

A specific transport system serving β-amino acids such as taurine was well characterized in the brush border membranes isolated from the kidney, the small intestine, and the placenta. In these tissues, the uptake system has higher affinity for taurine than for β-alanine. The affinity of the system for γ-aminobutyric acid, if any, is very small. The extent of inhibition of the uptake of radiolabeled taurine by unlabeled taurine, β-alanine, and γ-aminobutyric acid in the apical membrane vesicles from the retinal pigment epithelium indicates that the system has higher affinity for β-alanine and γ-aminobutyric acid than for taurine (Table 3). To compare the substrate affinities of the taurine uptake system in the pigment epithelium, the kidney, and the placenta, we studied the dose-dependent inhibition of radiolabeled taurine uptake by unlabeled taurine, β-alanine, and γ-aminobutyric acid under identical experimental conditions using apical membrane vesicles from the bovine retinal pigment epithelium and brush border membrane vesicles from the rat kidney and the human placenta. The data are given in Figure 6. The potency of inhibition in the pigment epithelium was in the following order: γ-aminobutyric acid > β-alanine > taurine.

Table 3. Effect of amino acids on taurine uptake*

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Taurine uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pmol/mg protein/30 s</td>
</tr>
<tr>
<td>Control</td>
<td>1.324 ± 0.124</td>
</tr>
<tr>
<td>Taurine</td>
<td>0.579 ± 0.051</td>
</tr>
<tr>
<td>Hypotaurine</td>
<td>0.119 ± 0.021</td>
</tr>
<tr>
<td>β-Alanine</td>
<td>0.178 ± 0.012</td>
</tr>
<tr>
<td>γ-Aminobutyric acid</td>
<td>0.430 ± 0.051</td>
</tr>
<tr>
<td>α-Aminoisobutyric acid</td>
<td>1.400 ± 0.092</td>
</tr>
<tr>
<td>α-Alanine</td>
<td>1.301 ± 0.102</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.379 ± 0.115</td>
</tr>
<tr>
<td>Proline</td>
<td>1.013 ± 0.135</td>
</tr>
</tbody>
</table>

* Taurine uptake was measured for 30 sec in the presence of a NaCl gradient. The concentration of radiolabeled taurine during uptake was 0.2 μM. The concentration of unlabeled amino acids was 100 μM. The results are given as means ± SE for four determinations.
Fig. 6. Dose-dependent inhibition of radiolabeled taurine uptake by taurine, β-alanine, and γ-aminobutyric acid in apical membrane vesicles of the bovine retinal pigment epithelium and in brush border membrane vesicles of the rat kidney and the human placenta. The membrane vesicles from the bovine retinal pigment epithelium (a), the rat kidney (b), and the human placenta (c) were preloaded with 300 mM mannitol, buffered with 10 mM HEPES/Tris, pH 7.5. The uptake buffer was 10 mM HEPES/Tris, pH 7.5, containing 150 mM NaCl. Uptake of radiolabeled taurine (0.2 μM) was measured with a 30-sec incubation. The concentrations of unlabeled taurine (●), β-alanine (©) and γ-aminobutyric acid (○) were varied from 0 to 25 μM. The results are given as means ± SE for four determinations in two different respective membrane preparations.

However, the order of the inhibitory potency was different in the kidney and the placenta (taurine > β-alanine > γ-aminobutyric acid). These results indicate that the uptake system responsible for taurine uptake in the pigment epithelial apical membrane vesicles is distinct from the uptake system for this amino acid in the brush border membrane of the kidney and the placenta.

Discussion

We investigated the characteristics of taurine uptake in the apical membrane vesicles isolated from the retinal pigment epithelium of bovine eyes. Our conclusions can be summarized as follows: (1) taurine uptake in these vesicles is energized by an inwardly directed NaCl gradient but not by a Na+ gradient alone, (2) two Na+ ions and one Cl− ion are cotransported with one taurine molecule, rendering the uptake system electrogenic, (3) the uptake system accepts the other β-amino acids such as hypotaurine and β-alanine, as well as γ-aminobutyric acid as substrates, and (4) the affinity of the uptake system for γ-aminobutyric acid is greater than that for taurine.

The presence of a carrier-mediated transport system for taurine in the retinal pigment epithelium was indicated by the observation that taurine accumulates in this tissue with a tissue-to-medium ratio of greater than 20. The characteristics of taurine uptake were studied with cultured retinal pigment epithelium. Accumulation of taurine inside the cells is Na+ and energy dependent and ouabain sensitive. The energy dependence and the ouabain sensitivity can be explained because of the involvement of a transmembrane Na+ gradient as the driving force in the uptake process. The anion requirement was not investigated in this study. The information obtained from this investigation however is limited because of the presence of a transport system in the apical and basolateral membrane of the pigment epithelium and the inability of the studies with cultured cells to differentiate the uptake processes localized at the two poles of the plasma membrane of the cell. Ours is the first report, to our knowledge, to use purified apical mem-
brane vesicles for taurine uptake studies. This experimental approach enabled us to investigate specifically the characteristics of taurine uptake that occur at the apical pole of the polarized pigment epithelial cell.

The general characteristics of taurine uptake such as the dependence on a NaCl gradient and the stoichiometry of Na\(^+\):Cl\(^-\):taurine observed in the apical membrane vesicles of the retinal pigment epithelium are similar to those described for taurine uptake in the brush border membrane vesicles of the kidney, the intestine, and the placenta.\(^{36-40}\) One notable difference, however, was the relative affinity of the uptake system for taurine and \(\gamma\)-aminobutyric acid. In the apical membrane of the pigment epithelium, the system responsible for taurine uptake exhibited a much greater affinity for \(\gamma\)-aminobutyric acid and \(\beta\)-alanine than for taurine. However, the system responsible for taurine uptake in the brush border membranes of the kidney, the intestine, and the placenta showed a greater affinity for taurine than for \(\beta\)-alanine and little, if any, for \(\gamma\)-aminobutyric acid. Thus, the taurine transport system present in the renal, intestinal, and placental brush border membranes truly conforms to the definition of a \(\beta\)-amino acid transporter. The observation that the uptake system for taurine in the pigment epithelial apical membrane accepts \(\gamma\)-aminobutyric acid as a better substrate than taurine indicates that this membrane does not possess the typical \(\beta\)-amino acid transporter. We speculate that the uptake of taurine in these membrane vesicles is done by a \(\gamma\)-aminobutyric acid transporter rather than a \(\beta\)-amino acid transporter.

\(\gamma\)-Aminobutyric acid is a potent inhibitory neurotransmitter, and this prompted numerous investigations on the transport of this amino acid in brain tissue. These investigations, based on pharmacologic and kinetic analyses, suggested the presence of at least two types of \(\gamma\)-aminobutyric acid transporters.\(^{41-44}\) Type A is present primarily in neuronal cell, and type B is present primarily in glial cells. The general properties shown by these two transporters are similar, and these properties include the absolute requirement for Na\(^+\) and Cl\(^-\), the electrogenicity, and the Na\(^+\):Cl\(^-\):\(\gamma\)-aminobutyric acid stoichiometry. However, the two systems can be distinguished on the basis of their pharmacology and substrate affinity. Type A is inhibited specifically by cis-3-aminocyclohexanecarboxylic acid and 2,4-diaminobutyric acid and has little or no affinity for \(\beta\)-alanine. Type B, however, is inhibited selectively by 4,5,6,7-tetrahydroisoxazolo[4,5-C]pyridine-3-ol and shows an appreciable affinity for \(\beta\)-alanine. There is no information available on the interaction of taurine with either transporter. Our studies with the transport system responsible for the uptake of taurine in the pigment epithelial apical membrane suggest that this transporter may be similar to the type B \(\gamma\)-aminobutyric acid transporter. The interaction of the transporter with \(\gamma\)-aminobutyric acid and \(\beta\)-alanine and its presence in the retinal pigment epithelium, a nonneuronal cell, support this suggestion. A detailed analysis of the pharmacology of the transporter is needed to establish its identity unambiguously.

Transport of taurine across the retinal pigment epithelium occurs in vivo in both directions, from the choroid to the neural retina and from the neural retina to the choroid. This indicates that the apical and basolateral membranes of the pigment epithelium must possess transport mechanisms for taurine. During the movement of taurine in the direction of choroid-to-retina, the basolateral transporter is responsible for the uptake of taurine from the choroidal blood into the cell, and the apical transporter is involved in the transfer of taurine from the cell to the neural retina. A reversal of the operational directionality of both transporters will result in the movement of taurine from the neural retina to the choroid. There is experimental evidence showing that exposure to light induces release of taurine from the neural retina.\(^{45-47}\) Taurine, like \(\gamma\)-aminobutyric acid, is an inhibitory neurotransmitter in the retina, and its concentration in the subretinal space between the neural retina and the pigment epithelium may play an important role in the modulation of the retinal function. The active uptake system for taurine present in the apical membrane of the pigment epithelium may participate in the removal of taurine that is released into the subretinal space during light stimulation. Thus, the taurine transport mechanisms localized at both poles of the retinal pigment epithelium are important for taurine homeostasis in the retina. We provided insight into the nature of the apical membrane taurine transport mechanism. Similar studies with purified membrane vesicles must be done to understand the nature of the basolateral membrane taurine transport mechanism.

Key words: retinal pigment epithelium, apical membrane vesicles, taurine uptake, energetics, kinetics

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