Accumulation of taurine by cultured retinal pigment epithelium of the rat

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Cultured rat retinal pigment epithelium actively accumulates taurine against a concentration gradient; an intracellular concentration of 12 mM is maintained at an extracellular taurine concentration of 0.12 mM. Taurine accumulation conforms to Michaelis-Menten kinetics for extracellular taurine concentrations between 0.006 and 0.05 mM; within that range the $K_m$ is 16 $\mu$M and the $V_{max}$ is 2.3 nmol./hr./ml of cells. Above an extracellular concentration of 0.5 mM the kinetics are complex. Accumulation is inhibited by reduced temperature and iodoacetate as well as by dinitrophenol in the absence of glucose. Ouabain, reduced extracellular sodium, and removal of extracellular potassium and calcium also inhibit taurine accumulation. The cells also release previously accumulated taurine at a half-time of 1 to 3 hours. The rapid accumulation and release of taurine by cultured retinal pigment epithelial cells is consistent with the idea that this tissue is a site for the transport of taurine in vivo.

Key words: taurine, retinal pigment epithelium, active transport, cell culture, rat, calcium.
Fig. 1. $^{14}$C-taurine accumulation as a function of time (1 to 60 minutes) and extracellular taurine concentration. Numbers to the right of each curve indicate the extracellular taurine concentration at which the measurements were made. Each point represents one culture. A, Cultured retinal pigment epithelium. B, Cultured scleral-choroidal fibroblasts.

Gate mechanisms of uptake. This study was also done to see if the cultured pigment epithelium concentration gradient for taurine is comparable to the known concentration gradients that exist between the retina and plasma in vivo in the rat.

Materials and methods

Cultures were prepared from single-cell suspensions of retinal pigment epithelium isolated from 7-day-old Long-Evans rats and were maintained with daily changes of taurine-free Ham's F-10 medium containing 20 percent dialyzed fetal bovine serum, 50 μg/ml gentamicin, and 100 μg/ml kanamycin. Only confluent cultures 8 to 12 days old were used to measure taurine accumulation. The surface area of each culture was 2.1 cm.$^2$ (Multivell Plates; Falcon Plastics, Oxnard, Calif.) and contained 55 to 80 μg of protein. For measurements of the endogenous taurine content of cultures grown with dialyzed serum, 3 to 4 cultures for each determination were homogenized in 0.9 percent NaCl, deproteinized with 0.05M 5-sulfosalicylic acid, and assayed for taurine on a Beckman 121 amino acid analyzer.

Taurine accumulation was measured as follows. Used medium of each culture was replaced with 0.5 ml. of fresh medium (Ham's F-10 plus 20 percent dialyzed serum without antibiotics) containing $^{14}$C-taurine (New England Nuclear, Boston, Mass.; 4.61 or 4.69 mCi./mmol.); the cultures were incubated in a rotating water shaker bath at 120 r.p.m., 37.5° C., and 5 percent CO$_2$ in air; the cultures were then quickly rinsed seven to 10 times in ice cold 0.9 percent NaCl, scraped, and homogenized. Aliquots of the homogenate were taken for protein determination and liquid scintillation counting. Initial and final medium taurine concentrations were also determined by scintillation counting. Accumulation of taurine by the cells is expressed as nanomoles per microliter of cells or millimolar concentration. Cell volume was estimated by assuming that cell density was 1 gm./cm.$^3$ and that protein comprised 8.1 percent of cell wet weight (the value reported for fresh bovine pigment epi-
Taurine accumulation by epithelium

To determine the kinetic constants for taurine uptake, pigment epithelial cells were incubated for 10 minutes in media containing \(^{14}\)C-taurine, 0.0056 to 40 mM. For measurement of long-term (12 to 48 hour) accumulation, \(^{14}\)C-taurine-containing medium was replaced at 4, 8, 12, 24, and 36 hours in order to approximate a constant external taurine concentration.

For efflux measurements in the absence of extracellular taurine, each of four cultures was preloaded with \(^{14}\)C-taurine under four different conditions (incubation for 1 hour at extracellular concentrations of 0.015 or 18.40 mM, and incubation for 26 hours at extracellular concentrations of 0.019 or 19.59 mM). At zero time of efflux, the cultures were rinsed, fresh taurine-free medium was added, and the cells were incubated in a shaker bath at 37.5° C and 5 percent CO\(_2\) in air. At intervals of 5 minutes for the first hour and 30 minutes thereafter, the medium was removed from each culture for scintillation counting and replaced with fresh taurine-free medium. At 3 hours, the remaining intracellular taurine concentration was measured as described above. Uptake and efflux rates were measured simultaneously by a double-label technique; cultures were preloaded with \(^{3}\)H-taurine (0.0006 mM, 749 mCi/mmole) for 15 minutes, rinsed, and incubated for 10 minutes with different concentrations of \(^{14}\)C-taurine. Final concentrations of \(^{3}\)H-taurine and \(^{14}\)C-taurine in both the cells and the media were determined as described above. Efflux rates were calculated from the fraction of total \(^{3}\)H-taurine released per minute and the mean endogenous taurine content of pigment epithelial cells.

Cultures of rat fibroblasts were prepared from explants of scleral-choroidal fragments remaining after removal of pigment epithelium. Portions of such primary cultures were trypsinized, replated, grown to confluence (8 days), and tested for taurine accumulation.

Inhibition of taurine accumulation was studied with an incubation time of 1 hour and an external taurine concentration of 0.02 mM. Three incubation media were used. The effects of reduced temperature, iodoacetate, ouabain, acetazolamide, and dinitrophenol were measured with F-10 medium plus 20 percent dialyzed serum. The effects of glucose and dinitrophenol on taurine accumulation were measured with Minimum Essential Medium (MEM)\(^{12}\) plus 20 percent dialyzed serum without glucose, with 6.1 mM glucose, with 6.1 mM glucose and dinitrophenol, and with dinitrophenol without glucose; the same set of experiments was also done with a balanced salt solution containing 127 mM NaCl, 3.82 mM KCl, 0.30 mM CaCl\(_2\), 0.62 mM MgSO\(_4\), 1.08 mM Na\(_2\)HPO\(_4\), 0.61 mM KH\(_2\)PO\(_4\), 14.3 mM NaHCO\(_3\), and 3.4 mM phenol red.\(^9\) The effects of altered cation concentrations were also measured in balanced salt solution containing 6.1 mM glucose. All media were without antibiotics and adjusted to pH 7.2 to 7.6 with 5 percent CO\(_2\) in air.

In order to be certain that taurine was not incorporated into macromolecules, pigment epithelial cells were incubated with \(^{14}\)C-taurine for 1 to 48 hours and treated with 5 percent trichloroacetic acid at 0° C; over 99 percent of the radioactivity was recovered in the supernatants. To rule out the possibility that significant amounts of taurine were metabolized to other substances, cultures were homogenized and assayed with a thin-layer chromatographic technique; 95 percent of the radioactivity migrated with a taurine standard.

**Results**

Accumulation of \(^{14}\)C-taurine by pigment epithelium is approximately linear for at least 1 hour at extracellular concentrations up to 0.1 mM (Fig. 1, A). At 1 hour the tissue-to-medium ratio of \(^{14}\)C-taurine is 43...
Fig. 3. Long-term taurine accumulation by cultured pigment epithelium. Numbers to the right of each curve indicate the extracellular taurine concentration at which the measurements were made. Each point represents one culture. $^{14}$C-taurine–containing medium was changed at 4, 8, 12, 24, and 36 hours.

Fig. 4A. Efflux of $^{14}$C-taurine as a function of time and of conditions of preloading with $^{14}$C-taurine. Each curve represents one culture. Preloading conditions are indicated for each curve as the preloading incubation time (hours) and the extracellular taurine concentration (mM).
at an extracellular taurine concentration of 0.023 mM and is 14 at an extracellular concentration of 0.109 mM. Endogenous taurine content of cells is 0.58 ± 0.26 mM (mean ± S.D.); the 14C-taurine accumulated by these cells in 1 hour equals or exceeds this amount at extracellular concentrations as low as 0.023 mM (Fig. 1, A). Within the concentration range of 0.02 to 0.1 mM, pigment epithelium accumulates taurine 10 to 15 times faster than scleralchoroidal fibroblasts cultured under identical conditions (Fig. 1, B). As the extracellular taurine concentration is increased, the difference between the two cell types decreases, and at 20 mM taurine, the accumulation is approximately equal in both types.

The double-reciprocal plot of taurine accumulation for pigment epithelium is linear for extracellular taurine concentrations between 0.005 and 0.05 mM, with a $K_m$ of 16 $\mu$M and a $V_{max}$ of 2.3 nmol/hr./$\mu$l of cells (Fig. 2). For extracellular taurine concentrations greater than 0.5 mM, the curve approaches the origin.

Accumulation by pigment epithelium has been measured at two extracellular taurine concentrations for 48 hours (Fig. 3). The maximum intracellular concentration of 14C-taurine is 9 to 12 mM after 12 to 24 hours. The maximum tissue-to-medium ratios are 432 at an extracellular concentration of 0.022 mM and 102 at an extracellular concentration of 0.12 mM.

The cells rapidly lose intracellular taurine in the absence of extracellular taurine (Fig. 4, A). The rate of taurine release is greater at higher intracellular taurine concentrations. The cells lose half their taurine in 1 to 3 hours. Efflux and uptake of taurine occur simultaneously (Fig. 4, B); the rate of efflux (0.0046 to 0.0079 nmol./min./$\mu$l of cells) is relatively constant and independent of extracellular taurine concentration, whereas rates of uptake increase from 0.0075 to 0.37 nmol./min./$\mu$l of cells as the extracellular concentration of taurine is increased from 0.0056 to 0.5 mM.

Reduced temperature results in a de-
Table II. Effects of ouabain and acetazolamide

<table>
<thead>
<tr>
<th>Addition</th>
<th>Taurine accumulation (% control) ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100 ± 7.3</td>
</tr>
<tr>
<td>Ouabain, 0.1 mM</td>
<td>67.2 ± 4.2*</td>
</tr>
<tr>
<td>Ouabain, 1.0 mM</td>
<td>25.4 ± 0.7†</td>
</tr>
<tr>
<td>Ouabain, 1.0 mM, preincubated 30 minutes</td>
<td>11.0 ± 0.3†</td>
</tr>
<tr>
<td>Acetazolamide, 1 mM</td>
<td>90.6 ± 7.9 N.S.</td>
</tr>
</tbody>
</table>

N.S., not significant.
*p ≤ 0.01.
†p ≤ 0.001.

Increase in taurine accumulation with a Q10 of 2.0 (Table I). Iodoacetate almost completely inhibits taurine accumulation. Dinitrophenol in the absence of glucose significantly reduces taurine accumulation; neither dinitrophenol nor deprivation of glucose has a significant effect separately. This occurs regardless of whether a complex medium containing amino acids and vitamins (F-10 or MEM plus serum) or a simple medium (balanced salt solution) is used. The effect is reversible; following a 1 hour treatment with dinitrophenol in the absence of glucose, taurine accumulation in fresh control medium is nearly equal to the untreated control (Recovery, Table I).

Ouabain, an inhibitor of sodium-potassium adenosinetriphosphatase (ATPase), significantly reduces taurine accumulation (Table II). Removal of potassium from the medium inhibits taurine accumulation (Table III). Ouabain, an inhibitor of sodium-potassium adenosinetriphosphatase (ATPase), significantly reduces taurine accumulation (Table II). Removal of potassium from the medium inhibits taurine accumulation (Table III). Ouabain, an inhibitor of sodium-potassium adenosinetriphosphatase (ATPase), significantly reduces taurine accumulation (Table II). Removal of potassium from the medium inhibits taurine accumulation (Table III). Ouabain, an inhibitor of sodium-potassium adenosinetriphosphatase (ATPase), significantly reduces taurine accumulation (Table II). Removal of potassium from the medium inhibits taurine accumulation (Table III).

Table III. Effects of sodium and potassium ions

<table>
<thead>
<tr>
<th>[K+] ([M])</th>
<th>[Na+] ([M])</th>
<th>Additions</th>
<th>Taurine accumulation (% control) ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.43</td>
<td>143.5</td>
<td>None</td>
<td>100 ± 8.7</td>
</tr>
<tr>
<td>0</td>
<td>143.5</td>
<td>None</td>
<td>13.8 ± 1.5*</td>
</tr>
<tr>
<td>4.43</td>
<td>14.3</td>
<td>Sucrose, 208 mM</td>
<td>0.9 ± 0.1*</td>
</tr>
<tr>
<td>4.43</td>
<td>14.3</td>
<td>Choline chloride, 127 mM</td>
<td>1.4 ± 0.1*</td>
</tr>
</tbody>
</table>

*p ≤ 0.001.

Discussion

These results demonstrate that cultured rat retinal pigment epithelium accumulates taurine against a concentration gradient. For extracellular concentrations below 0.05 mM, the double-reciprocal plot (Fig. 2) is linear and represents a high-affinity mechanism with a relatively low K_m of 16 μM. Comparison of pigment epithelium with fibroblasts shows that the high-affinity mechanism is much more active in pigment epithelium (Fig. 1); the rapid accumulation of taurine at low extracellular taurine concentrations is not simply a characteristic of all cultured cells.

At extracellular taurine concentrations above 0.5 mM, the double-reciprocal plot for pigment epithelium deviates from linearity. Therefore, in addition to the relatively high-affinity mechanism, there may be one or more relatively low-affinity mechanisms described by higher K_m's. A passive leak may also exist; a possibility consistent with the general mathematical models of Koch et al; the efflux studies provide evidence in support of a leak.

The efflux measurements (Fig. 4) suggest that intracellular taurine is exchanging rapidly with extracellular taurine in the steady state; this could provide...
Table IV. Effects of calcium and magnesium ions

<table>
<thead>
<tr>
<th>[Ca++] (mM)</th>
<th>[Mg++] (mM)</th>
<th>Additions</th>
<th>Taurine accumulation (% control) ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.30</td>
<td>0.62</td>
<td>None</td>
<td>100 ± 2.1</td>
</tr>
<tr>
<td>0</td>
<td>0.62</td>
<td>None</td>
<td>33.1 ± 1.9*</td>
</tr>
<tr>
<td>0.30</td>
<td>0</td>
<td>None</td>
<td>99.2 ± 12.9 N.S.</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>None</td>
<td>7.9 ± 2.0*</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>EDTA, 0.1 mM</td>
<td>1.6 ± 0.4*</td>
</tr>
</tbody>
</table>

N.S., not significant.
*p < 0.001.

Taurine accumulation by epithelium

a mechanism for rapid release of taurine from the pigment epithelium to the retina, which also has an active uptake mechanism for taurine.7, 14, 15 The efflux rate is independent of the uptake rate (Fig. 4, B), indicating that increases in uptake with increased extracellular taurine concentration are not due to exchange diffusion.

The effects of reduced temperature and metabolic inhibitors (Tables I and II) clearly demonstrate the active nature of taurine accumulation by cultured pigment epithelium. The temperature coefficient (Q10) value of 2 is characteristic of active transport processes, whereas values less than 1.5 are typical of passive diffusion.16 The inhibition of taurine accumulation in rat pigment epithelium by ouabain exhibits about the same dose response as does ouabain inhibition of partially purified rat heart sodium-potassium ATPase.17 The effect of ouabain and the requirement for extracellular potassium are consistent with the idea that sodium-potassium ATPase is involved in taurine accumulation. Glucose deprivation plus dinitrophenol was required to achieve significant inhibition, suggesting that ATP synthesis through either the glycolytic or oxidative pathways can be reduced to some degree without inhibiting taurine accumulation.

In conclusion, the taurine tissue-to-medium ratio of 102 observed in cultured rat retinal pigment epithelium in the presence of 0.12 mM taurine (i.e., comparable to the rat plasma level) is similar to in vivo retina-to-plasma ratios of 100 to 400 in the normal rat. Furthermore, the rapid accumulation and release of taurine by cultured retinal pigment epithelium are consistent with the idea that this tissue is a site for the transport of taurine in vivo.

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REFERENCES


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