Potassium Modulation of Taurine Transport across the Frog Retinal Pigment Epithelium

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ABSTRACT Net taurine transport across the frog retinal pigment epithelium-choroid was measured as a function of extracellular potassium concentration, \([K^+]_o\). The net rate of retina-to-choroid transport increased monotonically as \([K^+]_o\) increased from 0.2 mM to 2 mM on the apical (neural retinal) side of the tissue. No further increase was observed when \([K^+]_o\) was elevated to 5 mM. The \([K^+]_o\) changes that modulate taurine transport approximate the light-induced \([K^+]_o\) changes that occur in the extracellular space separating the photoreceptors and the apical membrane of the pigment epithelium. The taurine-potassium interaction was studied by using rubidium as a substitute for potassium and measuring active rubidium transport as a function of extracellular taurine concentration. An increase in apical taurine concentration, from 0.2 mM to 2 mM, produced a threefold increase in active rubidium transport, retina to choroid. Net taurine transport can also be altered by relatively large, 55 mM, changes in \([Na^+]_o\). Apical ouabain, 10^{-4} M, inhibited active taurine, rubidium, and potassium transport; in the case of taurine, this inhibition is most likely due to a decrease in the sodium electrochemical gradient. In sum, these results suggest that the apical membrane contains a taurine, sodium co-transport mechanism whose rate is modulated, indirectly, through the sodium pump. This pump has previously been shown to be electrogenic and located on the apical membrane, and its rate is modulated, indirectly, by the taurine co-transport mechanism.

INTRODUCTION

In the vertebrate retina the photoreceptor outer segments and the apical surfaces of the pigment epithelial cells share the same ionic environment. They can, therefore, alter the activity of one another by causing ion or metabolite concentration changes in the space that separates them. In frog, for example, it is now known that light-evoked changes in photoreceptor activity can alter the extracellular concentration of potassium ([K^+]_o), which then changes the membrane potential of the pigment epithelial cells. In particular, light causes a decrease in [K^+]_o that hyperpolarizes the apical
membrane of the epithelial cells and provides the principal voltage source of the c-wave of the electroretinogram (Oakley and Green, 1976; Oakley, 1977; Oakley et al., 1977).

Potassium may also affect pigment epithelial function in other ways. It is well known, for example, that sugar and amino acid transport across cell membranes can be significantly altered by changes in the extracellular concentration of potassium or sodium (Riggs et al., 1958; Crane, 1962, 1964; Schultz and Curran, 1970; Heinz, 1972). For potassium, extracellular concentration changes of 10–40 mM are usually required to cause sizable changes in the rate of nonelectrolyte transport. There exist some systems, however, for which changes of a few millimolar in [K+]o can significantly alter the rate of metabolite transport (Reddy, 1968; Dantzler, 1974). We were interested in determining, therefore, whether amino acid transport across the epithelium would be affected by changes in potassium concentration that were similar to those produced by light (Oakley and Green, 1976; Oakley, 1977).

The amino acid taurine was chosen for study because of its importance in the retina (Starr and Voaden, 1972; Pasantes-Morales et al., 1974; Hayes et al., 1975; Cunningham and Miller, 1976; Orr et al., 1976; Schmidt, 1978), and because it undergoes a large net transport across the retinal pigment epithelium in the apical → basal (retina → choroid) direction (Miller and Steinberg, 1976). Recently, it has been shown that there is a light-stimulated release of taurine from frog rod outer segments (Salceda et al., 1977).

METHODS

These studies were performed on the isolated retinal pigment epithelium-choroid of the bullfrog, Rana catesbeiana. The retinal pigment epithelium (RPE) consists of a single layer of cuboidal epithelial cells. The basal surface of the RPE faces the choroid, which consists mainly of blood vessels and melanocytes dispersed in a fibrous stroma. Individual RPE cells measure approximately 15 μm in width and depth (Porter and Yamada, 1960; Nilsson, 1964; Steinberg, 1973). The junctional complexes connecting these cells in frogs differ from those of other species by being absent at the cellular apices; they begin about halfway down the lateral surfaces of each cell (Porter and Yamada, 1960; Hudspeth and Yee, 1973). The apical surface of the RPE faces the sensory retina, and is covered with villous-like processes that are 60–90 μm long. These processes are closely apposed to the photoreceptors, and extend all the way down to their inner segments (Nilsson, 1964).

In these in vitro studies, the tissue was mounted as a sheet separating two separate compartments, each of which contained a modified Ringer's solution. The fluid bathing the apical surface was referred to as the apical solution; that bathing the basal surface was referred to as the basal solution. The composition of these two solutions could be separately controlled.

The bullfrogs were obtained from Californian and Midwestern suppliers, and kept from several days to several weeks in running tap water at 17.5 °C, on an alternating 12-h cycle of light and darkness. Pieces of pigment epithelium-choroid, 6.5 mm square, were isolated from dark-adapted eyes and mounted in a lucite chamber, so that the surface of the choroid and the apical surface of the pigment epithelium were immersed in separate 1.8-ml baths. The chamber design and the techniques used for dissecting the tissue and mounting it between two lucite plates were identical to those used previously (Miller and Steinberg, 1977 a, b).
The composition of the control bathing solution was 82.5 mM NaCl, 27.5 mM NaHCO₃, 2.0 mM KCl, 1.0 mM MgCl₂·6H₂O, 1.8 mM CaCl₂, 10.0 mM glucose; the solution was gassed with 95% O₂/5% CO₂ to a pH of 7.4 ± 0.1. The osmolarity of this solution was 227 mosM. In some experiments, the potassium concentration was varied by equimolar exchange of KCl and NaCl. In other experiments the concentration of potassium was increased by adding small amounts of KCl (0.1–5 mM) to the solutions bathing the apical or basal membranes. In all experiments “cold” taurine (0.1–5 mM) was added to one or both solutions. The sodium concentration was varied by equimolar replacement of NaCl and NaHCO₃ with tris chloride (tris [hydroxy-methyl] aminomethane hydrochloride) and choline bicarbonate, respectively.

Two pairs of Agar-Ringer bridges on each side of the tissue were used to monitor the transepithelial potential (TEP) and to pass current both for short-circuiting (short-circuit current, SCC) and to measure the transepithelial resistance (Rₜ). Short-circuit current was monitored continuously on a pen recorder. Readings of TEP were obtained hourly by briefly interrupting the short circuit, and Rₜ also was obtained at the same time by passing 1 µA pulses transepithelially and recording the changes in TEP.

For the determination of the transepithelial unidirectional fluxes of taurine, a tracer amount of the isotope [³⁵S]taurine or [³H]taurine, was added either to the solution facing the choroid—the basal solution—or to the apical solution. The fluxes, apical → basal (retina → choroid) or basal → apical (choroid → retina) were then measured by sampling the “cold” solution on the opposite side of the tissue every 40 min. The sample size was 100 µl, and it was replaced with an equal amount of “cold” solution. The fluid in each chamber was stirred and oxygenated by a stream of water-saturated gas bubbles.

Samples were assayed by conventional counting techniques, using a Packard Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). The unidirectional fluxes were calculated in nmol/cm²h from the rate of tracer appearance on the “cold” side and the specific activity of the “hot” side; the area refers to the area of the window between the two lucite chambers.

RESULTS
Potassium and Taurine Fluxes

We previously showed that the retinal pigment epithelium actively transports taurine in the retina-choroid direction and that this net transport takes place over a wide range of taurine concentrations, 1.7 × 10⁻⁴ to 3 × 10⁻²M (Miller and Steinberg, 1976). These experiments were performed with an external potassium concentration ([K⁺]₀) of 2.0 mM in both the apical and basal solutions. To determine whether unidirectional taurine fluxes were affected by a decrease in [K⁺]₀, we first performed experiments in which [K⁺]₀ was changed, in one or both solutions, to 0.2 mM. These data have been plotted in Figs. 1 and 2 (symbols) along with the data previously obtained at 2 mM [K⁺]₀ (lines). Fig. 1 shows that a 10-fold reduction in [K⁺]₀ did not appreciably change the choroid-retina taurine flux regardless of whether the [K⁺]₀ was altered in both solutions or in only the apical or basal solution. By contrast, Fig. 2 shows that there was a dramatic decrease in the retina-choroid flux when [K⁺]₀ was reduced on the apical side alone (□) or on both sides (○) of the tissue. This decrease, to approximately 20% of the level in 2.0 mM [K⁺]₀ (line), occurred across the whole range of taurine concentrations. The points
at 1.85 mM (marked by an arrow on the abscissa) represent a decrease in taurine flux of 70 nM/cm²h.

Fig. 2 also shows that the reduction of [K⁺]₀ in the basal solution alone (Δ) did not affect the retina-choroid flux, across the entire range of taurine concentrations. It is only apical [K⁺]₀, therefore, that affects the transepithelial movement of taurine.

Because the experiments in Fig. 2 each required a period of hours to complete, it was important to determine if the observed effects were reversible. That the tissues can regain their previous rate of taurine transport after readmission of potassium is demonstrated in Fig. 3. The retina-choroid flux in 0.3 mM [K⁺]₀ was only 10 nM/cm²h after 3 h, but a sudden increase in [K⁺]₀ to 2.0 mM in both solutions immediately increased the flux, as well as the short-circuit current, the flux reaching a steady state of 80 nM/cm²h after about 2 h. This is the rate expected for a tissue in 2.0 mM [K⁺]₀ that had not been exposed previously to low potassium (Figs. 2 and 4).

We next examined the effect of much smaller increases and decreases in [K⁺]₀ around the 2.0 mM point and at a fixed taurine concentration of 1.85 mM. The latter was chosen because it represented a point on the curves (Figs. 1 and 2), where the unidirectional flux was high but not maximal. Fig. 4 presents the data for a total of 66 experiments; 57 in the retina-to-choroid direction and 9 in the opposite direction. In most cases [K⁺]₀ was kept constant.
on both sides of the tissue during the course of the experiment. These experiments show that the retina-choroid taurine flux (○, □) is significantly altered by small changes in [K\(^+\)]\(_o\), while the choroid-to-retina flux (■, △, ●) was approximately constant over the entire range of [K\(^+\)]\(_o\) changes. The average retina-choroid flux was sensitive to [K\(^+\)]\(_o\) changes as small as 0.5 mM, the smallest increment tested, and there was a sharp fall in flux when [K\(^+\)]\(_o\) was decreased below 1.0 mM either in both solutions (○) or in the apical solution alone (□). We concluded, therefore, that from 0.2 to 5 mM, apical [K\(^+\)]\(_o\) is an important determinant of net taurine transport across the RPE.\(^1\)

![Figure 2](image-url)

**Figure 2.** Unidirectional, retina → choroid, taurine fluxes, as a function of taurine concentration, in 0.2 mM [K\(^+\)]\(_o\) (log-log plot). Potassium concentration at 0.2 mM on both sides of the tissue (○); or on only the retina side (□), or on the choroid side (△). The open circle and square at 1.85 mM (marked by an arrow on the abscissa) represent the average of three experiments (mean and SEM). Otherwise as Fig. 1.

Fig. 4 shows that the average flux was maximum at approximately 2 mM [K\(^+\)]\(_o\) and that this was not significantly altered when [K\(^+\)]\(_o\) was increased to 5 mM. In order to determine that there was a significant increase in flux between 1 and 2 mM [K\(^+\)]\(_o\), a separate series of experiments was performed in

\(^1\) In Figs. 3 and 5, a decrease in transepithelial resistance (\(R_t\)) accompanies the increase in [K\(^+\)]\(_o\) (see legends). This inverse relationship holds over the whole range of [K\(^+\)]\(_o\) changes and must result from a decrease in cell membrane (apical or basal) resistance, shunt resistance or a combination of the two (Miller and Steinberg, 1977 a). The decrease in \(R_t\) could also signify an altered tissue taurine permeability, which might explain the rise in retina to choroid flux as a function of [K\(^+\)]\(_o\) (Fig. 4). This seems very unlikely, however, since the choroid to retina fluxes were unaffected by apical or basal changes in [K\(^+\)]\(_o\).
which the average fluxes at these concentrations were both measured on a single tissue. The taurine flux in 1.0 mM [K+]o was measured first and then after reaching the steady state [K+]o was increased to 2.0 mM (Fig. 5). This was repeated on six tissues, and the results are summarized in Table I. In five out of six tissues, there was a significant increase in the steady-state retina-choroid flux at 2.0 mM [K+]o; the difference between the means was 30 nM/cm²h. A similar set of measurements was made between 2 and 3 or 5 mM [K+]o, and the results, plotted in Fig. 4, show that the retina-to-choroid taurine flux in 2 mM [K+]o was not appreciably different than in 3 or 5 mM [K+]o.

Sodium and Taurine Fluxes

Since, in many systems, the active accumulation of nonelectrolytes is coupled to the flow of sodium down its electrochemical potential gradient (Wheeler and Christensen, 1967; Schultz and Curran, 1970; Heinz, 1972; Kimmich et al., 1977; Schultz, 1977), we next examined unidirectional taurine fluxes as a function of external sodium concentration. The solid lines in Figs. 6 and 7
specify unidirectional taurine flux as a function of taurine concentration; in these experiments the external sodium concentration, $[\text{Na}^+]_o$, was set at 110 mM in both the apical and basal solutions. We first determined whether a large change in sodium concentration, from 110 to 11 mM, could affect the taurine unidirectional fluxes. Fig. 6 shows that there was no change in the choroid → retina flux when $[\text{Na}^+]_o$ was reduced to 11 mM on both sides or only on one side of the tissue. In contrast, Fig. 7 shows that the retina → choroid fluxes were much reduced in low sodium across the entire range of taurine concentrations. The reduction of $[\text{Na}^+]_o$ to 10% of its normal level reduced the taurine fluxes to approximately 3% of their value in 110 mM $[\text{Na}^+]_o$. The data at 1.85 mM taurine (marked by the arrow on the abscissa) indicated that most or all of this change was caused by the reduction of $[\text{Na}^+]_o$ at the apical side ($\square$) of the tissue. Fig. 8 shows the variation in retina-
FIGURE 5. Taurine flux in the retina → choroid direction with either 1.0 mM [K+]o or 2.0 mM [K+]o in the apical and basal solutions. The steady-state flux (first interrupted horizontal line) was first measured with 1.0 mM [K+]o in both solutions. [K+]o was then elevated to 2.0 mM in both solutions, which increased the mean unidirectional flux by 52 nM/cm²h (second interrupted horizontal line). Otherwise as in Fig. 3. During the first part of the experiment the average TEP was 12.0 mV and the average $R_t$ was 4.5 kΩ. After [K+]o was elevated to 2 mM, $R_t$ decreased to 3.8 kΩ and the average TEP fell slightly to 11.8 mV.

![Graph showing taurine flux in the retina → choroid direction with [K+]o concentrations of 1.0 mM and 2.0 mM.]

TABLE 1

| $1.0 \text{ mM [K}^+\text{]}_o$ | $2.0 \text{ mM [K}^+\text{]}_o$ |
|--------------------------------|---------------------------------|
| Expt. | TEP | $R_t$ | Flux | $R_t$ | TEP | Expt. | TEP | $R_t$ | Flux | $R_t$ | TEP |
|-------|-----|------|------|------|------|-------|-----|------|------|------|------|
| 1     | 11.2| 5.4  | 56   | 94   | 3.8  | 10.0  |
| 2     | 12.9| 4.6  | 97   | 149  | 3.9  | 11.6  |
| 3     | 12.5| 6.7  | 51   | 71   | 5.0  | 12.6  |
| 4     | 9.0 | 6.3  | 86   | 115  | 4.5  | 9.5   |
| 5     | 9.2 | 7.5  | 63   | 105  | 5.7  | 9.6   |
| 6     | 11.5| 6.3  | 65   | 69   | 5.7  | 12.4  |

Mean ± SEM 70 ± 7 101 ± 12

Elevation of taurine flux by $\Delta[K^+]_o$ in individual tissues. For each experiment columns 4 and 5 present the average taurine flux, in the retina → choroid direction, as a function of external potassium concentration. Potassium concentration was changed simultaneously on both sides of the tissue. Columns 2 and 7 and 3 and 6 are the average transepithelial potentials and resistances, respectively. The change in taurine flux produced by elevating $[K^+]_o$ is highly significant ($P < 0.001$, paired $t$ test).
to-choroid taurine flux as a function of $[Na^+]_o$, and at a fixed taurine concentration of 1.85 mM. These data demonstrate that relatively large changes in $[Na^+]_o$ (55 mM) are needed to reduce appreciably the taurine flux.

In many of the experiments (Fig. 4), $[K^+]_o$ was altered by exchanging NaCl for KCl, and it was important to determine whether the small increase in $[Na^+]_o$, for example, 1.8 mM for a $[K^+]_o$ decrease from 2.0 to 0.2 mM, caused the change in taurine flux. This was shown not to be the case because almost identical changes in taurine flux could be produced by holding $[Na^+]_o$ constant and increasing $[KCl]_o$, from 0.3 to 2.0 mM (Fig. 3). (This effect of increasing $[KCl]_o$ was not an osmotic one, since taurine fluxes were not altered by adding 3 mM choline chloride or tris chloride to the apical or basal solution.) In other experiments, small changes in $[Na^+]_o$ (10 mM or less) produced no measurable change in taurine transport.

**Taurine and Rubidium Fluxes**

It has been shown in other epithelia (Schultz and Curran, 1970; Ahearn, 1976; Schultz, 1977) that Na-nonelectrolyte (sugars and amino acids) interactions are reciprocal; that is, a change in the concentration of one of these
Figure 7. Unidirectional, retina → choroid, taurine fluxes as a function of taurine concentration, in 11 mM [Na⁺]₀. The sodium concentration was maintained at 11 mM on both sides of the tissue in one group of experiments (○). In other experiments, it was maintained at 11 mM on only one side of the tissue: (□) retina side, (△) choroid side; with 110 mM on the opposite side. Otherwise as in Fig. 6.

Figure 8. Taurine flux in the retina → choroid direction as a function of external sodium concentration. Each point without an error bar is the mean steady-state unidirectional flux from one tissue. There were at least four steady-state measurements per point, and the SEMs were never more than ± 0.1 log units. The symbol at 110 mM represents the average ± SEM for 10 tissues. The symbols are defined as in Fig. 7. In each experiment the taurine concentration was 1.85 mM.
substances can alter the active transport of the other substance. It was, therefore, of general interest to examine the possibility of a reciprocal interaction between \([\text{Na}^+]_o\) or \([\text{K}^+]_o\) and taurine. Of more specific interest, with regard to RPE function, is the possibility that changes in the concentration of taurine in the extracellular space between the rod photoreceptors and the apical membrane of the RPE could affect the transepithelial movements of sodium and/or potassium. We studied, therefore, the changes in unidirectional cation flux as a function of external taurine concentration. We found that the unidirectional sodium fluxes were not significantly affected, but there was a large effect on active rubidium transport.

The interaction between taurine and potassium transport was examined by measuring \(^{86}\text{Rb}\) unidirectional fluxes across the RPE as a function of extracellular taurine concentration. In these experiments both sides of the tissue were initially bathed in normal Ringer’s (Methods) containing no taurine. Flux measurements were made over a 2-3h period, and then taurine was added to the apical or basal solutions, and the experiment continued for another 2-3h. In Fig. 9 the \(^{86}\text{Rb}\) flux, retina to choroid, is plotted as a function of time. The initial steady-state flux in zero taurine Ringer’s was 81 nM/cm²h, and after 3 h 5 mM taurine was added to the solutions bathing both sides of the tissue. The flux quickly rose to 145 nM/cm²h. The transepithelial potential and resistance also decreased slightly. The decrease in TEP, noted in the legend to Fig. 9, was due to a depolarization of the apical membrane.

The rubidium fluxes were also measured at other taurine concentrations, and Fig. 10 is a summary of these data. Each data point represents the mean steady-state flux in a separate experiment. There were a total of 25 experiments, 17 in the retina-to-choroid direction (open symbols) and 8 in the choroid to retina direction (closed symbols). Fig. 10 shows that the choroid-to-retina flux of \(^{86}\text{Rb}\) was unaffected whether taurine was elevated on one or both sides of the tissue. In contrast, the retina-to-choroid flux of rubidium was unaffected whether taurine was elevated on one or both sides of the tissue. In contrast, the retina-to-choroid flux of rubidium was

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2 We assume, by analogy with many other systems, that a rise in cell sodium concentration will stimulate this pump (Schwartz et al., 1975; Joiner and Lauf, 1978). In nine experiments apical taurine (2 mM) increased the net choroid-to-retina sodium flux by an average of 110 nM/cm²h but this increase was not statistically significant. The presumed increase in Na-K pump rate, however, could have been masked by an alteration in apical and/or basal membrane sodium conductance.

3 Rubidium is a convenient substitute for potassium because it is cheaper and has a much longer half-life. This substitution is justified since rubidium and potassium compete for the same conductance channels in other systems (Solomon, 1952; Sjodin, 1959; Cavaggioni et al., 1973), and this seems to be true also at the apical and basal membrane of the RPE (unpublished observations). In addition, the RPE actively transports \(^{86}\text{Rb}\) and \(^{42}\text{K}\) in the retina-to-choroid direction; in both cases the active transport was inhibited by ouabain, acting at the apical membrane (Miller, S. S., and Steinberg, R. H., manuscript in preparation). This is also true in a variety of other systems (Bonting, 1970). We also bathed the tissue in RbCl Ringer’s and verified that the net taurine transport could be inhibited by reducing the concentration of rubidium from 2 to 0.2 mM. The net taurine flux was reduced by a factor of 2.5 as compared to 3.5 in potassium Ringer’s (Fig. 4).

4 Miller, S. S., and R. H. Steinberg. Unpublished observations.
elevated at every concentration tested, from 0.2 to 5 mM. Comparing the $^{86}$Rb unidirectional fluxes at zero taurine concentration shows that rubidium is actively transported across the retinal pigment epithelium at approximately 125 nM/cm²h. This active transport rate was stimulated by increasing the taurine concentration in one (apical) or both of the bathing solutions and the maximum stimulation occurred at a concentration of 1-3 mM. These data indicate that the $^{86}$Rb, taurine interaction occurred at the RPE apical membrane.

![Graph](image)

**Figure 9.** Rubidium flux in the retina → choroid direction with either 0 mM taurine or 5 mM taurine in the apical and basal solutions. These solutions contained 2 mM potassium and no "cold" rubidium. The steady-state flux was first measured with no taurine in the bathing solutions (first interrupted horizontal line) and at 3 h the taurine concentration was elevated to 5 mM in both solutions. The mean steady-state flux was increased by 64 nM/cm²h (second interrupted horizontal line). Otherwise as in Fig. 3. During the first part of the experiment the average TEP was 9.5 mV and the average $R_t$ was 4.7 kΩ. After the taurine concentration was elevated to 5 mM, these values decreased to 6.5 mV and 4.25 kΩ, respectively.

Information about the specificity of the taurine transport site was previously obtained by comparing the net transport of taurine and L-methionine across the RPE (Miller and Steinberg, 1976). The net transport of L-methionine was significantly smaller than taurine, did not saturate at high concentrations (1.5

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In Fig. 9 the tissue was bathed in Ringer's that contained 2 mM potassium and no "cold" rubidium. In the experiments summarized in Fig. 10, the Ringers contained 2 mM rubidium and no potassium. This difference in the composition of the Ringer's accounts for the difference in steady-state rubidium flux at 5 mM taurine (Miller, S. S., and R. H. Steinberg, manuscript in preparation).
X 10^{-2}M) and was not, in contrast to taurine, inhibited by apical ouabain. These results and others strongly suggested that taurine and L-methionine were transported across the RPE by different mechanisms.

The specificity of the taurine transport site has been elaborated on in the course of the present study by two kinds of experiments. As in other systems (Christensen et al., 1954; Christensen, 1964; Reddy, 1968), it was shown (seven experiments) that \(\beta\)-alanine, a \(\beta\)-amino acid, competitively inhibited net taurine transport. This transport was 60\% inhibited by 0.2 mM \(\beta\)-alanine and was 90\% inhibited when \(\beta\)-alanine was greater than or equal to 5 mM. The taurine concentration was 1.85 mM in these experiments. In the second class of experiments, the specificity of the interaction between rubidium and taurine was examined by measuring the retina to choroid flux of \(^{86}\)Rb before and after the addition of 2 mM L-methionine to the solutions bathing both sides of the tissue. In four experiments L-methionine caused no significant increase in the retina-to-choroid rubidium flux. This was in striking contrast to the effect of 2 mM taurine which increased the \(^{86}\)Rb flux by 250 nM/cm\(^2\)h (Fig. 10).

![Figure 10. Rubidium flux as a function of external taurine concentration.](image-url)

The open symbols represent retina \(\rightarrow\) choroid fluxes and the closed symbols choroid \(\rightarrow\) retina fluxes. The rubidium concentration was 2 mM in both solutions for all the experiments shown. The circles represent experiments in which the taurine concentration was the same on both sides of the tissue; the squares and triangles represent experiments in which the taurine concentration was varied in one solution only (apical or basal) and maintained at 0 mM in the opposite solution. There were at least five steady-state measurements per experiment; the standard error in each was typically 8–15 nM/cm\(^2\)h and never exceeded 30 nM/cm\(^2\)h. The open and closed circles with standard error bars represent an average of 17 and 8 experiments, respectively.
DISCUSSION

The retinal pigment epithelium actively transports taurine, potassium, rubidium, and sodium (Miller and Steinberg, 1976; Miller and Steinberg, 1977b; Fig. 10). The present study shows that: (a) small (1 mM) changes in extracellular potassium concentration modulate the active transepithelial movement of taurine; (b) there is a reciprocal interaction between taurine and potassium, that is, 1 mM changes in extracellular taurine concentration modulate the active transepithelial movement of rubidium (potassium); (c) relatively large (55 mM) changes in extracellular sodium concentration are required to alter active taurine transport, while the addition of taurine (2 mM) does not significantly alter active sodium transport; (d) these changes in ion or amino acid concentration are effective only from the apical (or neural retinal) side of the tissue; (e) the range of potassium ion concentration change, which modulates net taurine transport, approximates the narrow range known to be produced by light (Oakley and Green, 1976; Oakley, 1977).

Cation-Linked Transport of Organic Solutes

A mechanism for the coupling of amino acids and other metabolites to cation transport was first suggested by Riggs and his co-workers (1958). They showed that Ehrlich mouse acites tumor cells concentrated amino acids to many times that of the environment. On the basis of this data it was suggested that potassium efflux from the cell drove amino acid influx by exchange diffusion (counter-transport). They realized that the exchange diffusion could also have been driven by sodium influx (co-transport), and in recent years the sodium gradient hypothesis has received support in most, but not all, systems (Schultz and Curran, 1970; Heinz, 1972; Schultz, 1977). In general, the electrochemical gradients of both sodium and potassium, along with a direct link to metabolic processes (ATP hydrolysis), could be used to accumulate amino acids, sugars, and other metabolites.

The active accumulation of these solutes has been measured as a function of extracellular potassium concentration in a variety of preparations (Riklis and Quastel, 1958; Kleinzeller and Kotyk, 1961; Abadom and Scholefield, 1962; Bihler and Crane, 1962; Fox et al., 1964; Dantzler, 1974; Dantzler and Bentley, 1975). For example, in isolated guinea pig intestine potassium changes from 15 to 5 mM or 15 to 30 mM significantly inhibited the active absorption of glucose (Riklis and Quastel, 1958). These effects were apparently not due to permeability changes. As in the pigment epithelium, it was found that rubidium could effectively substitute for potassium in inhibiting transport. As another example, Sellström and Hamberger (1975) showed that uptake of y-aminobutyric acid (GABA) by glia-enriched fractions was maximal at 5.0 mM, the normal level of [K+]o, but the two comparison levels were 0 and 30 mM. It is characteristic of almost all of the above quoted studies that the potassium concentration changes were relatively large, compared to the present experiments, and therefore the cells were more vulnerable to nonspecific osmotic effects.

Reddy (1968) studied the effects of small changes in extracellular potassium
concentration on taurine accumulation in the rabbit ciliary body-iris preparation. He found that this accumulation could be increased monotonically, from approximately 3 to 100% of control, by increasing extracellular potassium in small steps, from 0 to 5 mM. In comparison, relatively large changes in extracellular sodium (148 → 73 mM) had a much smaller effect on taurine accumulation (28% of control). Similar relationships exist in the RPE between changes in \([K^+]_o\), \([Na^+]_o\), and taurine transport (Figs. 4 and 9). Reddy also showed that the active accumulation of taurine was inhibited by ouabain and β-alanine. This is also true for the RPE (Miller and Steinberg, 1976; Fig. 11) and further suggests a basic similarity in the underlying transport mechanism. This similarity would not be surprising since the retinal pigment epithelium and ciliary body are anatomical extensions of one another and are embryologically of similar origin.

**Na⁺- and/or K⁺-Linked Taurine Transport**

The dependence of taurine transport on small changes in \([K^+]_o\) could implicate a direct link between taurine movement and the potassium electrochemical gradient. If taurine is carried across the RPE apical membrane by a K⁺ counter-transport mechanism, which is driven by the apical K⁺ electrochemical gradient, then increasing that gradient should increase the net apical to basal transport of taurine. From electrophysiological experiments on this tissue, the potassium electrochemical gradient (2 mM K⁺ on both sides of the tissue) can be estimated as \(V_m - E_K = +12\) mV, driving K⁺ out of the cell. \(V_m\) is the apical membrane resting potential, and \(E_K = -100\) mV is the potassium equilibrium potential (Miller and Steinberg, 1977a; Oakley et al., 1978). Decreasing \([K^+]_o\) from 2 → 0.2 mM would increase this driving force by 40–50 mV. Therefore, a carrier mechanism which accumulates taurine by counter-transport of potassium would cause an increase in the net apical-to-basal transport of taurine, not the observed decrease. We next considered the possibility of a Na⁺ taurine co-transport mechanism.

If sodium and taurine movements are coupled by co-transport on a shared carrier, then the net taurine transport should be reduced by a decrease in the sodium electrochemical potential gradient (Alvaredo and Mahmood, 1974; Christensen et al., 1974; Heinz, 1972; Schultz and Curran, 1970; Ahearn, 1977). It is known that the apical membrane of the RPE contains a Na-K pump whose electrogenicity can be removed by a reduction in \([K^+]_o\) and/or a rise in \([K^+]_i\) (Miller et al., 1978; Oakley et al., 1978). It is possible, therefore, that lowering \([K^+]_o\) (Fig. 4) reduced Na-pump efflux, increased internal sodium, and thereby caused a reduction in the sodium electrochemical potential gradient across the apical membrane.

This mechanism does not require that the change in cell sodium be very large. The size of this change can be estimated by assuming a reasonable range of values for the cell sodium concentration (10-30 mM; Kernan and MacDermott, 1976; Zeuthen, 1978). For these concentrations the sodium equilibrium potential, \(E_{Na}\), would be 60 and 33 mV, respectively. These values can be used in Fig. 8 to calculate the reduction in \([Na^+]_o\), needed to produce
a given reduction in taurine flux. For example, a reduction of approximately 60 nM/cm²h was produced by a decrease in apical [K⁺]o from 2.0 to 0.2 mM. According to Fig 8, this decrease could also be achieved by reducing [Na⁺]o from 110 to 70 mM. In the case that cell sodium is 10 mM, this would decrease ENa from 60 to 49.5 mV. One can now calculate the increase in cell sodium that would be required to achieve this same reduction in ENa. From the Nernst equation, cell sodium would only have to increase from 10 to 15.7 mM. (In the case that cell sodium is 30 mM, the increase would be to 47 mM.)

Fig. 10 also can be understood in terms of the sodium gradient hypothesis. This understanding follows from Figs. 1 and 2 (solid lines), which show that net taurine transport is stimulated as the taurine concentration increases from 0 to 2 mM. Sodium co-transport into the cell, across the apical membrane, therefore, would also be stimulated. If this caused a rise in internal sodium, stimulating the Na-K pump, then one could expect an increase in the active retina-to-choroid rubidium (potassium) flux.

We have assumed here that the sodium pump and the Na⁺, taurine co-transport mechanism are spatially separate entities. This would not be true if there were a direct link between taurine transport and the apical membrane bound Na-K ATPase (or the Na-K pump). In order to distinguish between these possibilities, the following experiment was performed. A microelectrode was placed in a RPE cell and the concentration of taurine in the apical bath was increased from 0 to 2 or 10 mM. The apical membrane potential began to depolarize as soon as the first taurine molecules arrived at the apical surface (not shown). Since taurine is a neutral amino acid, this suggests the stimulation of a Na⁺, taurine carrier, but it is also consistent with a taurine co-transport mechanism involving Na-K ATPase. If the observed voltage response depended directly on the Na-K ATPase (or the Na-K pump), then the prior addition of ouabain, 10⁻⁴M, to the apical bath should have eliminated this response. In fact, it was not eliminated and its size was unchanged 10-15 min after the application of ouabain. This experiment suggests therefore that the carrier and the pump are located at separate sites on the apical membrane.

The Driving Force, Chemical or Electrical?
Since some co-transport systems seem to be mainly driven by chemical or electrical gradients while others have contributions from both (Geck and Heinz, 1976; Crane, 1977; Kimmich and Carter-Su, 1978), it was of interest to determine whether the RPE falls into the former or latter category. It has been shown that the net retina—choroid flux of taurine is ouabain inhibitable (Miller and Steinberg, 1976). In these experiments, however, the first flux measurements were obtained 20 min after the application of ouabain, so that the inhibition may have been due to a decrease in the apical membrane potential, the sodium chemical potential, or both. More recently, these measurements were repeated using a much larger (by a factor of 5) number of microcuries on the “hot” side. This allowed the first flux measurements to be made at a time when the apical membrane electrogenic pump was still
depolarizing. Previous electrophysiological experiments showed that the onset of this ouabain-induced depolarization coincided with the arrival of ouabain molecules at the apical membrane and that the time-course of this "fast" depolarization, 2½-3 min, was determined by the diffusion of ouabain molecules through the unstirred layer to the apical membrane (Miller et al., 1978). Since these experiments also indicated that the Na, K gradients did not start running down until 15 min after the application of ouabain, the flux measurements can provide evidence for the membrane potential contribution to taurine transport.

One of the experiments is shown in Fig. 11, where the average steady-state flux before ouabain was 87 nM/cm²h. After approximately 4 h, 10⁻⁴M ouabain was added to the apical bath (see figure legend for details), and a flux sample was taken 5 min after the SCC began to change. The flux had fallen to 38 nM/cm²h, which was significantly smaller than the pre-ouabain value. In five experiments the average pre-ouabain flux (±SEM) in the retina to choroid direction was 94 ± 12 nM/cm²h, and in the first flux period, 2½-5 min after ouabain began to alter the apical membrane potential, the average flux was 18 ± 12 nM/cm²h. The average basal to apical flux (five experiments) was 23.6 ± 2 nM/cm²h and was not significantly altered by ouabain. Therefore, the net decrease in taurine flux coincided in time with the arrival of ouabain molecules at the apical membrane. (In control experiments, without ouabain, the steady-state flux obtained from a 5-min sample was indistinguishable from the more usual 20- or 40-min samples.) This means that the apical membrane potential forms part of the driving force responsible for active taurine transport. But if the potential change were the only factor controlling taurine transport, one could not explain, in Fig. 4, the observed increase in taurine flux with the apical membrane depolarization that accompanies an increase in [K⁺]o (Miller and Steinberg, 1977 a). These data indicate, therefore, an electrical and chemical contribution to transepithelial taurine transport. At present, however, we cannot say to what extent each component is involved in the movement of taurine across the RPE.

**Taurine and [K⁺]o in the Dark and Light**

It is now well established that taurine is the most abundant amino acid in the vertebrate retina, that a very substantial portion of this amino acid is located in the photoreceptors, and that it is actively accumulated by the pigment epithelium (Pasantes-Morales et al., 1972; Cohen et al., 1973; Kennedy and Voaden, 1974; Orr et al., 1976; Lake et al., 1977; Pourcho, 1977; Voaden et al., 1977). In cat it has been shown that a dietary deficiency of taurine disrupts outer segment structure and eventually leads to photoreceptor cell death (Schmidt et al., 1976; Berson et al., 1976). Retinal activity also can be altered by small changes in extracellular taurine, 0.1-5 mM. For example, extracellular concentration changes in this range can reversibly alter b-wave and ganglion cell activity in the retina (Pasantes-Morales et al., 1974; Cunningham and Miller, 1976; Mandel et al., 1976).

In the dark-adapted frog retina, the concentration of potassium in the
subretinal space is approximately 3–4 mM (Oakley and Green, 1976; Oakley, 1977). A bright flash of light produces several effects in a dark adapted eye: (a) on a fairly rapid time scale (milliseconds), it severely reduces the entry of Na⁺ into the outer segments causing the photoreceptors to hyperpolarize (Bortoff, 1964; Hagins et al., 1970; Toyoda et al., 1970; Korenbrot and Cone, 1972; Brown and Pinto, 1974); (b) on a longer time scale (seconds), it causes a decrease in potassium concentration outside the photoreceptors (Cavaggioni et al., 1973; Oakley and Green, 1976; Oakley, 1977). According to the data summarized in Fig 4, the potassium decrease should alter the net transport of taurine out of the retina, across the pigment epithelium. The situation in vivo would be complex, however, since the past history of light exposure and presence or absence of other substances (taurine, for example) should also affect taurine transport. The past history of light exposure will set [K⁺]₀ at a particular level, and it will be the magnitude and duration of the change from this level that will determine the effect. An examination of Fig. 4, for

![Diagram](image-url)

**Figure 11.** Taurine flux in the retina → choroid direction. The taurine concentration was 2 mM in both solutions. Otherwise as in Fig. 3. Tracer, [³H]taurine (50–75 µCi) was added to the apical solution at t = 0, and the apical-to-basal flux was sampled at 40-min intervals; the taurine flux reached a steady-state at ≈2 h (interrupted horizontal line). The last pre-ouabain sample was taken at t = 4 h and is plotted at the midpoint of that sample period (t = 3⅓ h). Approximately 2 min prior to t = 4 h, 10⁻⁴M ouabain was added to the apical solution. This 2-min interval allowed the ouabain to reach the apical membrane and begin its effect on the membrane potential. The first post-ouabain sample period began with the onset of the change in TEP and was 5 min long. After this period the flux was sampled at 20-min intervals. Prior to the addition of ouabain, the average TEP was 11.3 mV and the average R𝑍 was 3.5 kΩ. 1 h after the addition of ouabain, the TEP decreased to 2 mV and R𝑍 rose to 4.1 kΩ.
example, shows that a 1.5 mM decrease in \([K^+]_o\) from 3.0 to 1.5 mM should have a considerably smaller effect on taurine transport than the decrease from 2.0 to 0.5 mM. Although the in vivo relationship between taurine and potassium is undoubtedly complex, it seems worthwhile to incorporate what is known about the accumulation and transport of these substances into some suggestions about the possible functional significance of taurine inside the photoreceptors and in the extracellular space surrounding them.

**Possible Functions of Taurine**

It has been shown in a variety of cells that osmotic swelling is counteracted by a substantial release of intracellular taurine (Fugelli and Zachariassen, 1976; Hoffman and Hendil, 1976). It is also known that light causes isolated outer segments to swell by approximately 6% (Enoch et al., 1973) and to release taurine (Salceda et al., 1977). This release may indicate the presence of an isoosmotic regulating mechanism that helps preserve osmotic balance during light stimulation.

The increase in extracellular taurine due to a light-evoked photoreceptor release might be magnified by an effect on pigment epithelial transport. A bright flash of light, for example, can lower \([K^+]_o\) below 1 mM (Oakley, 1977), which would decrease the net taurine transport across the RPE (Table I and Fig. 4), and tend to increase the concentration of taurine in the extracellular space between the photoreceptors and the RPE. According to the data summarized in Fig. 10, any increase of taurine concentration between 0 and 2 mM should lead to an increase in the active transport of \(K^+\) out of the retinal space and therefore help keep \([K^+]_o\) at a relatively low level.

After the light flash goes off, the taurine concentration outside the photoreceptors should fall since the net efflux of taurine from the outer segments decreased to its dark level (Salceda et al., 1977). This fall would be enhanced by the rise in \([K^+]_o\) (Oakley and Green, 1976; Oakley, 1977), which stimulates the transport of taurine out of the retinal space (Fig. 4). In order to assess critically the osmoregulatory function of taurine, one would need to compare, in the light and dark, the changes in extracellular ionic activity with the changes in intracellular and extracellular taurine activity. It would also be important to determine the mechanism that underlies the light-dependent movement of taurine across the outer segment membrane.

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