Effect of Taurine on the Isolated Retinal Pigment Epithelium of the Frog: Electrophysiologic Evidence for Stimulation of an Apical, Electrogenic Na⁺-K⁺ Pump

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Summary. The apical surface of the retinal pigment epithelium (RPE) faces the neural retina whereas its basal surface faces the choroid. Taurine, which is necessary for normal vision, is released from the retina following light exposure and is actively transported from retina to choroid by the RPE. In these experiments, we have studied the effects of taurine on the electrical properties of the isolated RPE of the bullfrog, with a particular focus on the effects of taurine on the apical Na⁺-K⁺ pump.

Acute exposure of the apical, but not basal, membrane of the RPE to taurine decreased the normally apical positive transepithelial potential (TEP). This TEP decrease was generated by a depolarization of the RPE apical membrane and did not occur when the apical bath contained sodium-free medium. With continued taurine exposure, the initial TEP decrease was sometimes followed by a recovery of the TEP toward baseline. This recovery was abolished by strophanthidin or ouabain, indicating involvement of the apical Na⁺-K⁺ pump.

To further explore the effects of taurine on the Na⁺-K⁺ pump, barium was used to block apical K⁺ conductance and unmask a stimulation of the pump that is produced by increasing apical $[K^+]_o$. Under these conditions, increasing $[K^+]_o$ hyperpolarized the apical membrane and increased TEP. Taurine reversibly doubled these responses, but did not change total epithelial resistance or the ratio of apical-to-basal membrane resistance, and ouabain abolished these responses.

Collectively, these findings indicate the presence of an electrogenic Na⁺/taurine cotransport mechanism in the apical membrane of the bullfrog RPE. They also provide direct evidence that taurine produces a sodium-dependent increase in electrogenic pumping by the apical Na⁺-K⁺ pump.

Key Words taurine · sodium-coupled transport · Na⁺,K⁺-ATPase · retinal pigment epithelium · retina

Introduction

The retinal pigment epithelium (RPE) separates the neural retina from the choroidal blood supply. Its apical surface faces the photoreceptor outer segments across the subretinal space, and its basal surface faces the choroid. Transport of fluid, ions, and other solutes by the strategically located RPE thus influences the environment of the photoreceptors. The electrical potential difference across the RPE also contributes importantly to late events in the electroretinogram (Steinberg, Linsenmeier & Griff, 1985).

In these experiments, we used electrophysiological techniques to study the response to taurine of the frog RPE. Taurine, an uncharged β-aminosulfonic acid, is particularly important for normal vision. It is highly concentrated (60–80 mM) in the outer nuclear layer of the vertebrate retina via a sodium-dependent uptake mechanism (Pasantes-Morales et al., 1972; Lake, Marshall & Voaden, 1975, 1977; Schmidt, 1980), and it is released into the subretinal space following light stimulation (Salceda, Lopez-Colome & Pasantes-Morales, 1977; Schmidt, 1978). Moreover, taurine deficiency causes photoreceptor degeneration, blindness, and ultimately nonrecordable electroretinograms in cats (Schmidt, Berson & Hayes, 1976), and has been associated with abnormal electroretinograms in humans (Geggel et al., 1985). Previous studies have demonstrated that the RPE actively transports taurine in the apical-to-basal direction via a mechanism that exhibits features suggestive of apical sodium-coupled uptake; that is, it is inhibited by reducing sodium concentration in the subretinal space or by ouabain (Miller & Steinberg 1979; Ostwald & Steinberg, 1981). While taurine-induced changes in the transepithelial potential (TEP) difference across the RPE have been previously identified (Miller & Steinberg, 1979), they have not been systematically investigated.

The purpose of these experiments was to study further the effects of taurine on the electrical prop-

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erties of the RPE, with a particular focus on the mechanisms responsible for taurine-induced changes in the TEP and effect of taurine on the Na$^+$-K$^+$ pump, which is present in the apical membrane of RPE cells (Miller, Steinberg & Oakley, 1978). We also used the accessibility of the apical Na$^+$-K$^+$ pump to demonstrate directly that taurine stimulates electrogenic Na$^+$-K$^+$ pump activity. Inasmuch as taurine serves as a model solute transported by a sodium-coupled mechanism, these observations are of general interest to transport by other epithelia.

Materials and Methods

PREPARATION AND SOLUTION

The chamber design and the techniques for dissecting and mounting the RPE of the bullfrog, *Rana catesbeiana*, were identical to those used by Miller and Steinberg (1977). In brief, a tissue consisting of RPE and choroid was dissected after first dark-adapting the animal to facilitate removal of the retina. The animal was decapitated, the eye enucleated, and the posterior half of the eye submerged in perfusate. The retina was gently peeled away from the RPE and the RPE-choroid was dissected free from the sclera and mounted between two Lucite plates.

The two sides of the tissue, retinal (apical) and choroidal (basal), were continuously superfused at 2–5 ml/min by a gravity-fed system from large reservoirs. The area of the tissue exposed to the perfusate was 0.07 cm^2. The control perfusate was a modified Ringer's solution having the following composition (mM): 82.5 NaCl, 27.5 NaHCO$_3$, 2.0 KCl, 1.0 MgCl$_2$, 1.8 CaCl$_2$, and 10.0 glucose bubbled with 95% O$_2$/5% CO$_2$, at pH 7.4. The apical and basal perfusates could be independently switched to test solutions with altered composition. In experiments involving taurine in a concentration of 4 mM or less, taurine was simply added to the perfusing solution. With 10 mM taurine, studies were performed both by simply adding taurine or adding taurine while removing 5 mM NaCl with equivalent results. Sodium-free solutions were prepared by replacing NaCl and NaHCO$_3$ isosmotically with either Tris base/Tris HCl (pH 7.4) and choline bicarbonate, respectively, or N-methyl-D-glucamine (NMDG) chloride and NMDG bicarbonate, respectively.

ELECTRODES

Conventional microelectrodes were made from 1.0-mm tubing (Omega Dot, Glass Co. of America, Millville, NJ), filled with 5 M K$^+$-acetate, and beveled to a resistance of 50–100 MΩ. Unity-gain preamplifiers (model 1090, Winston Electronics, San Francisco, CA) with input impedances of 10^{14} Ω were used to measure microelectrode potentials.

EQUIVALENT CIRCUIT (FIG. 1)

The equivalent circuit of the RPE has previously been presented in detail (Miller & Steinberg, 1977; Linsenmeier & Steinberg, 1983; Griff & Steinberg, 1984; Griff, Shirao & Steinberg, 1985). Briefly, the apical membrane is represented as a resistance (R_{ap})
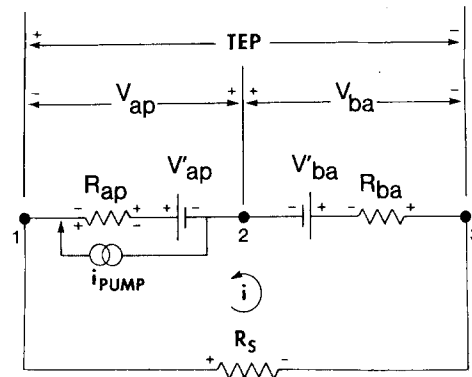

Fig. 1. Equivalent circuit for the isolated RPE-choroid. The apical membrane is represented by a resistor (R_{ap}) in series with a battery (V'_{ap}). Similarly, the basal membrane is represented by a resistor (R_{ba}) in series with a battery (V'_{ba}). The membrane resistances are shunted by a resistor (R_s). Due to the difference between V'_{ap} and V'_{ba}, a steady current (i) flows through the circuit. An additional current source from the electrogenic hyperpolarizing Na$^+$-K$^+$ pump is represented by i_{pump}. As a result of current flow across the apical and basal membrane resistances, the potentials recorded across them (V_{ap} and V_{ba}, respectively) differ from the batteries (V'_{ap} and V'_{ba}). The steady potential across the isolated RPE-choroid is called the transepithelial potential (TEP). The potentials V_{ap}, V_{ba}, and *TEP* are labeled with the polarity with which they are recorded. In all figures, these potentials are displayed with positive polarity upward. Extracellular electrodes were placed in the apical perfusate (position *1*) and in the basal perfusate (position *3*); a microelectrode was placed intracellularly (position *2*)

in series with a battery (V'_{ap}). Similarly, the basal membrane is modeled as a resistance (R_{ba}) in series with a battery (V'_{ba}). The two RPE membranes are connected by a resistor (R_s), which represents the parallel combination of the paracellular resistance across the intercellular junctional complexes, and a resistive pathway around the edge of the tissue. In control conditions, a steady current (i) flows through the circuit because V'_{ap} is greater in magnitude than V'_{ba}. This current hyperpolarizes the basal membrane and depolarizes the apical membrane (Miller & Steinberg, 1977). Thus, the potentials recorded by an intracellular microelectrode, V_{ap} and V_{ba}, differ in absolute magnitude from the membrane batteries. A change in one of the membrane batteries will also change the current (i) so that both membrane potentials will change. The change in membrane potential will be greater in magnitude for the membrane where the potential change originates; the smaller change at the other membrane is a passive "shunted" response. The TEP is the difference between V_{ba} and V_{ap}. The derivation of equations that describe these potential changes has been presented previously (Miller & Steinberg, 1977; Linsenmeier & Steinberg, 1983). In summary, for a potential generated at the apical membrane

$$V_{ap} = \Delta V'_{ap} \left(\frac{R_{ba} + R_s}{R_{ap} + R_{ba} + R_s} \right) \tag{1}$$

and

$$V_{ba} = \Delta V'_{ap} \left(\frac{R_{ba}}{R_{ap} + R_{ba} + R_s} \right). \tag{2}$$

The apical membrane also contains an electrogenic Na⁺-K⁺ pump that hyperpolarizes the apical membrane and is represented by i_{pump} in Fig. 1. Let us consider the effect of increasing the hyperpolarizing current across the apical membrane by stimulation of the Na⁺-K⁺ pump. Part of the pump current flows through R_{ap} hyperpolarizing the apical membrane, and part of it flows through R_s and then through R_{ba}, hyperpolarizing the basal membrane. The major fraction of the pump current flows across the apical membrane because R_{ap} is smaller than the sum of R_s and R_{ba} (Miller & Steinberg, 1977). The pump current hyperpolarizes the apical and basal membranes by

$$\Delta V_{ap} = i_{pump} \frac{R_{ap}(R_{ba} + R_s)}{R_{ap} + R_{ba} + R_s} \tag{3}$$

and

$$\Delta V_{ba} = i_{pump} \frac{(R_{ap})(R_{ba})}{R_{ap} + R_{ba} + R_s}. \tag{4}$$

The net effect, then, of stimulation of the apical pump current (i_{pump}), as observed in the present study, is hyperpolarization of the apical membrane, a lesser hyperpolarization of the basal membrane, and an increase in TEP.

In the present study, the net effect of taurine exposure is to depolarize the apical membrane and, to a lesser extent, the basal membrane because of shunting, and therefore to decrease TEP. Our data are consistent with activation by taurine of an apical depolarizing current associated with sodium-coupled taurine uptake.

Recording Configuration

Placement of electrodes and the recording configuration have been previously described (Griff, et al., 1985; Oakley, 1977). In brief, the TEP was measured differentially between two calomel electrodes that were connected to the apical and basal baths by a pair of agar-Ringer bridges. Apical (V_{ap}) and basal (V_{ba}) membrane potentials were recorded simultaneously by measuring differentially between an intracellular microelectrode and the apical and basal baths, respectively.

Current pulses (1.0 μA, 1.0 sec) were passed through the tissue via two silver-chlorided wires connected to each bath by a second pair of bridges. Two resistance ratios were determined from the appropriate current-induced voltage (iR) drops. The transepithelial resistance (R_T) is equal to

$$(R_s)(R_{ap} + R_{ba})/(R_{ap} + R_{ba} + R_s) \tag{5}$$

(see below) and is proportional to the iR drop across the RPE. The ratio of R_{ap} to R_{ba} (R_{ap}/R_{ba}) is proportional to the ratio of the iR drops across the apical and basal membranes.

The tissues used in these studies met the same minimum criteria for TEP, R_T, and RPE membrane potentials as in previous studies. The mean TEP (apical positive) in control Ringer's solution for the isolated RPE-choroid preparation was 8.8 ± 2.8 mV (SD) and the mean transepithelial resistance, R_T, was 2.8 ± 0.7 kΩ (n = 30 tissues). Intracellular recordings were made from 32 RPE cells with a mean apical resting potential (V_{ap}) of −85 ± 6 mV (SD).
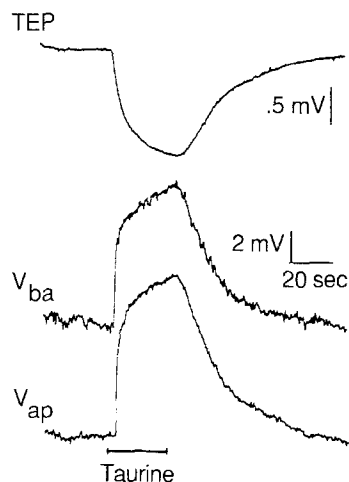

Fig. 2. Representative response of the isolated RPE-choroid to a relatively brief (30-sec) apical taurine exposure. RPE-choroid preparations were superfused over both the apical and basal surfaces with control Ringer's solution (see Materials and Methods). At the time indicated, the apical perfusate was switched to one in which taurine (10 mM) had been added. The top tracing represents the TEP, the middle and bottom tracings represent the potential difference between the intracellular electrode and the basal (V_{ba}) and apical (V_{ap}) baths, respectively

Results

Response of the RPE to Brief Taurine Exposure

Abrupt and transient exposure of the apical surface of the RPE to taurine (10 mM) produced a consistent decrease in TEP, with recovery upon taurine removal. In 22 tissues, this decrease in TEP averaged 1.34 ± −0.44 mV (mean ± SD) and was maximal within 2 min. Figure 2 depicts a typical response evoked by a 30-sec exposure to 10 mM taurine in the apical perfusate only. Taurine decreased the TEP and depolarized the apical membrane to a greater ($P < 0.01$) degree than the basal membrane (6.91 ± 2.56 vs. 5.46 ± 2.24 mV; n = 22). Indeed, since TEP equals V_{ba} minus V_{ap}, the consistent decrease in TEP following taurine exposure indicates that the fall in V_{ap} consistently exceeded the fall in V_{ba}. When the taurine was removed, the potentials returned to their control levels.

As described in Materials and Methods, the larger apical response indicates that the depolarization originates at the apical membrane; some of this depolarization is passively shunted to the basal membrane through a shunt resistance so that the basal membrane also depolarizes. This passive basal response also explains why the magnitude of the apparent change in V_{ap} considerably exceeded the
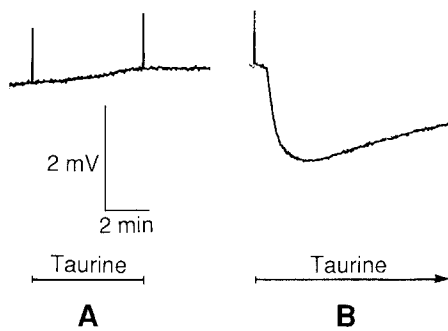
Fig. 3. Response of the isolated RPE-choroid to taurine in sodium-free (*A*) and control Ringer's (*B*) solutions. The RPE-choroid was initially superfused with Ringer's solution on both the apical and basal surfaces and the apical perfusate was then switched to one in which N-methyl-D-glucamine (NMDG) isosmotically replaced sodium. At the time indicated by the bar (*A*), the apical perfusate was switched to sodium-free solution with 10 mM taurine. Between the responses shown in (*A*) and (*B*), the apical perfusate was switched to taurine-free control Ringer's solution. At the time indicated by the bar in (*B*), the tissue was exposed again to 10 mM taurine, now in the presence of a normal sodium concentration

magnitude of the fall in TEP. If 10 mM taurine was introduced into the basal bathing solution only, no changes in potential were observed (*not shown*).

RESPONSE OF THE RPE TO TAURINE: EFFECT OF SODIUM SUBSTITUTION

This response is consistent with the presence of electrogenic, Na$^+$/taurine cotransport across the apical membrane. Analogous findings have been reported following acute exposure of amphibian and mammalian small intestine to amino acids or sugars known to be taken up via electrogenic sodium-coupled transport mechanisms (Rose & Schultz, 1971; Gunter-Smith, Grasset & Schultz, 1982; Grasset, Gunter-Smith & Schultz, 1983; Hudson & Schultz, 1984).

If the RPE response to apical taurine exposure actually reflects electrogenic taurine uptake, then elimination of sodium from the apical bath should blunt the tissue response to taurine. Figure 3 demonstrates that this is the case; the RPE-choroid failed to exhibit any TEP response to apical taurine in sodium-free (NMDG-substituted, *see* Materials and Methods) media, but subsequently responded normally to apical taurine in the presence of control Ringer's solution. In separate experiments (*not shown*), identical results were observed with medium in which sodium was replaced by Tris/choline (see Materials and Methods), and the findings were the same regardless of the exposure sequence; that is, taurine in sodium-free medium followed by
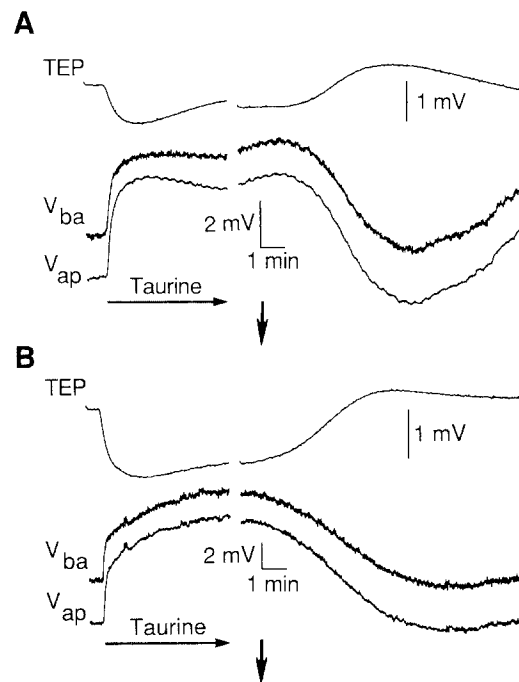

Fig. 4. Representative responses of the isolated RPE-choroid to a relatively prolonged (minutes) exposure to taurine. Experiments were performed as in Fig. 2. Those portions of the response coinciding with initial exposure to taurine (left panel, horizontal arrows) and taurine removal (right panel, vertical arrows) are shown. (*A*) Shows recordings of TEP and membrane potential in a tissue in which some recovery of TEP occurred in conjunction with apical membrane hyperpolarization during continued taurine exposure. (*B*) Shows similar recordings in a tissue in which the return of TEP toward baseline was accompanied by depolarizations of both membranes

taurine in Ringer's solution or vice versa. Finally, in .two tissues in which continuous intracellular recording was possible, sodium-free medium eliminated not only the TEP response to taurine, but the accompanying changes in V_{ap} and V_{ba} as well.

RESPONSE OF THE RPE TO MORE PROLONGED TAURINE EXPOSURE

When exposure to taurine was continued for several minutes, the initial, abrupt fall in TEP was followed by a variable phase. In some tissues, TEP returned toward baseline values (increased) despite continued taurine exposure, whereas in other tissues no return phase or a slower phase of continued TEP decrease was observed. After removal of taurine from the apical bath, TEP returned to normal over a period of several min, and a rebound increase in TEP above baseline was observed in some tissues. Figure 4 shows two examples of the variability in RPE membrane potential changes during responses

in which the TEP increased toward baseline during taurine exposure. Similar variability was also observed during responses in which the TEP continued to decrease (*not shown*). Figure 4*A* shows responses evoked by a 10-min exposure to 10 mM apical taurine. During the initial fall in TEP, the apical membrane depolarized relative to the basal membrane, consistent with the depolarization generated at the apical membrane. During the subsequent increase in TEP toward baseline in the continued presence of taurine, the apical membrane hyperpolarized relative to the basal membrane. This indicates that a slow hyperpolarization was generated at the apical membrane and passively shunted to the basal membrane. Figure 4*B* shows another example of a tissue response in which the initial fall in TEP was followed by a return toward baseline during taurine exposure. In this case, however, the TEP return (increase) is accompanied by a depolarization of the basal membrane relative to the apical membrane. This indicates that the TEP increase toward baseline is generated by a depolarization that originates at the basal membrane.

The changes in TEP, V_{ap}, and V_{ab} occurring in response to taurine exposure for several minutes were also accompanied, in some tissues, by a change in the ratio of the apical-to-basal membrane resistances (R_{ap}/R_{ba}). In seven tissues with continuous intracellular recordings that exhibited partial or complete TEP recovery despite continued exposure to taurine (Fig. 4), R_{ap}/R_{ba} averaged 0.30 ± 0.15 (range = 0.08–0.56) prior to taurine (10 mM) exposure and increased an average of 108 ± 38% (range = 58–146%) to 0.58 ± 0.22 (range = 0.19–0.88). R_T in these tissues (2.9 ± 0.7 KΩ, pre-taurine) either remained unchanged or decreased slightly (2.8 ± 0.7 KΩ post-taurine; 4.2% decrease overall). In contrast, in those tissues that exhibited minimal TEP recovery, R_{ap}/R_{ba} averaged 0.45 ± 0.52 (range = 0.23–0.88) prior to taurine (10 mM) exposure and increased only 14.2 ± 2% to 0.51 ± 0.42.

The source of the variability in the TEP and/or membrane responses was not obvious and the response exhibited by individual tissues occasionally changed during the course of an experiment. In this study, we chose to further examine the contribution of apical membrane hyperpolarization to the recovery in TEP toward baseline, after its initial fall, and to focus, in particular, on the effect of taurine on the activity of the apical Na$^+$-K$^+$ pump.

RESPONSE OF THE RPE TO TAURINE:
EFFECT OF Na$^+$-K$^+$-PUMP INHIBITORS

Figure 5 illustrates the response of a single tissue to taurine in the absence and again in the presence of
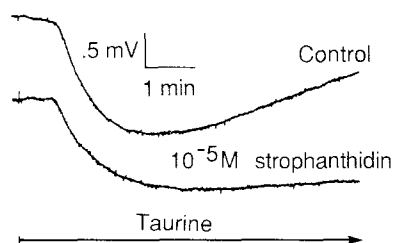

Fig. 5. Response of a single tissue to taurine in the presence and absence of 10^{-5} M strophanthidin. The RPE-choroid was first exposed to apical taurine in the absence of strophanthidin (*control*, top tracing). Following taurine removal, strophanthidin (10^{-5} M) was added to the apical perfusate (*not shown*), and the RPE-choroid was again exposed to 10 mM taurine (bottom tracing) in the continued presence of strophanthidin. This response was recorded from the same tissue as shown in Fig. 4*A*, in which the TEP return (increase) was generated by a slow apical hyperpolarization. The absolute value of the TEP in this RPE-choroid was 7.5 and 4.0 mV, respectively, prior to taurine exposure in control and strophanthidin-containing perfusate

strophanthidin (10^{-5} M). In the absence of strophanthidin, TEP returned toward baseline during continued taurine exposure. This increase in TEP was abolished by the presence of 10^{-5} M strophanthidin. Apical membrane hyperpolarization (e.g., Fig. 4*A*) during continued taurine exposure also was not observed in the presence of 10^{-5} M strophanthidin or 10^{-3} ouabain (three tissues studied; *not shown*).

EFFECT OF TAURINE ON THE Na$^+$-K$^+$ PUMP

The observations summarized above and, in particular, the effect of Na$^+$-K$^+$-pump inhibitors (Fig. 5), indicate that intact Na$^+$-K$^+$ pump function is necessary for the recovery in TEP in some tissues following the initial fall upon taurine exposure and for apical membrane hyperpolarization following the initial depolarization induced by taurine. The next series of studies was designed to study further the effect of taurine on the apical Na$^+$-K$^+$ pump and to explore directly the possibility that taurine exposure leads to stimulation of pump activity.

The RPE apical membrane has a large relative gK^+ so that apical membrane potential is influenced by the concentration of potassium in the apical bath $[K^+]_o$. Thus, increasing $[K^+]_o$ depolarizes the apical membrane and decreases the TEP, while lowering apical $[K^+]_o$ has the opposite effect (Miller & Steinberg, 1977). If, however, the apical membrane is first exposed to barium (BaCl$_2$), which blocks K$^+$ channels (e.g., Armstrong & Taylor, 1980; Eaton & Brodwick, 1980), then changing apical $[K^+]_o$ has the opposite effect on membrane potential. That is, increasing $[K^+]_o$ increases the apical membrane po-
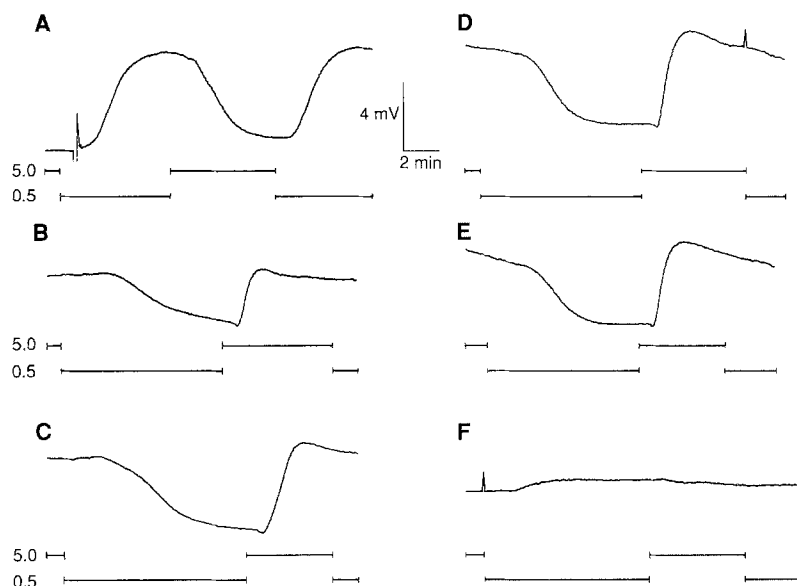
A

B

C

D

E

F

4 mV

2 min

5.0
0.5

Fig. 6. The effect of barium and varying taurine concentrations on the TEP response of the RPE-choroid to alternating apical $[K^+]_o$. RPE-choroid, initially superfused on both surfaces with Ringer's solution, was then exposed to alternating test solutions containing either 5 or 0.5 mM K. In (A), TEP increased when $[K^+]_o$ decreased. The same maneuver was then repeated in the presence of 0.5 mM apical barium ($BaCl_2$) (B), apical barium plus 0.5 mM taurine (C), apical barium plus 1.0 mM taurine (D), apical barium plus 4.0 mM taurine (E), and apical barium plus 4.0 mM taurine plus 0.5 mM ouabain (F). In the presence of barium (B–E), the TEP decreased when $[K^+]_o$ decreased, and the magnitude of this response was increased in the presence of taurine. TEP in this RPE-choroid ranged from 6.3 to 7.6 mV just prior to the responses shown in (A) to (E) and was 3.0 mV after ouabain exposure (F)

tential and decreasing $[K^+]_o$ decreases the apical membrane potential. This results from modulation of the contribution of the electrogenic Na^+-K^+ pump to apical membrane potential, which is unmasked in the presence of barium (Griff et al., 1985).

Figure 6A and B illustrate the TEP response of the RPE to changing apical $[K^+]_o$ in the absence and presence of apical barium, respectively. In Fig. 6A, a *decrease* in $[K^+]_o$ from 5.0 to 0.5 mM increased the TEP. When $[K^+]_o$ was increased back to 5.0 mM, TEP returned to baseline. Between the response shown in Fig. 6A and B the apical perfusate was switched to a solution containing 0.5 mM barium. This caused a decrease in the TEP of about 4 mV. The apical bath $[K^+]$ was again switched from 5.0 to 0.5 mM in the continued presence of barium, and, as shown in Fig. 6B, TEP now decreased. As discussed above, this decrease in TEP associated with decreasing $[K^+]_o$ in the presence of barium is due to a decrease in the activity of the electrogenic apical Na^+-K^+ pump.

Next, various concentrations of taurine were added to the apical bath along with barium, and, with each concentration, $[K^+]_o$ was again switched from 5.0 to 0.5 mM. As shown in Fig. 6C–E, taurine, in concentrations of 0.5, 1.0, and 4.0 mM, respectively, considerably exaggerated the changes in TEP associated with changing $[K^+]_o$, and these changes were in turn abolished by apical ouabain (Fig. 6F). Similar results were obtained in five tissues with varying concentrations of taurine and K^+ in the apical bath. Collectively, these findings are consistent with a taurine-induced stimulation of an apical, electrogenic Na^+-K^+ pump. The results in

Figs. 6F and 7D also indicate that the concentration of barium used in this study effectively blocked apical gK^+.

To more directly assess the effect of taurine on Na^+-K^+ pump function as well as the reversibility of any such affect, barium-treated RPE-choroid was again exposed to alternating apical $[K^+]_o$ in the presence and absence of 0.5 mM taurine while continuously recording from within the same RPE cell. In the recording shown in Fig. 7, $[K^+]_o$ was *increased* from 0.5 to 5.0 mM and then returned to 0.5 mM. This transient increase in apical $[K^+]_o$ was associated with a reversible increase in TEP. The intracellular recordings in Fig. 7A demonstrate that the increase in TEP was accompanied, as expected, by a hyperpolarization of the apical membrane relative to the basal membrane (Griff et al., 1985). As described in Materials and Methods, these results are consistent with the major part of the Na^+-K^+ pump current flowing across the apical membrane. Current also flows through the shunt and then across the basal resistance to cause the smaller basal hyperpolarization. Between the responses shown in Fig. 7A and B, 0.5 mM taurine was added to the apical perfusate in the continued presence of barium, and Fig. 7B shows the response evoked by an increase in $[K^+]_o$, again from 0.5 to 5.0 mM, in the presence of both barium and taurine. The intracellular recordings, Fig. 7A–C, show that the magnitude of this apical hyperpolarization was reversibly increased about twofold in the presence of taurine. Figure 7D shows that the response to changing apical $[K^+]_o$ was abolished when ouabain was added in the continued presence of both barium and taurine. The experiment depicted in Fig. 7 (with
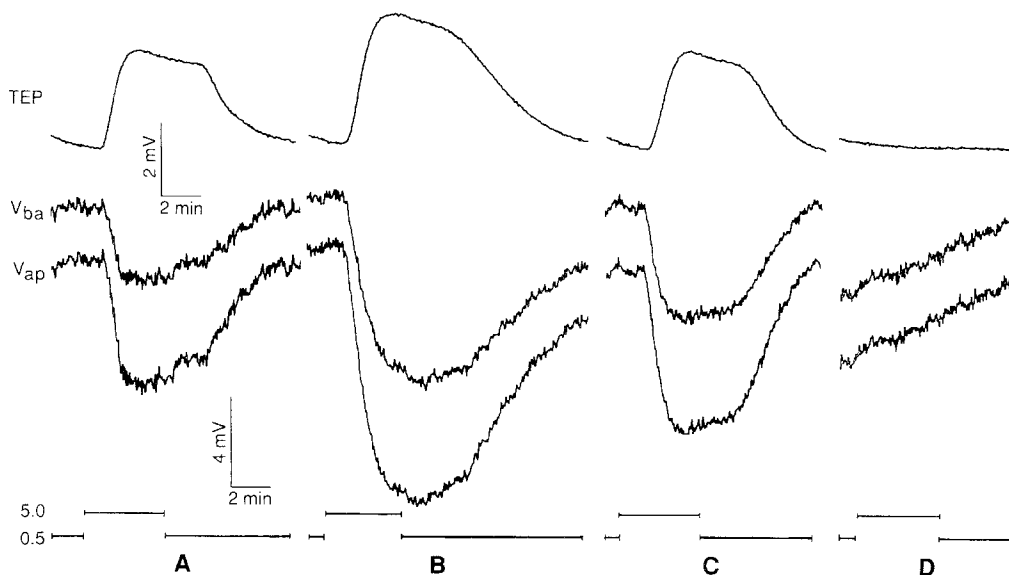
Fig. 7. Response of the barium-treated isolated RPE-choroid to changing apical $[K^+]_o$ in the presence and absence of taurine. The RPE-choroid was initially exposed to barium ($BaCl_2$) (0.5 mM) in the apical perfusate and apical $[K^+]_o$ was alternated between 0.5 and 5.0 mM (A). This same maneuver was repeated in the presence of apical (0.5 mM) taurine (B), again in the absence of taurine (C), and finally in the presence of both 0.5 mM taurine and 0.5 mM ouabain (D). In (D), both membrane potentials continuously depolarize as ionic gradients run down in the presence of ouabain; no $[K^+]_o$-evoked response is observed superimposed on this steady depolarization. TEP in this RPE-choroid ranged from 4.5 to 6.2 mV in (A) to (C) and was 2.2 mV after ouabain exposure (D).

TEP as well as intracellular recordings) was carried out in three different tissues with equivalent results.

Finally, to exclude the possibility that the enhanced apical hyperpolarization induced by increasing apical $[K^+]_o$ in the presence of barium and taurine was not simply due to an increased apical membrane resistance, which could account for an increase in the excursions in V_{ap} without an increase in pump activity, a similar experiment was performed (as in Fig. 7), while R_T and R_{ap}/R_{ba} were also measured. In a single tissue in which continuous intracellular recording with repeated current passage was possible, R_T and R_{ap}/R_{ba} were essentially identical in the presence and absence of taurine, despite taurine-induced enhancement of the apical hyperpolarization (Fig. 8). Collectively, these observations indicate that taurine stimulates a ouabain-sensitive, electrogenic apical pump—presumably the Na⁺-K⁺ pump.

Specificity of the Taurine Effect

Because the present studies as well as previous findings (Miller & Steinberg, 1977) suggest that a sodium-dependent uptake step mediates the active apical-to-basal transport of taurine, it might be expected that the RPE would respond similarly to other substrates transported via a sodium-coupled

mechanism. This was indeed the case. Alanine (10 mM), which is known to be taken up by a sodium-coupled mechanism and to enhance sodium entry into mammalian cells (Van Dyke & Scharschmidt, 1983; Grasset et al., 1983), produced changes in TEP, V_{ap}, and V_{ba} in two tissues (Fig. 9) very similar to those caused by taurine.

Discussion

The present studies have characterized the effect of taurine on the isolated RPE-choroid, with a particular focus on the determinants of taurine-induced changes in TEP and the effect of taurine on the apical Na⁺-K⁺ pump. The major findings are as follows: (i) Acute and continued exposure of the RPE to taurine in the apical (but not basal) bath produces an abrupt fall in the normally apical positive TEP followed by a variable degree of TEP recovery. (ii) The initial fall in TEP results from apical membrane depolarization. It persists in the presence of strophanthidin or ouabain, but does not occur upon apical exposure to taurine in sodium-free medium. (iii) The variable TEP recovery is accompanied by an increase in R_{ap}/R_{ba} in some tissues and is blocked by strophanthidin or ouabain. (iv) Apical taurine increases the magnitude of the RPE response evoked by changing apical $[K^+]_o$ in the continued presence
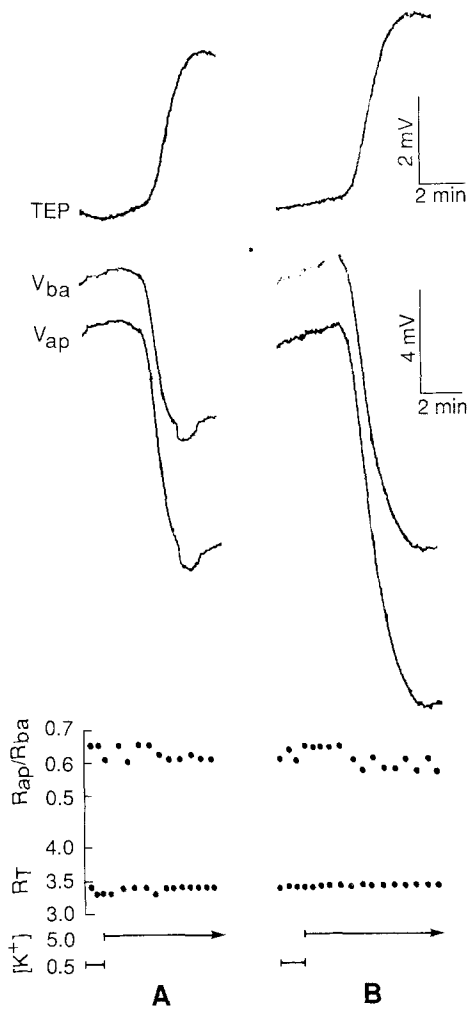
Fig. 8. Response of the barium-treated isolated RPE-choroid to changing apical $[K^+]_o$ in the absence and presence of 1 mM taurine. A single RPE-choroid was initially exposed to barium $(BaCl_2)$ (0.5 mM) in the apical perfusate in the absence of taurine, and apical $[K^+]_o$ was changed from 0.5 mM to 5.0 mM (A). Between the responses shown in (A) and (B), the tissue was exposed to 1 mM taurine, and the maneuver was repeated, now in the presence of taurine (B). The ratio of apical-to-basal membrane resistance R_{ap}/R_{ba} and total tissue resistance (R_T) were calculated from repeated passage of current through the tissue (see Materials and Methods). TEP in this RPE-choroid ranged from 4.4 to 5 mV at 5 mM apical $[K^+]$ during the experiment depicted in (A) and (B)

Fig. 9. Response of the isolated RPE-choroid to 10 mM alanine. The RPE-choroid was superfused over both the apical and basal surfaces with control Ringer's solution (see Materials and Methods). At the time indicated, the apical perfusate was switched to one to which alanine (10 mM) had been added. As in previous figures, the top tracing represents the TEP; the middle and bottom tracings represent the potential difference the intracellular electrode and the basal (V_{ba}) and apical (V_{ap}) baths, respectively

of barium, a response due to stimulation of the Na^+-K^+ pump. Before discussing the implications of these findings with respect to RPE function *in situ* or other epithelia, it is appropriate to consider the electrical response of the RPE-choroid to taurine and taurine transport in some detail.

The response of the RPE-choroid to taurine exposure consists of at least two phases. The first phase consists of a fall in the normally 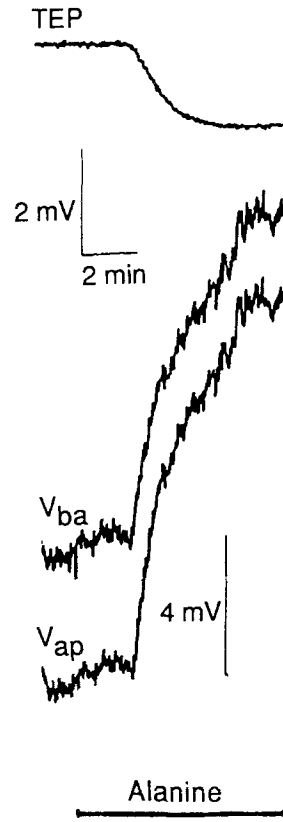apical positive TEP attributable to a predominant apical membrane depolarization. As would be expected from the equivalent circuit analysis of the RPE-choroid (*see* Materials and Methods) and as has been noted in the response of other epithelia to actively transported solutes (Gunter-Smith et al., 1982; Grasset et al., 1983), the magnitude of the apical membrane depolarization considerably exceeds that of the fall in TEP, reflecting a simultaneous and presumably passive or "shunted" depolarization of the basal membrane. This phase persists in the presence of ouabain but is absent in sodium-free media and is very likely attributable to an influx of positive charge across the apical membrane in the form of Na^+ accompanying the uptake of taurine, a neutral amino acid, via a symport mechanism. The present findings, as well as previous observations (Miller & Steinberg, 1979), are all consistent with this formulation, and taurine is known to be taken up via a sodium-coupled mechanism in other tissues (Bucuvalas, Goodrich & Suchy, 1987). Moreover, the

TEP response to alanine, which is also taken up by a sodium-coupled mechanism in a wide variety of tissues (Grasset et al., 1983; Van Dyke & Scharschmidt, 1983) is very similar to that of taurine.

The second (i.e., later) phase of the tissue response to continued taurine exposure is both more complex and variable, and likely represents concurrent basolateral as well as apical membrane events. One late event occurring in response to continued taurine exposure, apical membrane hyperpolarization, was a focus of our study. This hyperpolarization was readily apparent within minutes in some tissues (e.g., Fig. 3A), but not in others (e.g., Fig. 3B). However, it is tempting to speculate that it was present in all tissues, but was occasionally masked by basal membrane events. This tentative conclusion is supported by our observation that the taurine-induced stimulation of the Na$^+$-K$^+$ pump evident with alternating apical [K$^+$]$_o$ in the presence of barium (Figs. 6 and 7) was present in every tissue examined.

Apical membrane hyperpolarization during the second phase of the tissue response is very likely attributable to activation of the apical Na$^+$-K$^+$ pump, and we speculate that this Na$^+$-K$^+$ pump activation results from taurine-induced stimulation of sodium influx, with a resultant increase in intracellular [Na$^+$] (Skou, 1965; Garay & Garrahan, 1973; Shimazaki & Oakley, 1984). This formulation is based on the following evidence: (i) apical membrane hyperpolarization and an increase in TEP is the expected response of the RPE to activation of an apical electrogenic pump, based on equivalent circuit analysis (Fig. 1); (ii) the response was blocked by ouabain or strophanthidin, indicating that intact Na$^+$-K$^+$ pump function is required for the response; (iii) separate studies (discussed below) provide direct evidence for electrogenic Na$^+$-K$^+$ pump stimulation by taurine; and (iv) studies in other cell types have demonstrated an increase in Na$^+$-K$^+$ pump activity, measured as short-circuit current or as ouabain-suppressible ^{86}Rb uptake, in response to enhanced uptake of sodium, and the increased Na$^+$-K$^+$ pump activity in these tissues exhibited a sigmoidal relationship to intracellular sodium activity (Van Dyke & Scharschmidt, 1983; Turnheim, Thompson & Schultz, 1987). It has recently been reported that cAMP stimulates the Na$^+$-K$^+$ pump in frog RPE (Hughes et al., 1988), and the present findings do not exclude the possibility that cAMP mediates the effect of taurine on pump function. Further examination of the mechanism by which taurine stimulates pump function will require direct measurement of intracellular Na$^+$ and cAMP.

Changes in basal membrane potential also contributed to the second or later phase of the tissue response to taurine. For example, in some tissues, depolarization of the basal membrane clearly contributed to the late recovery in TEP observed during prolonged taurine exposure (Fig. 3B). Moreover, in those tissues in which TEP recovery occurred, an increase in R_{ap}/R_{ba} was observed. Because simultaneously measured R_T either did not change or decreased slightly, the increase in R_{ap}/R_{ba} suggests that R_{ba} decreased. The mechanism of this response was not explored in these studies; however, several possibilities exist. For example, enhanced basal potassium conductance and efflux have been observed in other tissues in association with enhanced Na$^+$-coupled solute transport (Grasset et al., 1983; Lau, Hudson & Schultz, 1984), and taurine has previously been shown to stimulate the apical-to-basal transport of potassium several-fold in the RPE (Miller & Steinberg, 1979). Recent observations from this laboratory (J. Immel & R. Steinberg, unpublished observations) showed that K$^+$ in the space between the RPE and choroid of isolated tissue, measured with K$^+$-selective electrodes, was almost 1.0 mM higher than in the basal solution, suggesting that the choroid represents a significant diffusion barrier for potassium. Thus, taurine may act by increasing the apical-to-basal potassium flux and the potassium concentration in the subchoroidal space, thereby causing basal membrane depolarization. Alternatively, as recently proposed for cAMP (Hughes et al., 1988), basal membrane depolarization may result from an increase in the conductance of the basal membrane to anions.

Our observations regarding the effect of taurine on the apical Na$^+$-K$^+$ pump in the RPE also merit consideration in relation to studies in other epithelia. In previous studies in other tissue types, an increase in activity of the (basal) Na$^+$-K$^+$ pump has been inferred from indirect evidence; including changes in short circuit current and intracellular Na$^+$ activity induced by actively transported solutes or the effects of metabolic inhibitors or ouabain on the tissue response (Rose & Schultz, 1971; Gunter-Smith et al., 1982; Grasset et al., 1983; Hudson & Schultz, 1984). Alternatively, electrogenic Na$^+$-K$^+$ pump activity in the basolateral membrane has been estimated from measurements of basolateral electromotive forces and resistance (Lewis & Wills, 1983). However, we are not aware of a previous study in which changes in electrogenic pump activity have been directly demonstrated to occur in association with exposure to a solute known to be taken up via a Na$^+$-coupled mechanism. Indeed, Grasset et al. (1983) have pointed out that the delayed electrical response (TEP recovery and basal membrane hyperpolarization) of Necturus small intestine following exposure to alanine could be ex-

plained by an increase in basal gK^+ alone without necessarily invoking Na^+-K^+ pump stimulation. In these studies, we have exploited the accessibility of the apical RPE Na^+-K^+ pump to rapid solution changes and the novel maneuver of blocking apical gK^+ while changing apical $[K^+]_o$ (Griff et al., 1985) to demonstrate directly taurine-induced *stimulation* of a ouabain-sensitive, electrogenic apical Na^+-K^+ pump. While it is appropriate to consider alternative explanations for our findings, they seem unlikely. For example, the possibility that taurine does not affect the pump but instead increases apical membrane resistance so as to increase the *IR* (voltage) drop across the apical membrane appears to be excluded by direct measurement of R_T and R_{ap}/R_{ba} in the presence and absence of taurine (Fig. 8). Thus, direct stimulation of the Na^+-K^+ pump, likely due to alteration of $[Na^+]_i$ and/or other events such as altered cAMP, or cell swelling (Lau et al., 1984; Hughes et al., 1988), emerges as the most plausible explanation for the findings shown in Figs. 6–8.

Finally, it is appropriate to consider the implications of these findings with respect to the function of the RPE in vivo. In the dark-adapted frog retina, $[K^+]_o$ in the subretinal space is approximately 3–4 mm (Oakley & Green, 1976; Oakley et al., 1977). A flash of light produces, within seconds, a decrease in $[K^+]_o$ in the subretinal space (Oakley & Green, 1976) and a presumed release of taurine into the subretinal space (Salceda et al., 1977; Schmidt, 1978). The light-evoked $[K^+]_o$ decrease itself inhibits the Na^+-K^+ pump as shown by experiments using barium in a retina-RPE preparation (Griff & Steinberg, 1984). With maintained illumination, $[K^+]_o$ reaccumulates. The situation is thus complex, and the electrical response of the RPE *in situ* would appear to depend upon the normal sequence of events as well as the magnitudes of the decrease in the concentration of potassium and increase in the concentration of taurine. At present, the timing of the presumed light-evoked taurine changes relative to the potassium changes are unknown, as is the concentration of taurine in the subretinal space in vivo during light or dark conditions. However, because taurine is the most abundant amino acid in the vertebrate retina and reaches intracellular concentrations of 60–80 mm, it seems quite possible that the same effect on Na^+-K^+ pump activity of the type observed in these in vitro studies at an extracellular taurine concentration of 0.5 mm also occurs in vivo.

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