

# Response Properties of the Toad Retinal Pigment Epithelium

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Electrophysiologic responses were recorded *in vitro* from the retina of the toad, *Bufo marinus*. In the isolated retinal pigment epithelium (RPE)-choroid preparation, a decrease in the  $K^+$  concentration outside the apical membrane evoked a sequence of trans-epithelial (TEP) and membrane potential changes. A hyperpolarization originating at the RPE apical membrane that increased the TEP was followed by a delayed hyperpolarization originating at the basal membrane that decreased the TEP. This response sequence differed from the well-studied bullfrog RPE in exhibiting potentials generated at the basal membrane; it more closely resembled responses from the reptilian and avian RPE. The toad direct-current electroretinogram recorded from a neural retina-RPE-choroid preparation also differed from other amphibians and was more similar to responses recorded in reptiles, birds, and mammals. In this initial study, the electrophysiologic characteristics of the RPE's  $Na^+/K^+$  pump were examined. Contrary to a previous report, ouabain, a specific inhibitor of the pump, depolarized the apical membrane and decreased the TEP.  $Ba^{2+}$  was used to block apical  $K^+$  conductance and unmasked a modulation of the  $Na^+/K^+$  pump by subretinal  $K^+$ . Decreasing the  $K^+$  concentration in the presence of  $Ba^{2+}$  depolarized the apical membrane and decreased the TEP. These responses were blocked by ouabain, indicating that the  $K^+$  concentration decrease slowed the  $Na^+/K^+$  pump. Invest Ophthalmol Vis Sci 31:2353-2360, 1990

The retinal pigment epithelium (RPE) consists of a single layer of cells whose apical membrane faces the neural retina and whose basal membrane faces the choroid. This report describes responses from the toad, *Bufo marinus*, and establishes the toad retina as a novel amphibian model. Two different RPE response patterns have been evoked by light stimulation of the vertebrate neural retina and recorded in the direct-current electroretinogram (DC-ERG), depending on the species. In fish and amphibians that have been studied previously (carp, eel, frog, and newt), the DC-ERG is relatively simple; after the a- and b-waves of the ERG, the c-wave rises to a peak and then slowly decays toward the dark-adapted baseline.<sup>1,2</sup> In reptiles, birds, and mammals, after the peak of the c-wave, there is a trough, the fast-oscillation trough, and then a slow rise to the light peak.<sup>1,3-5</sup> Intracellular RPE recordings in the cat, chick, and gekko show that the fast-oscillation trough and the light peak are generated by potentials that originate primarily at the RPE basal membrane.<sup>3,5</sup> However, the study of basal membrane mechanisms has been

hampered by lack of a suitable *in vitro* preparation. In RPE-choroid preparations from the lizard, *Gekko gekko*, the choroid prevents free diffusion of substances from the choroidal superfusate to the basal membrane, making it difficult to study basal membrane mechanisms further.<sup>6</sup> The chick retina is prone to retinal spreading depression.<sup>5,7</sup> In this report, I show that responses of the toad RPE are similar to those of the gekko RPE and that substances can reach the basal membrane through the choroid. Thus, the toad RPE will be an important new model for studying responses that originate at the basal membrane. The toad RPE has not been studied recently, in part because anomalous transport properties had been observed. A previous report indicated that ouabain, a specific inhibitor of the  $Na^+/K^+$  pump, did not alter the trans-epithelial potential (TEP) of the toad,<sup>8</sup> as had been observed in all other vertebrate RPEs studied. It was, therefore, critical to reexamine the  $Na^+/K^+$  pump of the toad RPE and to establish its electrophysiologic characteristics.

## Materials and Methods

### Preparation

*B. marinus* toads were obtained from Delta Biologicals (Tucson, AZ), kept in large tanks, and fed a diet of crickets. Animals were killed by decapitation, pithed, and enucleated under dim red light. All pro-

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Supported by a grant from the National Institutes of Health (EY05893).

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cedures adhered to the ARVO Resolution on the Use of Animals in Research. The eyes were sectioned behind the lens and the posterior portion of the eye submerged in perfusate. The neural retinal-RPE-choroid was dissected free from the sclera, mounted in a chamber, and stimulated with light, as described previously for the chick and gecko.<sup>5,9</sup> For experiments using the isolated RPE, animals were dark-adapted for 12 hr before each experiment to facilitate removal of the neural retina from the RPE; the remaining RPE-choroid was then mounted as previously described for the bullfrog.<sup>10</sup> The area of exposed tissue was 0.07 cm<sup>2</sup>.

Both the apical (or retinal) and choroidal surfaces of the tissue were continuously superfused at 2–5 ml/min by a gravity-feed system from two large reservoirs in which each solution was bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. The volume of each side of the chamber was 2.5 ml. The control perfusate was a modified Ringer's solution having the following composition: 82.5 mM NaCl, 27.5 mM NaHCO<sub>3</sub>, 2.0–5.0 mM KCl, 1.0 mM MgCl<sub>2</sub>, 1.8 mM CaCl<sub>2</sub>, 10.0 mM glucose, pH 7.4. Test solutions were prepared by adding substances in the following concentrations: 0.1–0.3 mM ouabain, 0.01 mM strophanthidin, 0.2 or 1.0 mM BaCl<sub>2</sub>, 0.1 mM dinitrophenol (DNP), 0.1 mM 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS).

### Electrodes and Recording

The placement of electrodes and recording electronics were similar to previously published methods.<sup>6,10</sup> In brief, the potential across the tissue was measured by a pair of calomel electrodes connected to the apical (or retinal) and choroidal chambers by agar-Ringer bridges. Conventional microelectrodes were used to measure simultaneously the apical membrane potential,  $V_{ap}$ , recorded differentially between the microelectrode and the apical bath, and the basal membrane potential,  $V_{ba}$ , recorded differentially between the microelectrode and the choroidal bath. The polarity of each membrane potential was inside with respect to outside.

Two resistance ratios were calculated from potential changes induced by injecting 1.0  $\mu$ A current pulses across the isolated RPE. The total resistance across the epithelium,  $R_t$ , was calculated from the current-induced change in TEP. The ratio of the apical to basal resistances,  $a$ , was calculated from the ratio of the current-induced changes in apical and basal membrane potential.

All signals were continuously monitored on a storage oscilloscope (model 5111; Tektronix, Beaverton, OR) and a chart recorder (model 220; Gould Elec-

tronics, Cleveland, OH). Selected responses were stored digitally at 20 kHz using a video cassette recording system (model PCM-8; Medical Systems, Greenvale, NY). Responses were also stored using a computer (model 158; Zenith Data Systems, St. Joseph, MI), an analog to digital board (DASH-16; Metrabyte, Taunton, MA), and a data acquisition program (HSDAS; Quinn-Curtis, Newton Centre, MA). The current-induced potentials that were used to calculate resistances were digitized on the computer at 100 Hz; responses to solution changes were digitized at 2 Hz; the DC-ERG was digitized at 1 Hz; and the faster components of the ERG were redigitized at 60 Hz. The digitized responses were plotted on a digital plotter (model 7550A; Hewlett Packard, San Diego, CA) using a spreadsheet program (Lotus 1-2-3; Lotus Development, Cambridge, MA).

### Analysis of Intracellular Recordings

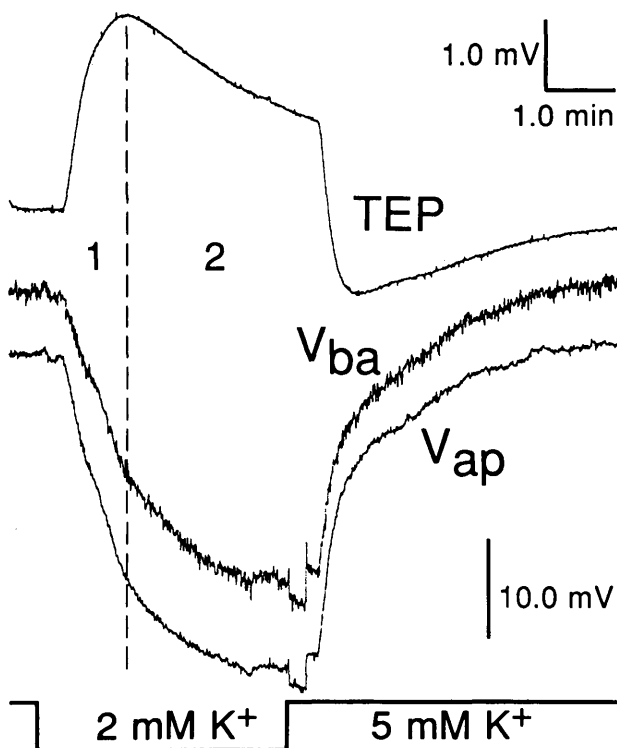
Analysis of RPE membrane potentials has been discussed previously<sup>9,10</sup> and are only briefly described here. The resting RPE apical membrane potential of all species examined is larger (more negative inside) than the basal membrane potential so that the resting TEP is positive when measured outside the apical membrane (subretinal space) with respect to the choroid. The TEP is the difference between the basal and apical membrane potentials:  $TEP = V_{ba} - V_{ap}$ . Thus, a voltage change generated at either membrane will alter the TEP. For example, the TEP will decrease if the apical membrane depolarizes or if the basal membrane hyperpolarizes. Analysis of intracellular responses is complicated, however, because the two membranes are electrically connected via a shunt resistance. For example, even if a change in potential is generated at only one membrane, the other membrane will polarize in the same direction because current flows through the shunt and across that membrane. The membrane generating the voltage change during a specified time period can be identified because it has a larger change in potential and therefore polarizes relative to the other membrane.

### Results

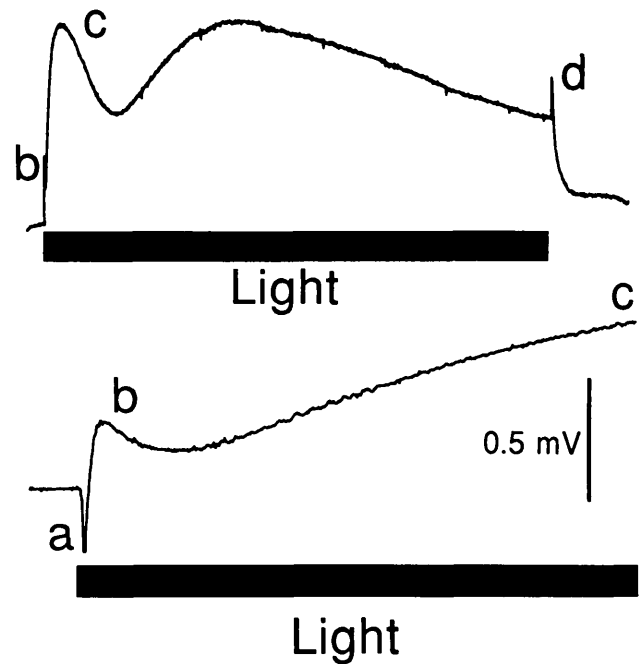
These results are based on recordings from 37 isolated RPE-choroid preparations from 29 toads. The mean TEP was  $23.4 \pm 6.4$  mV (mean  $\pm$  standard deviation), and the mean trans-epithelial resistance,  $R_t$ , was  $3.4 \pm 0.8$  kOhms ( $238 \pm 56$  ohms-cm<sup>2</sup>). The mean apical membrane potential from 41 intracellular recordings from 17 tissues was  $-86.0 \pm 13.2$  mV. The basal membrane was depolarized relative to the apical by an amount equal to the TEP. The mean ratio of apical to basal resistance,  $a$ , in 25 cells from

14 tissues was  $0.54 \pm 0.19$ . Only tissues where TEP was greater than 10 mV and  $R_t$  was greater than 3.0 kOhms at the start of the experiment were used in this study.

Figure 1 shows responses evoked from the toad RPE by decreasing the potassium concentration outside the apical membrane, apical  $[K^+]_o$  from 5 to 2 and back to 5 mM. Decreasing apical  $[K^+]_o$  caused a TEP increase (period 1) followed by a delayed TEP decrease (period 2) while the apical surface was still bathed with the low  $[K^+]_o$  solution. The amplitude of the delayed TEP decrease was variable. When the solution was switched back to 5 mM  $[K^+]_o$ , a TEP decrease was followed by a delayed TEP increase back to baseline. These responses differed from those recorded under similar conditions in the bullfrog RPE where decreasing apical  $[K^+]_o$  caused a monophasic TEP increase, and returning to the control



**Fig. 1.**  $[K^+]_o$ -evoked responses. The transepithelial potential (TEP), apical membrane potential ( $V_{ap}$ ), and basal membrane potential ( $V_{ba}$ ) were measured simultaneously while the apical perfusate was switched from 5 mM  $K^+$  to 2 mM  $K^+$  and back to 5 mM  $K^+$ . Initial values of TEP,  $V_{ap}$ , and  $V_{ba}$  were 7, -71, and -64 mV, respectively. Decreasing  $[K^+]_o$  caused an initial TEP increase (period 1), generated by a hyperpolarization of the apical membrane, followed by a delayed TEP decrease (period 2), generated by a delayed hyperpolarization of the basal membrane. When the apical superfusate was switched back to 5 mM  $K^+$ , a TEP decrease (undershoot) was followed by a slower TEP increase to the baseline. The second solution change caused a moment of instability in the intracellular recordings.



**Fig. 2.** The DC-ERG of the toad. The DC-ERG was recorded as a change in the potential across the retina-RPE-choroid preparation. The top trace shows the response evoked by a 1500-sec light stimulus (bar). The bottom trace shows the initial 20 sec of the response; the a-, b-, c-, and d-waves are indicated.

$[K^+]_o$  caused a monophasic TEP decrease.<sup>9,10</sup> Decreasing  $[K^+]_o$  in the choroidal superfusate caused a small TEP decrease but no delayed TEP increase.

The intracellular recordings in Figure 1 show that a larger hyperpolarization of the apical membrane, accompanied by a smaller hyperpolarization of the basal membrane generated the TEP increase (period 1). This indicates that the hyperpolarization originated at the apical membrane. During the subsequent TEP decrease (period 2), both membranes continued to hyperpolarize, but during this period the basal membrane hyperpolarized relative to the apical membrane so that the TEP decreased. This indicates that the TEP decrease in period 2 was caused by a delayed hyperpolarization that originated at the basal membrane. When the solution was switched back to 5 mM  $[K^+]_o$ , a TEP decrease generated by a depolarization that originated at the apical membrane was followed by a TEP increase generated by a delayed depolarization that originated at the basal membrane. This sequence of membrane-potential changes differed from the  $K^+$ -evoked response in bullfrog and was similar to that in the gecko.<sup>9</sup> These results prompted an examination of the light-evoked response of the toad.

Figure 2 shows responses evoked by a 1500-sec stimulus ( $500 \pm 10$  nm) that delivered  $5.7 \times 10^7$  quanta  $\text{sec}^{-1} \mu\text{m}^{-2}$  to the neural retina-RPE choroid

preparation of the toad. The initial response (bottom traces) consisted of the a-, b-, and c-waves of the ERG. After the peak of the c-wave at about 45 sec (top traces), the potential dipped to a trough (fast-oscillation trough) at about 220 sec, and then increased slowly to a second peak at about 550 sec, after which the potential slowly decreased while the light remained on. At stimulus off, a rapid increase (d-wave) was followed by a decrease that reached a minimum about 950 sec after the light was turned off and slowly returned to the dark-adapted potential (not shown). Similar light-evoked responses were observed in 17 preparations.

A fast-oscillation trough and light peak, responses generated by the RPE in other species,<sup>3-5</sup> have not been found in other amphibians, and this has prompted a detailed examination of the toad RPE. In this study, two approaches were used to examine the apical  $\text{Na}^+/\text{K}^+$  pump in the toad RPE. First, the effects of ouabain on the TEP and the membrane potentials of the toad RPE were investigated. Next, modulation of the pump by  $[\text{K}^+]_o$  was measured electrophysiologically using  $\text{Ba}^{2+}$  to unmask a pump response.<sup>11</sup>

Figure 3 shows that switching the apical perfusate

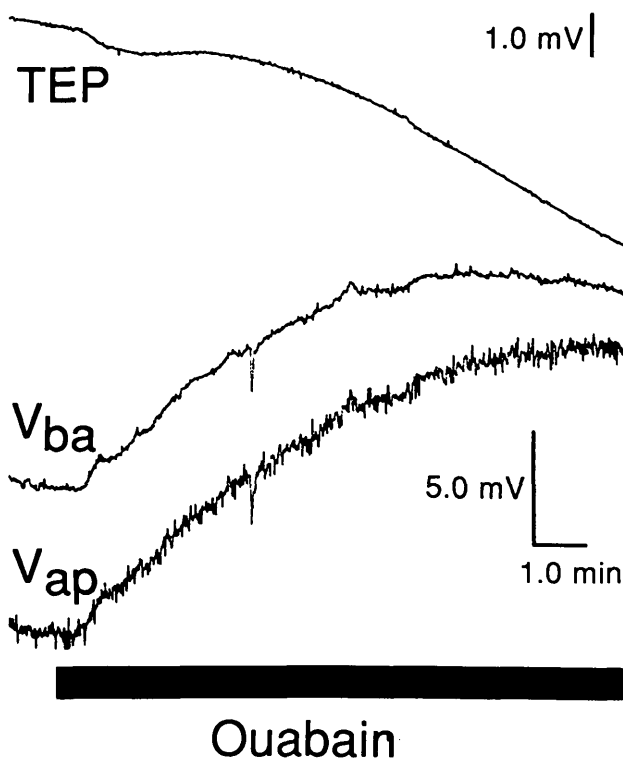


Fig. 3. Effects of ouabain. TEP,  $V_{ap}$ , and  $V_{ba}$  were measured simultaneously while the apical perfusate was switched to a test solution containing 0.2 mM ouabain. Initial values of TEP,  $V_{ap}$ , and  $V_{ba}$  were 17, -73, and -56 mV, respectively. Ouabain caused  $V_{ap}$  to depolarize relative to  $V_{ba}$ , resulting in a decrease in TEP.

to a test solution containing 0.2 mM ouabain decreased the TEP from 10 to 6.4 mV in about 10 min. The intracellular recordings show that both membranes depolarized, with the apical membrane depolarizing relative to the basal membrane. This indicates that the TEP decrease was caused by a depolarization that originated at the apical membrane. For six tissues, the mean apical depolarization was  $7.2 \pm 2.7$  mV. In two of these tissues, including the example shown in Figure 3, there was a small initial fast apical depolarization, as has been shown in bullfrog.<sup>12</sup> In the other tissues, a fast component could not be distinguished.

The effects of ouabain were partly reversible in the toad RPE, as has been observed in the rabbit RPE.<sup>13</sup> In seven tissues where the ouabain test solution was switched back to control Ringer's, the TEP recovered to  $78 \pm 18\%$  of the original value. In three of these, intracellular recordings during at least part of the recovery showed that the recovery was generated by a hyperpolarization of the RPE apical membrane. Strophanthidin ( $10^{-5}$  M), another cardiac glycoside that reversibly inhibits the  $\text{Na}^+/\text{K}^+$  pump in toad rods,<sup>14</sup> was ineffective in decreasing TEP in five of six tissues. In one tissue strophanthidin reversibly decreased TEP 12% from 25 to 22 mV. Ouabain (0.2 mM) applied only to the choroidal superfusate had no effect on the TEP ( $n = 2$ ).

Figure 4 shows that switching the apical superfusate to a test solution containing 0.2 mM  $\text{BaCl}_2$  decreased the TEP, and the intracellular recordings show that the apical membrane depolarized relative to the basal membrane. Similar results with either 0.2 or 1 mM  $\text{Ba}^{2+}$  were recorded in seven cells in seven different tissues. These results indicate that the decrease in TEP was caused by a depolarization that originated at the apical membrane, and the results are consistent with the idea that  $\text{Ba}^{2+}$  blocked one or more apical potassium conductances.

Further evidence that  $\text{Ba}^{2+}$  blocked an apical potassium conductance was obtained by passing current across the tissue and monitoring  $R_i$  and  $a$ . During the first several minutes of  $\text{Ba}^{2+}$  exposure,  $R_i$  and  $a$  both increased. For nine cells from eight tissues,  $R_i$  increased  $13 \pm 11\%$ , and  $a$  increased  $117\% \pm 83\%$ . The increase in  $R_i$  indicates that at least one of the RPE component resistances must have increased. An increase in  $a$  would occur if the apical membrane resistance increased or if the basal resistance decreased. An increase in apical membrane resistance is the simplest way to account for the increase in both  $R_i$  and  $a$ .

With prolonged exposures to  $\text{Ba}^{2+}$ , both  $R_i$  and  $a$  began to decrease. For four cells from four tissues,  $R_i$  decreased  $16 \pm 14\%$  from its maximum in  $\text{Ba}^{2+}$  and  $a$

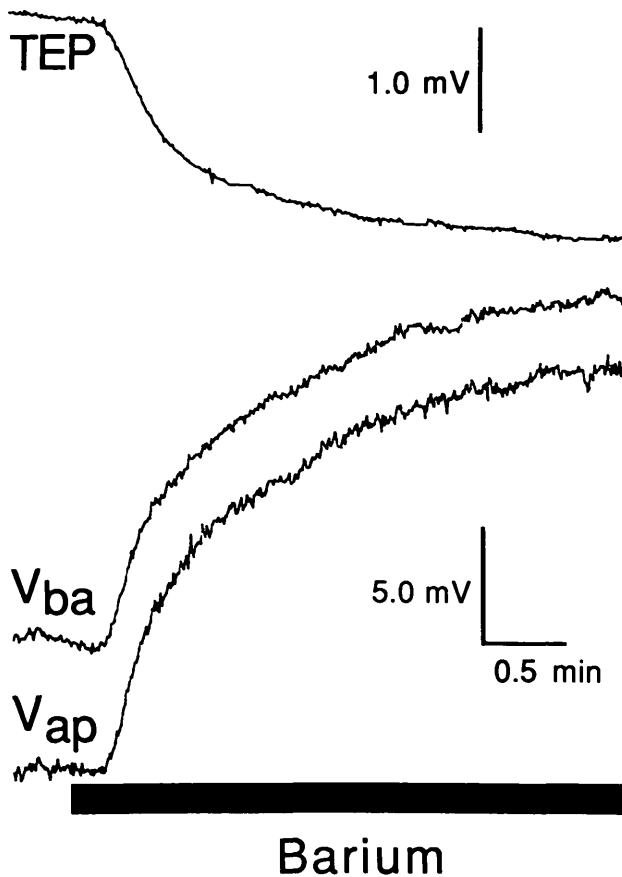


Fig. 4. Effects of barium. TEP,  $V_{ap}$ , and  $V_{ba}$  (initial values 12, -80, and -68 mV, respectively) were measured simultaneously while the apical perfusate was switched to a test solution containing 0.2 mM  $BaCl_2$ .  $Ba^{2+}$  caused  $V_{ap}$  to depolarize relative to  $V_{ba}$  so that the TEP decreased.

decreased  $36 \pm 17\%$ . The simplest explanation for both these changes is a decrease in basal membrane resistance. However, there was no obvious correlation between tissues that exhibited this apparent decrease in basal resistance and those that showed a biphasic response to  $Ba^{2+}$ .

In four of 18 experiments,  $Ba^{2+}$  caused a biphasic response. The TEP decreased during the first 2-3 min and then began to recover (increase) in the continued presence of apical  $Ba^{2+}$ . Intracellular recordings in each of the four tissues showed that the TEP decreased because the apical membrane depolarized relative to the basal membrane. During the subsequent TEP recovery, both membranes continued to depolarize, with the basal membrane depolarizing relative to the apical. This indicates that the second phase of the  $Ba^{2+}$ -evoked response, the TEP increase, was generated by a depolarization that originated at the basal membrane.

Figure 5 shows responses evoked by decreasing apical  $[K^+]_o$  from 3 to 1 mM and back, concentrations chosen so that the results here could be easily

compared with previously published experiments.<sup>11</sup> In the control condition (A), decreasing apical  $[K^+]_o$  primarily caused an increase in TEP. The intracellular recordings show that the apical membrane hyperpolarized relative to the basal membrane indicating that the TEP increase was generated by a hyperpolarization that originated at the apical membrane. A small delayed basal hyperpolarization that decreased the TEP from its peak was also present. When the apical superfusate was switched back to 3 mM  $K^+$ , an apical depolarization that decreased TEP was followed by a small delayed basal depolarization that increased TEP to baseline.

The responses shown in Figure 5B were recorded in the continued presence of 0.2 mM  $Ba^{2+}$  (bar). Adding  $Ba^{2+}$  itself caused the apical membrane to depolarize relative to the basal membrane so that the TEP decreased (Fig. 4). In the presence of apical  $Ba^{2+}$ , decreasing  $[K^+]_o$  from 3 to 1 mM  $K^+$  caused an initial decrease in TEP rather than an increase. The intracellular recordings show that the apical membrane depolarized relative to the basal membrane, indicating that the TEP decrease was generated by a depolarization that originated at the apical membrane. Similar results were obtained in six cells from five tissues. In an additional five tissues where only TEP was measured,  $Ba^{2+}$  reversed the polarity of the  $K^+$ -evoked change in TEP compared with the control response. Decreasing apical  $[K^+]_o$  from 12.5 to 5 mM

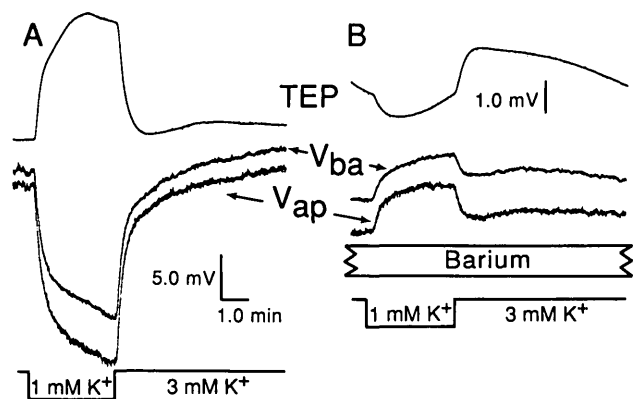


Fig. 5. Effects of barium on  $[K^+]_o$ -evoked responses. TEP,  $V_{ap}$ , and  $V_{ba}$  (initial values 16, -80, and -64 mV, respectively) were recorded simultaneously while the solution perfusing the apical membrane was switched from 3 mM  $K^+$  to 1 mM  $K^+$  and back to 3 mM  $K^+$ . The responses in A, recorded in control conditions, show that decreasing  $[K^+]_o$  primarily caused  $V_{ap}$  to hyperpolarize relative to  $V_{ba}$  so that the TEP increased. When  $[K^+]_o$  was switched back to 3 mM  $K^+$ , both membrane potential and the TEP recovered. The responses in B were recorded in the continued presence of 0.2 mM  $BaCl_2$  (bar) in the apical perfusate; in  $Ba^{2+}$ , TEP,  $V_{ap}$  and  $V_{ba}$  were 13, -65, and -52 mV, respectively. Decreasing  $[K^+]_o$  in the presence of  $Ba^{2+}$  primarily caused  $V_{ap}$  to depolarize relative to  $V_{ba}$  so that the TEP decreased. When  $[K^+]_o$  was switched back to 3 mM  $K^+$ , both membrane potential and the TEP recovered.

in the presence of 1.0 mM  $Ba^{2+}$  reduced, but did not reverse, the control TEP increase.

In four of the tissues, including the one shown in Figure 5, the  $K^+$ -evoked responses in the presence of  $Ba^{2+}$  were biphasic; decreasing  $[K^+]_o$  from 3 to 1 mM caused an initial TEP decrease followed by a TEP increase. In three other tissues, the biphasic component was very small, and in three other tissues, the response was a monophasic TEP decrease. Intracellular recordings during responses that exhibited a biphasic waveform (Fig. 5B) showed that the basal membrane depolarized relative to the apical membrane during the TEP increase. This indicates that the second phase of the response was generated by a depolarization that originated at the basal membrane. The mechanisms underlying this basal membrane response are unknown.

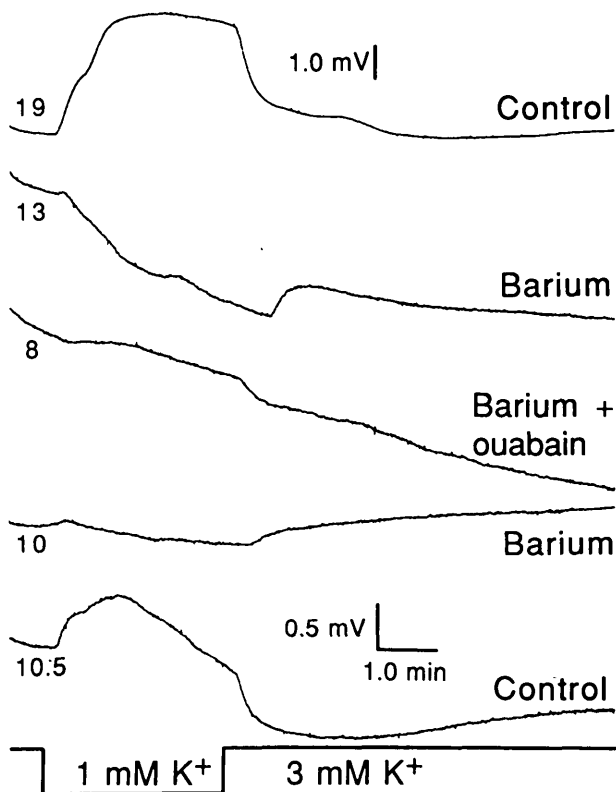


Fig. 6. Effects of barium and ouabain on  $[K^+]_o$ -evoked responses. TEP was recorded while the solution perfusing the apical membrane was switched from 3 mM  $K^+$  to 1 mM  $K^+$  and back to 3 mM  $K^+$  under several conditions. The absolute TEP (mV) is shown at the start of each record. In the control, lowering  $[K^+]_o$  increased TEP and when  $[K^+]_o$  was switched back to 3 mM, TEP decreased back to baseline. In the presence of 0.2 mM  $Ba^{2+}$ , which itself decreased the TEP to 13 mV, lowering  $[K^+]_o$  decreased TEP. In the presence of both 0.2 mM barium and 0.2 mM ouabain, lowering  $[K^+]_o$  caused a small TEP increase, which again became a TEP decrease when the ouabain was washed out. When control perfusate (no barium or ouabain) was reintroduced, the control  $[K^+]_o$ -evoked response recovered.

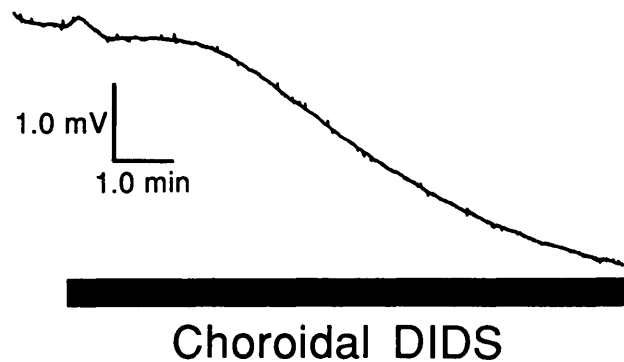


Fig. 7. Effects of choroidal DIDS. The TEP (initial value, 14 mV) was recorded while switching the choroidal superfusate to a test solution containing 0.1 mM DIDS. DIDS caused an irreversible TEP decrease.

Figure 6 demonstrates that the TEP decrease evoked by lowering apical  $[K^+]_o$  in the presence of  $Ba^{2+}$  was due to slowing the  $Na^+/K^+$  pump. This figure shows TEP responses evoked by lowering  $[K^+]_o$  from 3 to 1 mM and back to 3 mM in control and several experimental conditions. In the control, as described above, reducing  $[K^+]_o$  caused the TEP to increase; in the presence of  $Ba^{2+}$ , the TEP decreased. Addition of ouabain in the continued presence of  $Ba^{2+}$  in the apical superfusate eliminated this TEP decrease, suggesting that the TEP decrease is a "pump response." In the presence of both barium and ouabain, lowering  $[K^+]_o$  evoked a small TEP increase, suggesting that 0.2 mM  $Ba^{2+}$  did not block all of the apical  $K^+$  conductance. When ouabain was washed out, the pump response recovered, and when the  $Ba^{2+}$  test solution was again replaced with control Ringers, the control  $K^+$ -evoked response recovered. A reduction or elimination of the pump response with ouabain was demonstrated in three tissues.

The TEP responses caused by test substances in the choroidal superfusate were examined to determine whether such agents can diffuse to the RPE basal membrane. Figure 7 shows that switching the choroidal superfusate to a test solution containing 0.1 mM DIDS caused the TEP to decrease irreversibly ( $n = 10$ ). Similar results have been seen in the chick where DIDS may block a basal membrane chloride conductance.<sup>15</sup> Switching the choroidal superfusate to a test solution containing 0.1 mM DNP caused a reversible TEP decrease ( $n = 17$ ). In some tissues switching the choroidal superfusate did not change the TEP ( $n = 3$ , DIDS;  $n = 6$ , DNP).

## Discussion

These experiments indicate that the toad, *B. marinus*, is an important animal model for the study of RPE function. Figure 1 shows that the toad RPE ex-

hibits a delayed hyperpolarization that originates at the basal membrane when the  $[K^+]_o$  outside the apical membrane (analogous to the subretinal space) is decreased. This delayed basal hyperpolarization follows a hyperpolarization generated by the apical membrane that increases the TEP. The TEP starts to decrease when the delayed basal hyperpolarization becomes dominant. The variability in the TEP response reflects, at least in part, the relative amplitudes of the hyperpolarizations generated at the apical and basal membranes (Griff, in preparation). Figure 2 shows that the DC-ERG of the toad exhibits a fast-oscillation trough and light peak after the c-wave. Similar DC-ERGs have been recorded from reptiles, birds, and mammals<sup>1,4</sup> where both the fast-oscillation trough and the light peak are produced primarily by potentials originating at the RPE basal membrane.<sup>3,5</sup> The relationship between light-evoked changes in subretinal  $K^+$  and the electrophysiologic responses from the toad RPE must now be analyzed in detail.

Two approaches were used to establish that the toad RPE, like all other RPEs studied to date, has an apical, electrogenic  $Na^+/K^+$  pump. When ouabain was added to the apical superfusate, a depolarization generated at the apical membrane decreased the TEP (Fig. 3). This response was due in part to the elimination of the electrogenic  $Na^+/K^+$  pump's contribution to the resting apical membrane potential. The apical (and basal) depolarizations also probably reflected a reduction of the equilibrium potentials across each membrane as intracellular ion concentrations changed. Concentration changes are likely because the time-course of the ouabain-induced response was relatively slow even with rapid exchange of solution at the apical surface. The  $Na^+/K^+$  pump is presumably located only at the apical membrane since ouabain added to the choroidal superfusate had no effect on the TEP. Both DIDS and DNP reduced the TEP when introduced only into the choroidal superfusate (Fig. 7), indicating that substances can diffuse through the choroid to the basal membrane in toad preparations.

The effects of changing extracellular potassium in the presence of  $Ba^{2+}$  provided further evidence for an apical electrogenic  $Na^+/K^+$  pump in the toad.  $Ba^{2+}$  itself locked apical  $K^+$  conductances as evidenced by an apical depolarization (Fig. 4) and an increase in the apparent apical membrane resistance. In some tissue, apical  $Ba^{2+}$  may also block a basal  $K^+$  conductance. Lowering  $[K^+]_o$  from 3 to 1 mM (or from 5 to 2 mM) in the presence of  $Ba^{2+}$  caused an apical depolarization that decreased TEP (Fig. 5). This response is the polarity expected if reducing  $[K^+]_o$  slowed an apical, electrogenic, hyperpolarizing  $Na^+-K^+$  pump.<sup>11,12</sup> Furthermore, the TEP decrease was elimi-

nated or reduced by subsequent exposure to ouabain (Fig. 6). With higher  $K^+$  concentrations,  $Ba^{2+}$  did not reverse the  $[K^+]_o$ -evoked TEP response, suggesting that  $K^+$  competes with  $Ba^{2+}$  at the  $K^+$  channel.

The demonstration of a  $[K^+]_o$ -evoked, delayed basal hyperpolarization in the isolated RPE-choroid preparation and the fast-oscillation and light peak in the DC-ERG suggest that the toad RPE is more similar to the RPEs of reptiles, birds, and mammals than to amphibians. An important advantage of the toad over the gecko preparation is that test substances in the choroidal superfusate can diffuse to the RPE basal membrane. Important advantages of the toad over the chick retina include the large retinal cells, particularly photoreceptors, characteristic of the amphibian retina<sup>16,17</sup> and the stability of an in vitro preparation from a cold-blooded vertebrate where problems such as retinal spreading depression are not common.<sup>18</sup> Thus, the toad will be particularly useful for studying interactions between cells of the neural retina and the RPE.

**Key words:** retinal pigment epithelium, retina,  $Na^+/K^+$  pump, ouabain, animal models

### Acknowledgments

The author thanks Laura McKibben for technical assistance and Burks Oakley II for comments on the manuscript.

### References

1. Kikawada N: Variations in the corneo-retinal standing potential of the vertebrate eye during light and dark adaptations. *Jpn J Physiol* 18:687, 1968.
2. Oakley B II and Steinberg RH: Effects of maintained illumination upon  $[K^+]_o$  in the subretinal space of the frog retina. *Vision Res* 22:767, 1982.
3. Steinberg RH, Linsenmeier RA, and Griff ER: Retinal pigment epithelial cell contributions to the electroretinogram and electrooculogram. *In Progress in Retinal Research*, Osborne NN and Chader GJ, editors. New York, Pergamon Press, 1985, pp. 33-66.
4. Valetton JM and van Norren D: Intraretinal recordings of slow electrical responses to steady illumination in monkey: Isolation of receptor responses and the origin of the light peak. *Vision Res* 22:393, 1982.
5. Gallemore RP, Griff ER, and Steinberg RH: Evidence in support of a photoreceptor origin for the "light-peak substance." *Invest Ophthalmol Vis Sci* 29:566, 1988.
6. Griff ER: Metabolic inhibitors reversibly alter the basal membrane potential of the gecko retinal pigment epithelium. *Exp Eye Res* 50:99, 1990.
7. Van Harreveld A: Two mechanisms for spreading depression in the chicken retina. *J Neurobiol* 9:419, 1978.
8. Lasansky A and de Fisch FW: Potential, current, and ionic fluxes across the isolated retinal pigment epithelium and choroid. *J Gen Physiol* 49:913, 1966.
9. Griff ER and Steinberg RH: Changes in apical  $[K^+]$  produce

- delayed basal membrane responses of the retinal pigment epithelium in the gecko. *J Gen Physiol* 83:193, 1984.
10. Miller SS and Steinberg RH: Passive ionic properties of frog retinal pigment epithelium. *J Membr Biol* 36:337, 1977.
  11. Griff ER, Shirao Y, and Steinberg RH: Ba<sup>2+</sup> unmasks K<sup>+</sup> modulation of the Na<sup>+</sup>-K<sup>+</sup> pump in the frog retinal pigment epithelium. *J Gen Physiol* 86:853, 1985.
  12. Miller SS, Steinberg RH, and Oakley B II: The electrogenic sodium pump of frog retinal pigment epithelium. *J Membr Biol* 44:259, 1978.
  13. Frambach DA, Valentine JL, and Weiter JJ: Initial observations of rabbit retinal pigment epithelium-choroid-sclera preparations. *Invest Ophthalmol Vis Sci* 29:814, 1988.
  14. Torre V: The contribution of the electrogenic sodium-potassium pump to the electrical activity of toad rods. *J Physiol* 333:315, 1982.
  15. Gallemore RP and Steinberg RH: Effects of DIDS on the chick retinal pigment epithelium: I. Membrane potentials, apparent resistances and mechanisms. *J Neurosci* 9:1968, 1989.
  16. Fain GL and Lisman JE: Membrane conductances of photoreceptors. *Prog Biophys Mol Biol* 37:91, 1981.
  17. Griff ER and Pinto LH: Observations on rod coupling in the isolated retina of *Bufo marinus*. *Vision Res* 24:149, 1984.
  18. Mori S, Miller WH, and Tomita T: Müller cell function during spreading depression in frog retina. *Proc Nat Acad Sci USA* 73:1351, 1976.

### Correction

In the articles "Changes in Intermediate Filament Immunolabeling Occur in Response to Retinal Detachment and Reattachment in Primates," and "Immunocytochemical Identification of Muller's Glia as a Component of Human Epiretinal Membranes," by Christopher Guérin et al, which appeared in the August 1990 issue of *Investigative Ophthalmology and Visual Science*, Figures 1 (page 1485), 3 (page 1487), 5 (page 1480), and 6 (page 1489) were incorrect. Corrected reprints are available from the corresponding author. The publisher regrets any inconvenience this error may have caused.