Potassium-evoked Responses from the Retinal Pigment Epithelium of the Toad Bufo marinus

EDWIN R. GRIFF

Department of Biological Sciences, University of Cincinnati ML 006, Cincinnati, OH 45221 0006, U.S.A.

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Changes in the apical and basal membrane potentials and the resultant changes in the transepithelial potential were recorded from the isolated retinal pigment epithelium of the toad Bufo marinus while the potassium concentration superfusing the apical membrane was changed. Lowering apical potassium caused an initial apically-generated hyperpolarization that increased the transepithelial potential which was usually followed by a delayed basally-generated hyperpolarization that decreased the transepithelial potential. Light evoked a similar pattern of apical and basal responses in a preparation of neural retina-retinal pigment epithelium-choroid. The delayed basal hyperpolarization was accompanied by an apparent increase in basal membrane resistance, and was inhibited by adding the anion transport blocker 4,4'-diisothiocyanatostilbene-2,2'-disulfonylic acid or the metabolic inhibitor dinitrophenol to the solution superfusing the choroidal side of the retinal pigment epithelium (RPE). The results suggest that a change in the chloride equilibrium potential or chloride conductance of the basal membrane mediates the delayed basal response.

Key words: retinal pigment epithelium; RPE; electrophysiology; potassium; basal membrane; Bufo marinus.

1. Introduction

The retinal pigment epithelium (RPE) is a single layer of cells separating the neural retina from the choroidal blood supply. In common with all epithelia, each RPE cell has two distinct membranes: an apical membrane that faces the photoreceptors across a subretinal space and a basal membrane that faces the choroid. Recordings from the eyes of several vertebrates suggest that there are at least two patterns of RPE electrical responses evoked by light. In the best studied amphibian model, the bullfrog Rana catesbeiana, a relatively simple pattern was observed in which changes in the trans-epithelial potential were generated only by the RPE apical membrane and followed the time-course of light-evoked changes in subretinal potassium (e.g. Oakley, 1977; Steinberg and Miller, 1979). In the RPEs of mammals, birds and reptiles, more complex responses have been found, with several slower potentials generated by the RPE basal membrane following an initial apically-generated response (Steinberg, Linsenmeier and Griff, 1985; Gallemore and Steinberg, 1989b). In these animals the potentials generated by the basal membrane contribute to the fast-oscillation trough and the light peak of the DC-ERG (Linsenmeier and Steinberg, 1982, 1984; Griff and Steinberg, 1982, 1984; Van Norren and Heynen, 1986). Preliminary results suggested that the toad, Bufo marinus, exhibited a complex pattern of RPE responses more closely resembling those of a reptile (gecko) than the bullfrog (Griff and Law, 1989). Unlike the gecko, however, basal membrane mechanisms can be studied by manipulating the composition of the solution superfusing the choroidal surface of the tissue (Griff, 1990b). In this paper an isolated, superfused RPE preparation of Bufo marinus is used to study responses evoked by lowering the potassium concentration outside the apical membrane, apical \([K^+]_0\), in the range that mimics the light evoked decrease in potassium concentration in the subretinal space.

2. Materials and Methods

Preparation and Recording

Bufo marinus were obtained from Delta Biologicals (Tucson, AR), kept in large tanks and fed a diet of crickets. Prior to an experiment, a toad was dark-adapted for 12 hr to facilitate removal of the neural retina from the RPE: the animal was then decapitated, pithed and enucleated under dim red light. Procedures 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 retina was removed and the remaining RPE-choroid was mounted as previously described for bullfrog (Miller and Steinberg, 1977). The area of exposed tissue was 0.07 cm². Mounted tissues were maintained in a beaker of bubbled Ringer's until tested. A typical experiment lasted 8–14 hr, but some tissues exhibited stable responses 24 hr after dissection.

Mounted tissues were transferred to a chamber in which the apical and choroidal surfaces were continuously superfused at 2–5 ml min⁻¹ by a gravity feed system from two large reservoirs; each solution was...
bubbled with 95% O₂ and 5% CO₂. The volume of each side of the chamber was 2.5 ml. The control superfusate was modified Ringer’s solution with the following composition (mM): 82.5 NaCl; 27.5 NaHCO₃; 1.0-5.0 KCl; 1.0 MgCl₂; 1.8 CaCl₂; 10.0 glucose; pH 7.4. Test solutions were prepared by adding 0.1 mM dinitrophenol or 0.01-0.01 mM DIDS (4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid). The concentration of K⁺ in the choroidal superfusate was 2.0 mM for most experiments; for the experiment shown in Fig. 6, choroidal K⁺ was 3.0 mM.

The placement of electrodes and recording electronics were similar to previously published methods (Miller and Steinberg, 1977; Griff, 1990a). In brief, the potential across the tissue was measured by a pair of calomel electrodes connected to the apical and choroidal chambers by agar-Ringer bridges, and conventional microelectrodes were used to simultaneously measure the apical and basal membrane potentials, Vᵦ and Vᵦ, respectively (see Fig. 1). The total resistance across the epithelium, Rᵦ, and the ratio of the apical to basal resistances, α, were calculated from potential changes induced by injecting 10-μA current pulses across the isolated RPE. Instrumentation for monitoring and recording responses has been described previously (Griff, 1990a). Digitized responses were plotted using a technical graphics and data analysis program (Axum, Trimetrix Inc., Seattle WA).

Equivalent Electrical Circuit

The equivalent electrical circuit of the RPE (Fig. 1) provides a way to understand how changes in the apical and/or basal membrane potential alter the TEP. This circuit has been analyzed in detail by Miller and Steinberg (1977), Oakley (1977), Griff and Steinberg (1982), and Linsenmeier and Steinberg (1983) and relevant equations can be found in those papers.

In brief, the RPE apical and basal membranes are each represented by a battery in series with a resistance and the two membranes are electrically connected (or shunted) by the finite resistance of a paracellular shunt, Rₛ. At rest, the apical membrane is more hyperpolarized than the basal membrane so that current flows through the shunt and around the RPE circuit. The shunt current produces passive voltages across the resistances of each membrane (V = iR): a change in shunt current, due to a change in either membrane’s potential (battery) or a change in one of the circuit resistances, will cause a change in these passive voltages at both membranes. For example, if the apical membrane hyperpolarizes, an increase in current flowing through the shunt will passively hyperpolarize the basal membrane. The potential at the membrane where the polarization originates (the membrane generating the change), in this example an apical hyperpolarization, will always be larger than the passive potential shunted to the other membrane.

3. Results

The results are based primarily on recordings from 62 isolated RPE-choroid preparations from 38 toads. For tissues where the apical superfusate contained 2 mM K⁺ at the start of the experiment (n = 27), the mean initial TEP (± S.D.) was 17.8 ± 6.4 mV, and the mean transepithelial resistance, Rᵦ, was 3.3 ± 6.5 kΩ. Intracellular recordings were obtained from 43 cells in 29 tissues with a mean apical membrane potential, Vᵦ, of 73 ± 7.4 mV and a mean value of α of 0.5 ± 0.16. For tissues where the apical superfusate contained 5 mM K⁺ at the start of the experiment (n = 29), the mean TEP was 21.9 ± 7.2 mV, the mean Rᵦ was 3.3 ± 0.7 kΩ, the mean Vᵦ was 69 ± 7.4 mV (n = 25 cells), and the mean value of α was 1.1 ± 0.8. Light-evoked responses were recorded from six cells in five neural retina-RPE choroid preparations.

Response Patterns Evoked by [K⁺]

Several different response patterns, characterized by changes in RPE potentials (Figs 2-4) and RPE resistance (Fig. 6, Table I), were observed when the potassium concentration in the apical superfusate, [K⁺], was reduced, usually from 5 to 2 mM, and then returned to 5 mM. For most responses (Case 1), lowering [K⁺], caused the TEP to initially increase to a peak and then decrease toward, and sometimes below, the baseline, while [K⁺], was kept at 2 mM [Figs 2 (lower trace), 3(A), 6, 7(A), and 8(A)]. When [K⁺], was switched back to 5 mM, the TEP decreased to a minimum and then increased back to the baseline. In other responses (Case 2), lowering [K⁺], caused a TEP...
increase that occurred in several phases. When [K⁺]o was switched back to 5 mM, the TEP decreased toward the baseline in several phases, with a relatively rapid decrease followed by a much slower decrease [Figs 2 (upper trace) and 4]. Other responses evoked by lowering [K⁺]o were intermediate between the above cases; the TEP increased to a plateau after which the TEP either increased slightly and/or decreased slightly. When [K⁺]o was switched back to 5 mM, TEP decreased back to baseline in several phases sometimes separated by a phase when the TEP transiently increased [Fig. 2 (middle trace)].

The specific response pattern at the start of a particular experiment was not correlated with the initial TEP or $R_2$, or with a subjective evaluation of the overall dissection or the relative ease with which the neural retina separated from the RPE. Tissues that exhibited a Case 1 pattern at the start, repeatedly showed this pattern throughout the experiment; a Case 1 pattern never spontaneously changed to a Case 2 pattern. On the other hand, in some tissues exhibiting a Case 2 pattern at the start of an experiment, the pattern spontaneously changed to an intermediate and then to a Case 1 pattern.

Figure 3 (A) presents intracellular recordings during a Case 1 response evoked by lowering apical [K⁺]o from 5 to 2 mM; the TEP first increased (period 1) and then decreased toward the baseline (period 2). During period 1, the apical and basal membranes both hyperpolarized; since the apical membrane hyperpolarization was larger than that of the basal membrane, the TEP increased. Such membrane potential changes would occur if a hyperpolarization originated at the apical membrane and was passively shunted to the basal membrane via the shunt resistance.
Fig. 3. [K\(^+\)]\(_o\)-evoked responses with a delayed basal response (Case 1). A. The TEP, apical membrane potential (\(V_{ap}\)), and basal membrane potential (\(V_{bs}\)) were recorded simultaneously while the apical superfusate was switched from 5 to 2 mM [K\(^+\)]\(_o\). Lowering [K\(^+\)]\(_o\) initially increased the TEP (period 1) because the apical membrane hyperpolarized relative to the basal membrane. This period was followed by a delayed TEP decrease (period 2), during which the basal membrane hyperpolarized relative to the apical membrane. Starting values of TEP, \(V_{ap}\), and \(V_{bs}\) were 22, -75, and -53 mV, respectively. The vertical position of the traces in this and other figures is arbitrary. B. TEP, \(V_{ap}\), and \(V_{bs}\) (starting values 19, -82, -63 mV, respectively) were simultaneously measured in a different tissue while the apical superfusate was switched from 2 to 5 mM [K\(^+\)]\(_o\). Increasing [K\(^+\)]\(_o\) caused an initial TEP decrease (period 3), as the apical membrane depolarized relative to the basal membrane, followed by a TEP increase (period 4) during which the basal membrane depolarized relative to the apical membrane. The time marker applies to both (A) and (B). The extra noise on one of the intracellular traces in this and other figures is an artifact of the recording electronics.

During period 2, both membranes continued to hyperpolarize but the hyperpolarization of the basal membrane was now larger than that of the apical membrane so that the TEP decreased. These membrane potential changes during period 2 would occur if a hyperpolarization originated (was generated) at the basal membrane and passively shunted to the apical membrane. Since this basally-generated hyperpolarization became evident after the apically-generated hyperpolarization, it has been called the delayed basal hyperpolarization (Griff and Steinberg, 1984; Linsenmeier and Steinberg, 1984).

Figure 3(B) shows the RPE responses evoked by raising apical [K\(^+\)]\(_o\), from 2 to 5 mM. During period 3,
[K⁺]-EVOKEO RESPONSES OF TOAD RPE

**Figure 5.** Light-evoked RPE responses. Light onset caused a rapid increase (b-wave) followed by a slower, larger increase (c-wave) in the trans-tissue potential (TTP) recording across a neural retina-RPE-choroid preparation. In (A) a microelectrode positioned in the subretinal space simultaneously recorded the transretinal (Vₜₐₛ) and the transepithelial (TEP) potentials. All potentials are presented retinal side positive. In (B), a microelectrode positioned in an RPE cell simultaneously recorded the apical membrane potential contaminated with small potentials across the neural retina (Vₜ₉₋₁) and the basal membrane potential (Vₑ). During the TTP increase (period 1), the apical membrane hyperpolarized relative to the basal membrane; during the subsequent TTP decrease both membranes continued to hyperpolarize (period 2). In both (A) and (B), the lower trace shows the timing of a 3 min light stimulus.

The apical membrane depolarized relative to the basal membrane so that the TEP decreased. This suggests that a depolarization during this period was generated by the apical membrane and passively shunted to the basal membrane. During period 4, the basal membrane depolarized relative to the apical membrane so that the TEP now increased. This suggests that a depolarization during this period was generated by the basal membrane and passively shunted to the apical membrane.

Figure 4(A) shows an example of a Case 2 response where lowering [K⁺] from 5 to 2 mM caused a multiphasic increase in TEP. The intracellular recordings show that the apical membrane hyperpolarized relative to the basal membrane during the entire response, suggesting that a multiphasic hyperpolarization generated by the apical membrane dominated all phases of the TEP increase. There is no indication of a basally-generated hyperpolarization in these responses. When the superfusate was switched back from 2 to 5 mM [K⁺], there was a multiphasic decrease in TEP. As shown in Fig. 4(B), during this response the apical membrane depolarized relative to the basal membrane, indicating that a depolarization generated by the apical membrane dominated the entire response.

Figure 5 shows that the RPE response pattern evoked by light onset in a preparation of toad neural retina-RPE-choroid resembles the Case 1 response pattern evoked by decreasing [K⁺]. In Fig. 5(A), the trans-tissue potential (TTP), showing b- and c-waves in response to 3 min of illumination, are presented along with simultaneous measurements of the transretinal, Vₑ, and transepithelial (TEP) components recorded with a microelectrode positioned in the subretinal space. The increase in TTP to the c-wave peak (period 1) was caused by an increase in TEP; during this period, Vₑ decreased; the decrease in TEP from the c-wave peak was generated by a decrease in TEP. The changes in subretinal [K⁺] that mediate these potentials have been shown to follow the approximate timecourse of Vₑ (Oakley, 1977; Griff and Steinberg, 1984).

Figure 5(B) shows light-evoked intracellular RPE responses, recorded in another neural retina-RPE-choroid preparation. Initially (period 1), the apical membrane hyperpolarized relative to the basal membrane, causing an increase in TEP (not recorded in this tissue) that in turn caused the increase in TTP to the c-wave peak. During period 2, both membranes continued to hyperpolarize, but now the basal membrane hyperpolarized relative to the apical membrane, causing the TEP and TTP to decrease. With longer and/or more intense stimuli, this TTP decrease forms a dip, the fast-oscillation trough, between the c-wave and the light peak (Griff, 1990b; Griff and Law, in preparation). The pattern of RPE responses evoked by light were similar to the Case 1 pattern evoked by lowering apical [K⁺], in the isolated RPE-choroid preparation where an apically-generated hyperpolarization that increased TEP was followed by a basally generated hyperpolarization that decreased TEP [Fig. 3(A)].

**RPE Resistance Changes Evoked by [K⁺].**

To investigate further the mechanisms that contribute to K⁺-evoked, RPE responses, current pulses were injected across the isolated RPE-choroid to calculate the transepithelial resistance, Rₑ, and the ratio, a, of apical to basal resistance. Table I compares Rₑ and a evoked by lowering apical [K⁺], for Case 1 and...
Fig. 6. Time-course of [K+]o-evoked RPE resistance changes. TEP, Vap, and Vba (starting values 11, 66, and 55 mV, respectively) were recorded simultaneously while the apical superfusate was switched from 3 to 1 and back to 3 mM [K+]o. Voltage responses were similar to those described above in Fig. 3. The trans-tissue resistance, R, (○), and the ratio, a, of apical to basal membrane resistance, Rap/Rba (△), were calculated from current-induced voltage changes (transients in voltage responses) and are plotted as a function of time. When [K+]o was lowered, R, increased and a decreased.

Fig. 7. Effects of choroidal DIDS. TEP, Vap, and Vba were recorded simultaneously while the apical superfusate was switched from 5 to 2 and back to 5 mM [K+]o with control choroidal superfusate (A: starting values, 10, -85, -75 mV, respectively), and with 0.05 mM DIDS in the choroidal superfusate (B: starting values 5, -80, -75 mV, respectively; responses from a different RPE cell in the same tissue as A, 42 min after switching to DIDS). In the presence of choroidal DIDS, the TEP decrease during period 2 was replaced by a TEP increase. The time marker applies to both (A) and (B).

Case 2 responses. The different patterns of membrane potential changes were accompanied by different patterns of resistance changes.

For Case 1 responses (Table 1A), R, increased in 29 out of 33 tissues when the apical superfusate was switched from 5 to 2 mM, with a mean increase of 15% from 3.8 to 4.1 kΩ. The ratio a decreased in four out of five tissues with a mean decrease of 38%. An increase in R, accompanied by a decrease in a can be most simply explained by an increase in the basal membrane resistance, Rba. For Case 2 responses, where a basally-generated response was not evident (Table 1B), R, increased slightly (2%), and there was no statistically significant difference between the mean values of R, in 5 and 2 mM [K+]o. In four out of six responses from four tissues, however, the mean value of a decreased by 19%, from 0.9 to 0.7. A decrease in a without a change in R, could occur if the apical and basal membrane resistances were both changing, with the apical resistance decreasing and the basal resistance increasing.

Figure 6 shows the time-course of the changes in RPE resistance during a Case 1 response pattern evoked by lowering apical [K+]o. In this example, [K+]o was switched from 3 to 1 and back to 3 mM: the TEP and membrane potential responses were similar to
those in Fig. 3, where [K']o was switched between 5 and 2 mM. Lowering [K']o from 3 to 1 mM initially caused the apical membrane to hyperpolarize relative to the basal membrane so that the TEP increased (period 1). Subsequently (period 2), the basal membrane hyperpolarized relative to the apical membrane, so that the TEP decreased. When the apical [K']o was switched back to 3 mM, the apical membrane depolarized relative to the basal so that the TEP increased back to the control level. As expected from the results in Table 1A, lowering apical [K']o increased R, (filled circles, lower traces) and decreased the ratio a (open triangles, lower traces). These resistance changes occurred primarily during period 2, when the delayed basal hyperpolarization dominated the response. When [K']o was switched back to 3 mM, R, and a returned to their control values.

Experimental Manipulations

Switching the choroidal superfusate to a test solution containing the anion transport blocker DIDS (4,4'-disothiocyanatostilbene-2,2-disulfonate; Knauf, Law, and Marchant, 1983) or the metabolic uncoupler DNP (dinitrophenol; Slater, 1967) selectively inhibited the delayed basally-generated responses. Figure 7 shows K+-evoked TEP and intracellular responses in control [Fig. 7(A)] and during superfusion of the choroidal surface with a test solution containing DIDS [Fig. 7(B)]. In the control, lowering apical [K']o, from 5 to 2 mM caused a Case 1 response pattern consisting of a hyperpolarization originating at the apical membrane that increased the TEP (period 1), followed by a delayed hyperpolarization originating at the basal membrane that decreased the TEP (period 2). When the superfusate was switched back to 5 mM [K']o, the TEP and membrane potentials returned toward their control levels.

The solution superfusing the choroidal surface was then switched to a test solution containing 0.05 mM DIDS. This caused a slow decrease in the TEP (seven of 12 tissues, mean 18 ± 15%) during which the basal membrane hyperpolarized relative to the apical membrane (n = 2). This indicates that DIDS caused a hyperpolarization that originated at the basal membrane. DIDS also caused an increase in R, (13 ± 10%, n = 6), and a decrease in the ratio a (50%, n = 1) suggesting that DIDS increased the apparent resistance of the basal membrane, as previously observed in chick (Gallemore and Steinberg, 1989a) and bovine RPE (Joseph and Miller, 1991). The apical solution was then switched from 5 to 2 mM [K']o, while the DIDS test solution continued to superfuse the choroidal side [Fig. 7(B)]. Lowering [K']o, now caused a multiphasic TEP increase (Case 2 response). SITS (4-acetamido-4'-isothiocyanostilbene, 0.1 mM) added to the choroidal superfusate had a similar effect (n = 1).

Figure 7(B) also shows intracellular recordings during the [K']o-evoked response in the presence of choroidal DIDS. Initially (period 1), the apical membrane hyperpolarized relative to the basal so that the TEP increased. At the beginning of period 2, both membranes hyperpolarized at about the same rate, so that there was little change in the TEP, and later in period 2, the apical membrane hyperpolarized relative to the basal membrane so that the TEP slowly increased. When the solution was switched back to 5 mM [K']o, the apical membrane depolarized relative to the basal so that the TEP decreased. Thus, choroidal DIDS blocked the delayed basal responses, changing the response from a Case 1 to a Case 2 pattern. The effects of DIDS were irreversible in the toad; when the choroidal superfusate was switched back to control, neither the TEP nor the [K']o-evoked responses recovered. Choroidal DIDS also caused a reduction in the magnitude of the apical and basal membrane hyperpolarizations during period 1. This reduction would occur if choroidal DIDS reduced the apical potassium conductance as reported by Gallemore and Steinberg (1989a) in the chick RPE. However, in the toad, DIDS-evoked changes in apical membrane potential and resistance that would be consistent with a decrease in apical potassium conductance did not dominate the effects of DIDS on a basal chloride conductance.

Figure 8(A) compares TEP responses evoked by decreasing [K']o, from 5 to 2 mM in the control choroidal superfusate, after switching to a choroidal test solution containing 0.1 mM DNP, and after returning to the control choroidal superfusate. In the control (upper trace), lowering [K']o, caused an initial TEP increase, followed by a delayed TEP decrease. After apical [K']o, was switched back to 5 mM, the choroidal superfusate was switched to the DNP test solution. In the continuing presence of choroidal DNP (middle trace), lowering apical [K']o, caused an initial TEP increase, but the delayed TEP decrease was blocked; similar results were obtained in five other tissues. The normal Case 1 increase in R, was also blocked in the presence of DNP (n = 2). When the choroidal test solution was switched back to control Ringer's and [K']o, was lowered, the Case 1 response pattern recovered [Fig. 8(A) lower trace].

Figure 8(B) shows that DNP added to the apical superfusate was also effective in reversibly blocking delayed TEP responses evoked by changing apical [K']o. In this example, [K']o, was switched from 2 to 5 and back to 2 mM. In control (upper trace), raising [K']o, caused an initial TEP decrease that was followed by a delayed TEP increase. The apical superfusate was then switched to a test solution containing 0.1 mM DNP. In the continued presence of apical DNP (middle trace), raising apical [K']o, caused an initial TEP decrease, but the subsequent delayed TEP increase was blocked. Similar results were obtained in two
other tissues. The DNP test solution was then switched back to control Ringers; raising [K+] again evoked a response consisting of a TEP decrease followed by a delayed TEP increase (lower trace). Addition of 0.1 mM cyanide to the apical superfusate instead of DNP had similar effects (n = 2).

4. Discussion

This paper presents an electrophysiological characterization of apical and basal membrane responses from the toad RPE evoked by changing [K+] in the apical superfusate. When [K+] was lowered to mimic the subretinal [K+] decrease evoked by light in preparations where the neural retina is attached (e.g., Oakley and Green, 1976), an initial apically-generated hyperpolarization [period 1 in Figs 3(A), 4(A), 6, 7(A) and 7(B)] was most often followed by a hyperpolarization that originated at the basal membrane. This delayed basally-generated hyperpolarization was evident when it became larger than the apically-generated response and caused the TEP to decrease from its peak [period 2 in Figs 3(A), 6 and 7(A)]. Light stimulation of a toad neural retina-RPE-choroid preparation [Fig. 5(B)] caused a similar sequence of RPE membrane responses that initially increased the transepithelial potential to the c-wave peak and then decreased the potential to produce a fast oscillation trough (Griff and Law, in preparation).

Figure 6 shows that the delayed basal hyperpolarization was accompanied by an increase in the transepithelial resistance, R, and a decrease in the ratio of apical to basal membrane resistance, a. This conclusion is also supported by the data in Table I, though the decrease in a was statistically significant only when expressed as a percent change. These resistance changes suggest that the delayed basal hyperpolarization is accompanied by an apparent increase in basal membrane resistance. Similar basal membrane responses have been demonstrated in isolated RPE preparations of lizard, chick, and bovine (Griff and Steinberg, 1984; Gallemore and Steinberg, pers. commun.; Joseph and Miller, 1991). An increase in basal resistance could cause a basal hyperpolarization by decreasing the conductance of an ion whose equilibrium potential across the basal membrane is more positive than the resting potential. An increase in basal resistance could also result from a basal membrane hyperpolarization as a voltage-dependent conductance.

Inhibition of the delayed basal hyperpolarization by choroidal DIDS (Fig. 7) suggests the involvement of anion transport across the basal membrane. Choroidal DIDS itself caused a hyperpolarization of the RPE basal membrane, an increase in R, and decrease in a, as shown previously in the chick RPE (Gallemore and Steinberg, 1989a), suggesting that DIDS primarily blocks a basal membrane anion conductance in the toad. Recently, Fujii et al. (1990) made measurements of intracellular chloride activity in the toad RPE and have shown that the equilibrium potential for chloride, ECl, at the basal membrane is more positive than the resting basal membrane potential. Thus, the inhibition of the delayed basal hyperpolarization by DIDS is consistent with the hypothesis that a decrease in basal chloride conductance causes the delayed basal hyperpolarization.

Alternatively, the delayed basal hyperpolarization could result from a decrease in intracellular chloride concentration. which would make ECl, more negative:
DIDS could inhibit this response by blocking basal chloride conductance. Lowering [K+]o outside the apical membrane could cause such a decrease in intracellular chloride by reducing potassium/chloride cotransport across the apical membrane. The demonstration of a net chloride flux in the retina to choroid direction implies that an apical chloride transporter is present (Lasansky and deFisch, 1966: Miller and Steinberg, 1982). The sensitivity of the apical membrane to furosemide and/or bumetanide indicates the presence of a Na+, K+, Cl- cotransport system in some species (Frambach and Misfeldt, 1983; Adorante and Miller, 1990).

Figure 8 shows that DNP also inhibits the delayed basal responses, as shown previously in the gecko (Griff, 1990a); in toad, DNP was effective when added to either the apical or choroidal superfusates. DNP itself caused primarily a hyperpolarization of the RPE basal membrane that decreased the TEP (unpubl. res.), as it did in the gecko (Griff, 1990a), but the mechanism of this effect is unknown. If DNP reduced ATP (by uncoupling of oxidative phosphorylation), active transport of ions would likely be reduced, and it is tempting to speculate that this would decrease intracellular chloride activity by blocking active chloride uptake at either the apical and/or basal membrane.

In some [K+]o-evoked responses, the initial apically-generated hyperpolarization that increased TEP was followed by a slower apical hyperpolarization that further increased TEP [Case 2 response: Figs 2 and 4(A)], instead of a basally-generated hyperpolarization that decreased the TEP. A similar multiphasic TEP increase was observed after the delayed basally-generated hyperpolarization was blocked with DIDS [Fig. 7(B)], suggesting that the slow or delayed apically-generated hyperpolarization was present in the control, but was masked by the delayed basal hyperpolarization. Case 2 response patterns evoked in control conditions may also occur because the delayed basal mechanism is blocked. This is supported by an observation that DIDS had no effect on a Case 2 response (n = 1). The mechanism that causes the slow or delayed apical hyperpolarization in a Case 2 response pattern is unknown, and difficult to study since this pattern changes to a Case 1 pattern. Slow apically-generated hyperpolarizations have been reported in the bullfrog RPE when [K+]o was switched very rapidly (La Cour, Lund-Anderson, and Zeuthen, 1986), and have been observed in the gecko when the delayed basal hyperpolarization was blocked (Griff, unpubl. res.).

In summary, this paper demonstrates that the toad exhibits a delayed basal hyperpolarization, evoked either by lowering apical [K+]o, or by light, similar to responses in reptiles (gecko), birds (chick), and mammals (bovine). Although the detailed mechanisms are not well understood, results with DIDS suggest a basal membrane chloride channel is involved. The toad also exhibits a delayed apical hyperpolarization, which is not obvious in the control responses in other species. Important advantages of toad preparations for further study of these responses are the large retail cells, the relative ease of maintaining amphibian tissues in vitro, and the extensive published data on toad photoreceptor mechanisms that contribute to the light-evoked decrease in subretinal [K+]o (Shimazaki and Oakley, 1984: 1986; Torrec, 1982).

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References


