

# Effects of Hypoxia on Potassium Homeostasis and Pigment Epithelial Cells in the Cat Retina

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**ABSTRACT** Intracellular recordings show that light-evoked hyperpolarizations of the apical and basal membranes of the cat retinal pigment epithelium (RPE) are altered by mild hypoxia. RPE cells, like glia, have a high  $K^+$  conductance, and measurements with  $K^+$ -sensitive microelectrodes show that the hypoxic changes in the RPE cell are largely the result of changes in extracellular  $[K^+]$  in the subretinal space ( $[K^+]_o$ ) rather than direct effects on RPE cells. During hypoxia, light-evoked  $[K^+]_o$  responses and membrane responses have longer times to peak, slower and less complete recovery during illumination, and larger amplitudes. In addition to the effects on light-evoked responses, hypoxia causes a depolarization of first the apical and then the basal membranes of RPE cells under dark-adapted conditions. The basal depolarization is accompanied by a decrease in basal membrane resistance. These depolarizations appear to be caused by a rapid increase in  $[K^+]_o$  at the onset of hypoxia, which is maximal in dark adaptation, and smaller if the retina is subjected to maintained illumination. All of the effects are graded with the severity of hypoxia and can be observed at arterial oxygen tensions as high as 65 mmHg, although the threshold may be even higher. We argue that the origin of hypoxic  $[K^+]_o$  changes is probably an inhibition of the photoreceptors'  $Na^+/K^+$  pump. This work then suggests that photoreceptors are more sensitive to hypoxia than previously believed, and that the high oxygen tension normally provided by the choroidal circulation is necessary for normal photoreceptor function.

## INTRODUCTION

Several slow electrical potentials recorded from the cat eye are affected by surprisingly mild episodes of hypoxia, during which the arterial oxygen tension ( $P_aO_2$ ) is 60–80 mmHg (Linsenmeier et al., 1983; Niemeyer et al., 1982). These signals form part of the DC-recorded electroretinogram (DC-ERG) and include potentials that develop over a time frame of several seconds (c-wave) to minutes

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(light peak). These slow events arise partially or entirely from several light-evoked responses of the retinal pigment epithelial (RPE) cells (Steinberg et al., 1970; Griff and Steinberg, 1982*a*, 1984; Linsenmeier and Steinberg, 1982, 1984; Valeton and van Norren, 1982), and the sensitivity of the DC-ERG to hypoxia suggested that RPE cells must be affected. However, since RPE cells respond to light indirectly as a result of photoreceptor activity, hypoxia could have affected RPE responses by altering photoreceptor function or by affecting the RPE cells themselves. Recordings of the DC-ERG could not discriminate between these possibilities, since they were made with an electrode in the vitreous humor. In addition, vitreal ERG recordings may contain potentials arising in the neural retina that can add to the signals generated across the RPE. Thus, the vitreal recordings suggested that the RPE-photoreceptor region was rather sensitive to hypoxia, but did not answer questions about the cellular location and mechanisms of these changes.

In this paper, we investigate the origin of these hypoxic effects by using intraretinal and intracellular recordings. Changes in RPE membrane potentials were found, as expected from previous work on the DC-ERG. Two of the light responses that are affected, the apical membrane hyperpolarization and the delayed basal membrane hyperpolarization, are responses of the RPE to light-evoked changes in  $K^+$  in the subretinal space ( $[K^+]_o$ ) (Oakley and Green, 1976; Oakley, 1977; Steinberg et al., 1980; Griff and Steinberg, 1984), so we sought the origin of the RPE effects by recording subretinal  $[K^+]_o$  during hypoxia. In addition to explaining the RPE changes,  $[K^+]_o$  recordings provide a link to the behavior of photoreceptors during hypoxia, since the photoreceptors have been shown to be of primary importance in causing the light-evoked changes in  $[K^+]_o$  (Oakley et al., 1979).

The behavior of  $[K^+]_o$  during hypoxia is also of interest when considering the retina as a model for brain tissue. A total lack of oxygen, produced as ischemia or anoxia, leads to relatively large increases in brain  $[K^+]_o$  (e.g., Vyskocil et al., 1972; Morris, 1974; Hansen, 1977; Sick et al., 1982), but the effects of graded hypoxia have been studied very little. We have the opportunity to study changes produced in the retina in a glial-like cell, the RPE cell (Steinberg et al., 1983), by the activity of a neuron, the photoreceptor, under conditions of relatively mild hypoxia.

We first describe the effects of hypoxia on the dynamics of light-evoked changes in RPE membrane potentials during illumination and show how these are related to changes in  $[K^+]_o$ . We then consider the effects of hypoxia on  $[K^+]_o$  and membrane potentials during steady darkness or steady illumination. This paper focuses on those effects of hypoxia at the cellular level that are related to  $[K^+]_o$ . In another paper, we will consider the effect of hypoxia on the slowest RPE response, the light peak, and relate the findings to the DC-ERG.

## METHODS

### *Preparation and Recording*

The experiments were performed on 26 adult cats. Methods for animal preparation, recording, and stimulation are described fully in Linsenmeier and Steinberg (1982, 1983,

1984). Briefly, animals were anesthetized during surgery with sodium thiamylal (Surital) or sodium pentothal. During the experiments, the animals were either anesthetized with urethane (200 mg/kg loading dose followed by 10–20 mg/kg·h<sup>-1</sup>) or rendered decerebrate by several bilateral pontine pretrigeminal electrical lesions placed stereotaxically. In most experiments, eye movements were suppressed with gallamine triethiodide (Flaxedil, 10 mg/kg·h<sup>-1</sup>), but in a few experiments, pancuronium bromide (Pavulon, 0.1–0.2 mg/kg·h<sup>-1</sup>) was used. These differences in procedure appeared not to influence the results.

The cat's rectal temperature was maintained between 37 and 40°C, and arterial pressure and expired CO<sub>2</sub> were monitored. Arterial pH, PCO<sub>2</sub> and PO<sub>2</sub> were measured intermittently with a blood-gas analyzer and were controlled by adjustments of ventilatory rate, stroke volume, and the composition of the inspired gas. Ordinarily, the inspired gas was room air, supplemented when necessary with oxygen. Hypoxia was induced by adding N<sub>2</sub> to the inspired mixture, and hypercapnia was induced by adding CO<sub>2</sub>.

Single-barreled glass microelectrodes were used for membrane potential recording. They were filled with 5 M K-acetate and were beveled to have resistances of 30–60 × 10<sup>6</sup> Ω. The reference electrode was an Ag/AgCl plate behind the eye (retrobulbar electrode). Double-barreled electrodes were used for measurements of [K<sup>+</sup>]<sub>o</sub> and were made essentially as described by Steinberg et al. (1980). Full details are available on request. Each barrel was 0.7 mm in diameter. One barrel was made potassium-selective by injecting 0.2 μl of a 0.2% solution of tri(*N*-butyl)-chlorosilane in CCl<sub>4</sub>, heating, and then injecting Corning 477317 ion-exchange resin (Corning Medical and Scientific, Medfield, MA) after the electrode had cooled. This barrel was then filled with 100 mM KCl. The other barrel, containing 5 M LiCl and 2 mM KCl, was used as a reference for the K<sup>+</sup> measurements, and also recorded intraretinal ERGs when referenced to the retrobulbar electrode. K<sup>+</sup> electrodes were beveled against a plate surfaced with 1 μm diamond or 0.3 μm AlO<sub>2</sub> dust, with the reference barrel down, until the resistance of the reference barrel was 20–40 × 10<sup>6</sup> Ω. At this time, the resistance of the K<sup>+</sup> barrel was 10–40 × 10<sup>9</sup> Ω (measured with an FD-223 amplifier [W-P Instruments, Inc., New Haven, CT]) and the tip opening, measured perpendicular to the shank of the electrode, was 2–4 μm. K<sup>+</sup> electrodes were routinely tested at room temperature by measuring their response to a solution change from 1 mM KCl, 111 mM NaCl to 10 mM KCl, 102 mM NaCl in a test chamber having a low volume and high flow rate. The mean response of electrodes that were acceptable was 40.1 mV (±3.2 mV SD for 45 electrodes). This is less than ideal Nernstian behavior because Na<sup>+</sup> interference is most noticeable at low K<sup>+</sup> concentrations. Since we were most interested in comparing normoxic and hypoxic responses, electrodes were usually not tested in other solutions, but electrodes that were calibrated more fully gave a response of 52–54 mV to a solution change from 10 to 100 mM KCl (again with constant K<sup>+</sup> plus Na<sup>+</sup>). This indicates that their selectivity for K<sup>+</sup> over Na<sup>+</sup> was good. K<sup>+</sup> electrodes were stable for about a week after construction. In the figures, V<sub>K<sup>+</sup></sub> is given in millivolts and an estimate of [K<sup>+</sup>]<sub>o</sub> is given in millimolar. This estimate assumes that (a) the complete calibration done for several electrodes applies to each electrode, and (b) the normoxic dark-adapted concentration of K<sup>+</sup> in the subretinal space is 5.5 mM (Steinberg et al., 1980).

All electrodes were introduced into the intact right eye of the cat through a guard needle, and were positioned in or near the area centralis. Electrodes were advanced to the subretinal space by monitoring the local ERG during penetration. One of two unity-gain high-impedance preamplifiers (model 1090; Winston Electronics, San Francisco, CA; or model FD-223; W-P Instruments, Inc.) amplified the signals. An additional electrode, a chlorided silver wire, was used to record the vitreal ERG with respect to the retrobulbar electrode. All signals were stored on magnetic tape for later analysis. [K<sup>+</sup>]<sub>o</sub> and membrane potential measurements were made on different cats.

Intraretinal measurements were complicated by the tendency of the retina to move or swell during hypoxia such that the RPE came closer to the electrode in the subretinal space. This was signaled by increased pulsation in the normally quiet subretinal space, or occasionally by inadvertent penetration of an RPE cell or the choroid. It was sometimes necessary, therefore, to withdraw the electrode as much as 50  $\mu\text{m}$  during hypoxia, and advance again following hypoxia. We think it unlikely, however, that these stability problems introduced significant artifacts. Damage to the RPE reduced the size of the local ERG, and when this occurred the measurements were excluded. Also, as shown below, extracellular  $\text{K}^+$  measurements and RPE cell membrane potential recordings were consistent and provided a check on each other. Finally, the changes observed would not be expected simply from electrode movement, since the hypoxic waveforms are unlike normoxic ones at any retinal depth.

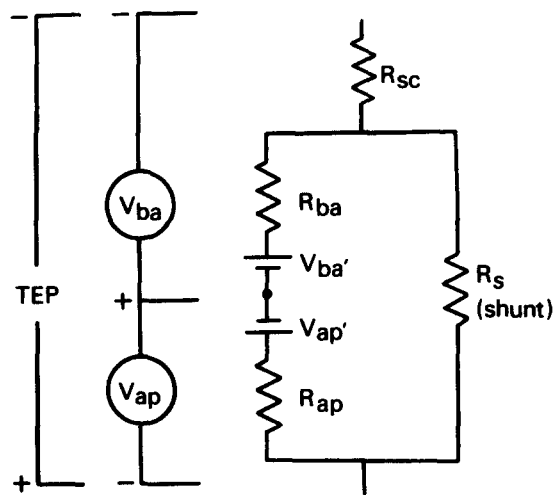


FIGURE 1. Equivalent circuit of the retinal pigment epithelium.  $R_{ap}$ , resistance of the apical membranes;  $R_{ba}$ , resistance of the basal membranes;  $R_s$ , resistance of the paracellular (shunt) pathway;  $R_{sc}$ , resistance of the sclera and choroid.  $V_{ap}'$  is a battery at the apical membrane;  $V_{ba}'$  is a battery at the basal membrane;  $V_{ap}$  is the measured potential across the apical membrane;  $V_{ba}$  is the measured potential across the basal membrane.

The stimuli were diffuse white light as previously described. The illumination was generally approximately at rod saturation,  $8.2 \log \text{quanta}(507)/\text{deg}^2 \cdot \text{s}^{-1}$  (Linsenmeier and Steinberg, 1982), but could be adjusted with neutral density filters.

#### RPE Circuit

The RPE can be viewed as a set of three resistances (Fig. 1):  $R_{ap}$  for the apical membrane, facing the photoreceptors,  $R_{ba}$  for the basal membrane, facing the choroid, and  $R_s$  for the paracellular (shunt) pathway between cells.  $R_s$  also includes the shunt pathway through other parts of the eye. Potential changes can originate from changes in the batteries at either membrane,  $V_{ap}'$  or  $V_{ba}'$ . The two measured membrane potentials,  $V_{ap}$  and  $V_{ba}$ , are influenced not only by the batteries, however, but by current flow around the circuit from the battery at the opposite membrane. For a potential change originating at one membrane (a change in  $V_{ap}'$  or  $R_{ap}$ , for example) the current flow through  $R_s$  (shunt

current) will reduce the potential change at that membrane from the value that would be observed if  $R_s$  were infinite, and will produce a passive voltage drop at the opposite membrane. The measured potential change is always larger at the membrane where the potential change originates, and is of the same sign (hyperpolarizing or depolarizing) at both membranes. In the cat,  $R_{ap}$  is smaller than  $R_{ba}$  (Linsenmeier and Steinberg, 1983), so that passive voltage changes ( $iR$  drops) across the apical membrane are smaller than those across the basal membrane. This is important in understanding the recordings, because events originating at the apical membrane cause large passive voltage changes at the basal membrane, while events originating at the basal membrane cause only small passive changes at the apical membrane. These concepts are developed more fully by Miller and Steinberg (1977), Griff and Steinberg (1982a), and Linsenmeier and Steinberg (1982, 1984).

Fig. 1 also illustrates that  $V_{ba}$  is actually recorded between the microelectrode inside the cell and an electrode behind the eye. Thus, it includes a passive voltage drop across the scleral resistance,  $R_{sc}$ .  $V_{ap}$  is obtained by measuring the potential between the subretinal space and the retrobulbar electrode (the transepithelial potential or TEP), and subtracting from this a recording of  $V_{ba}$  in response to the same stimulus obtained at a different time. In some cases,  $V_{ap}$  cannot be determined in this way because exactly comparable recordings of  $V_{ba}$  and TEP are not available. It is often possible to draw qualitative conclusions about  $V_{ap}$ , however, from the direction of changes in  $V_{ba}$  and TEP. It can be shown, for example, that a depolarization of the basal membrane accompanied by an increase in TEP implies that the depolarization originated at the basal membrane, while a basal depolarization accompanied by a decrease in TEP implies that the depolarization originated at the apical membrane and was shunted to the basal membrane.

Absolute values of the resistances in the circuit of Fig. 1 are difficult to measure, but changes in these resistances can be detected. In order to do this, pulses of constant current (0.2–0.5 s duration; 25–84  $\mu$ A) are passed across the eye, between a second vitreal electrode and a second retrobulbar electrode. The resulting voltage drops can be monitored between the microelectrode and the retrobulbar recording electrode and are proportional to the resistance of structures between the recording electrodes. If the microelectrode is in the subretinal space, we can obtain  $R_{sc}$ , the resistance of the sclera, plus  $R_t$ , the equivalent resistance of the RPE:

$$R_t = R_s(R_{ap} + R_{ba}) / (R_s + R_{ba} + R_{ap}) \quad (1)$$

(Miller and Steinberg, 1977).

## RESULTS

### *Responses to Illumination*

**CHANGES IN RPE MEMBRANE POTENTIALS IN RESPONSE TO ILLUMINATION** The RPE cell's response following illumination of the photoreceptors is rather complex. Three distinct responses have been described, one generated by the apical membrane, and two generated at the other side of the cell, at the basal membrane. These responses follow one another with progressively slower time courses. Here we will present data concerning only the apical response and the first basal response, because these have been shown to depend for their generation on the light-evoked change in  $[K^+]_o$  in the subretinal space.

The first response is the well-known hyperpolarization of the apical membrane that leads to the c-wave of the ERG (Steinberg et al., 1970). Fig. 2A ( $V_{ap}$ ) illustrates this response during 60 s of illumination in normoxia. As previously

described, the apical hyperpolarization peaks in  $\sim 4$  s, and then recovers toward the dark-adapted level, reaching a plateau after  $\sim 3$  min. This response is caused by changes in the  $K^+$  equilibrium potential across the apical membrane, as  $[K^+]_o$  decreases and partially recovers while illumination is maintained (Oakley and Green, 1976; Oakley, 1977; Steinberg et al., 1980).

At the basal membrane (Fig. 2A,  $V_{ba}$ ), a similar response occurs, but its time course is not identical to that of the apical response. A large part of the basal response depends upon shunting of the apical response (see Methods), but the time course of  $V_{ba}$  differs from  $V_{ap}$ , because the basal membrane generates an additional potential change. This basal event is called the delayed basal hyperpolarization (Griff and Steinberg, 1984; Linsenmeier and Steinberg, 1984). An estimate of its size and time course, when separated from the shunted response at the basal membrane by methods described previously (Linsenmeier and Steinberg, 1984), appears as the isolated basal trace during normoxia.<sup>1</sup> The delayed basal hyperpolarization is smaller than the apical hyperpolarization, and peaks in  $\sim 20$  s, before slowly recovering toward a plateau. As discussed in detail below, this response probably results from changes in intracellular  $[K^+]$  that are initiated by the light-evoked changes in subretinal  $[K^+]_o$ .

Both the apical and the delayed basal membrane events were altered by hypoxia. Fig. 2B shows these effects at an arterial oxygen tension ( $P_aO_2$ ) of 38 mmHg and Fig. 2 (bottom) shows a superposition of the normoxic and hypoxic responses. Observe that the hypoxic apical response had a longer time to peak, a larger peak amplitude, and a less complete recovery. Similar effects were observed in this animal with 1 and 2 log units less illumination, and in two other animals. Effects of hypoxia on the delayed basal hyperpolarization were qualitatively very similar to the effects on the apical hyperpolarization, since during hypoxia the delayed basal hyperpolarization was larger and peaked later. It is not apparent here, but in experiments in which illumination was maintained longer, the recovery from the delayed basal hyperpolarization was also less complete during hypoxia.

**CHANGES IN  $[K^+]_o$  IN RESPONSE TO ILLUMINATION** Since the apical and the delayed basal hyperpolarizations depend upon light-evoked changes in  $[K^+]_o$  in the subretinal space, we investigated whether the effects of hypoxia on membrane potentials could be accounted for by hypoxic changes in light-evoked  $[K^+]_o$  responses. The light-evoked change in subretinal  $[K^+]_o$  ( $V_{K^+}$ ) in response to 60 s of illumination is shown in Fig. 3. As previously described,  $[K^+]_o$  first fell to a minimum and subsequently reaccumulated to a level that was less than the dark-adapted concentration (Steinberg et al., 1980). At the offset of illumination,  $[K^+]_o$  overshot the dark-adapted level before returning to baseline.

<sup>1</sup> Shunting of the apical response to the basal membrane is estimated by finding the scaling factor needed to superimpose the first 2 s of  $V_{ap}$  on  $V_{ba}$ . Theoretically, this is given by  $V_{ba} = [R_{ba}/(R_{ba} + R_s)]V_{ap}$ . If  $R_{ba}$  decreases during hypoxia, one would expect this shunting factor to decrease. In two pairs of normoxic/hypoxic records, including that shown in Fig. 2, the shunting factor was unchanged, and in a third pair of records it decreased from 0.65 to 0.58 during hypoxia. Based on changes in the c-wave, which provide an index of changes in  $R_{ba}$  during hypoxia, we can conclude that it was only in this last case that  $R_{ba}$  actually changed.

Superimposed on the normoxic response is a response to the same stimulus conditions during hypoxia. The hypoxic response was 12% larger, had a slower time to peak (17 vs. 9 s for the normoxic response), and recovered more slowly and less completely during illumination. At the offset of light, the overshoot was smaller during hypoxia. These  $[K^+]_o$  responses were altered, therefore, in the same way as the corresponding  $V_{ap}$  responses in Fig. 2, which provides strong evidence that the changes in apical membrane potential were caused by changes

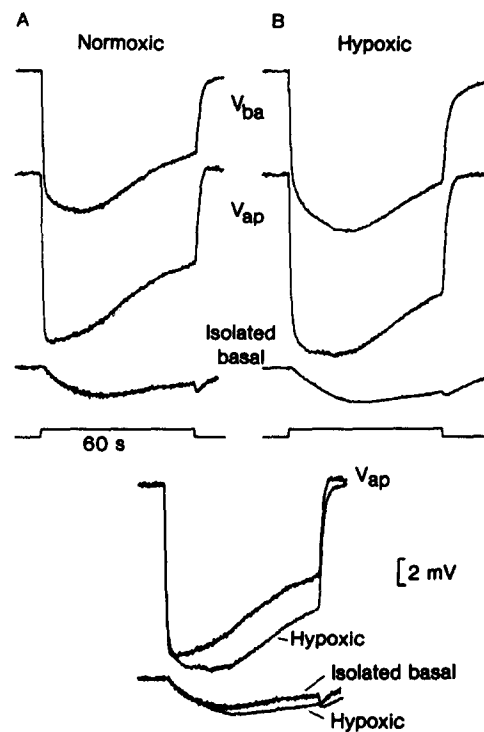


FIGURE 2. Normoxic and hypoxic responses of an RPE cell to 60 s of illumination at  $8.3 \log q/\text{deg}^2 \cdot \text{s}^{-1}$ .  $V_{ba}$  is basal membrane potential,  $V_{ap}$  is apical membrane potential (transepithelial minus  $V_{ba}$ ), and "isolated basal" is an estimate of the potential that is generated at the basal membrane ( $V_{ba}$  minus the shunted apical potential). (A) Before hypoxia. (B) During hypoxia ( $P_aO_2 = 38 \text{ mmHg}$ ). The apical and isolated basal responses of A and B are superimposed at the bottom of the figure. (Cat 77.)

in  $[K^+]_o$ . Hypoxia had similar effects on  $V_{K^+}$  at all illuminations studied ( $7.2\text{--}9.2 \log q/\text{deg}^2 \cdot \text{s}^{-1}$ ), and all effects were reversible at the end of hypoxia.

Although we did not determine the threshold for the hypoxic effect precisely, it was first observed at a rather high  $P_aO_2$  ( $\sim 65 \text{ mmHg}$ ) and was generally graded with the degree of hypoxia. Fig. 4A shows the graded nature of the effect on the early part of the response. These responses superimposed only for the initial 2 s, as shown more clearly in Fig. 13. Following this, the  $[K^+]_o$  responses became

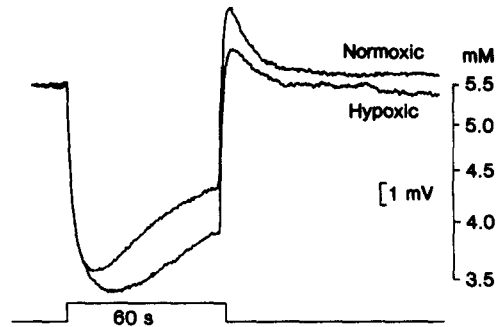


FIGURE 3. Effect of hypoxia on light-evoked  $[K^+]_o$  measured in the subretinal space. Stimuli had the same illumination and duration as in Fig. 2.  $V_{K^+}$  is the difference in voltage between the  $K^+$ -specific and reference barrels of the micro-electrode. Calibration in millimolar is approximate, based on previous measurements of the dark-adapted subretinal  $[K^+]_o$  (Steinberg et al., 1980) and the in vitro response of an average electrode used in this study. The calibration applies only to the normoxic response, since the baseline  $[K^+]_o$  was higher in hypoxia (see text).  $P_aO_2$  during hypoxia was 31 mmHg. The time to peak of the normoxic response in some experiments was longer than the 4 s reported by Steinberg et al. (1980; Figs. 3 and 4A, but not Figs. 4B or 13). The reason for this difference is not known. (Cat 96.)

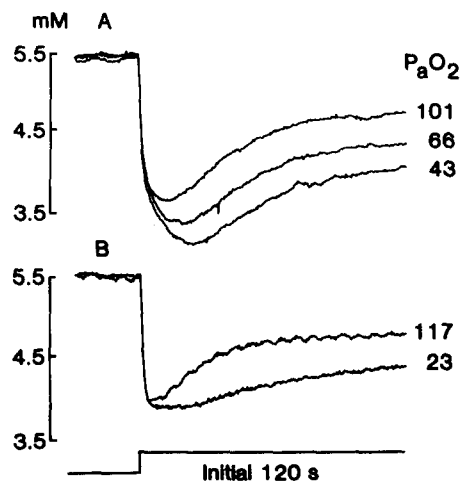


FIGURE 4. Effect of hypoxia on the initial 120 s of the response of subretinal  $[K^+]_o$  to 5 min illumination at  $8.3 \log q/\text{deg}^2 \cdot \text{s}^{-1}$ . (A) Progressive increase in amplitude and time to peak, and delay of recovery during hypoxia. The top trace was recorded before hypoxia, the middle one during hypoxia, and the bottom one during the same episode of hypoxia after the inspired  $PO_2$  had been reduced still further. (B) Flattening of the peak of the response during hypoxia. Arterial oxygen tension ( $P_aO_2$ ) during each response is given to the right of each record. (Cats 96 and 99.)



apparently larger, as the time to reach the minimum progressively lengthened with the degree of hypoxia. While the time to peak increased in most experiments, as in Fig. 4A, the peak was occasionally more flattened than delayed, as shown in Fig. 4B. In such experiments, the effect of hypoxia appeared to be predominantly on the time course of  $[K^+]_o$  reaccumulation, and less on the time to peak and amplitude of the responses.

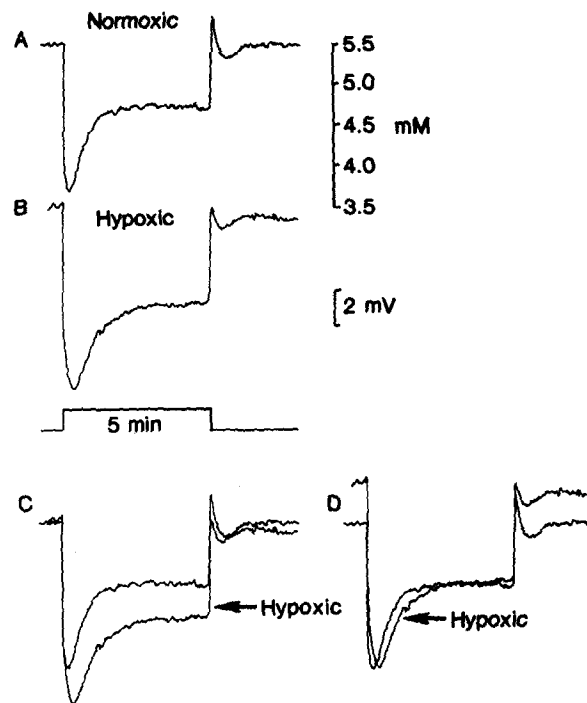


FIGURE 5. Response of subretinal  $[K^+]_o$  to 5 min illumination at  $8.3 \log q/\text{deg}^2 \cdot \text{s}^{-1}$ . (A) Before hypoxia. (B) During hypoxia ( $P_{iO_2} = 43 \text{ mmHg}$ ). These are the same responses shown in the upper and lower traces of Fig. 3A. (C) Superposition of A and B at the dark-adapted baseline. (D) Superposition of A and B at the plateau during illumination. Recovery is not shown, but the recovered response was nearly identical to A. (Cat 96.)

Fig. 5 presents  $V_{K^+}$  responses to 5 min of illumination to show more clearly the plateau during illumination. The major effects are the same as observed in Fig. 3, as illustrated by the superposition of Fig. 5C, where the responses are superimposed as if the dark-adapted level of  $[K^+]_o$  were the same during normoxia and hypoxia. By doing this, the peak and plateau portions of the hypoxic response both appeared to reach lower values of  $[K^+]_o$  than the normoxic response. On the average ( $n = 8$  episodes of hypoxia), the peak response was 16% larger and the plateau of the response was 2 mV ( $\sim 0.5 \text{ mM}$ ) larger during hypoxia. As will be shown below, however,  $[K^+]_o$  in the dark was elevated during hypoxia, and it is more accurate, but probably still not exact, to superimpose the responses at

their light-adapted plateaus, as in Fig. 5D.<sup>2</sup> Now both the plateaus and the minima reach the same level during normoxia and hypoxia. The peak of the overshoot also reaches the same value in both conditions, but is a smaller overshoot in hypoxia relative to the dark level. The superposition in Fig. 5D suggests that much of the change in the response could be due simply to a different baseline during hypoxia, but there is still a change in the time course near and following the peak of the response that cannot be explained on this basis.

**HYPOXIC  $[K^+]_o$  AND THE DELAYED BASAL HYPERPOLARIZATION** The effects of hypoxia on  $[K^+]_o$  appeared to account directly for the effects on the light-evoked response of the apical membrane, but there were also hypoxic changes in the delayed basal hyperpolarization (Fig. 2). These changes are probably also attributable to the new time course of subretinal  $[K^+]_o$  during hypoxia. Before presenting the evidence for this, it is first necessary to review our current understanding of this delayed basal response.

The mechanism of the delayed basal hyperpolarization has been studied in *in vitro* preparations from the gecko eye, where this response is also observed. A delayed basal hyperpolarization could be produced in an RPE-choroid preparation (i.e., no neural retina) by making a solution change that mimicked the light-evoked  $[K^+]_o$  decrease. Simply decreasing  $[K^+]_o$  from 5 to 2 mM outside the apical membrane produced a delayed basal response. Furthermore, when intracellular  $K^+$  ( $[K^+]_i$ ) was prevented from changing during the change in apical  $[K^+]_o$ , the delayed basal hyperpolarization did not develop (Griff and Steinberg, 1982*b*, 1984). These results suggested that the delayed basal response depends on a decrease in  $[K^+]_i$  that results from the decrease in  $[K^+]_o$ .<sup>3</sup>

If the rate-limiting step in the generation of the delayed basal hyperpolarization in cat is a change in  $[K^+]_i$ , then the time course of the delayed basal hyperpolarization would be predictable from  $[K^+]_i$ . The change in  $[K^+]_i$ , in turn, could be predictable simply from the change in  $[K^+]_o$ . Since  $V_{ap}$  has the same time course as  $[K^+]_o$ , it may then be possible to predict the time course of the delayed basal hyperpolarization from  $V_{ap}$ . The delayed basal hyperpolarization actually looks like a filtered version of  $V_{ap}$ , and such a description is accurate, as shown in Fig. 6. The top traces are the actual delayed basal hyperpolarizations of Fig. 2, and the middle traces show the output of a filter with a single low-pass stage when  $V_{ap}$  is the input. The filter model is described in the Appendix. By a suitable adjustment of the two parameters, the model fits the normoxic basal response reasonably well. Of more importance in the present context is that the model also fits during hypoxia with the same choices of parameters. That is, one transfer

<sup>2</sup> The exact positioning of these responses could not be determined because the  $V_{K^+}$  signal drifted slowly, and it was impossible to separate this from slow actual changes in  $[K^+]_o$ .

<sup>3</sup> The expected decrease in  $[K^+]_i$  should decrease the apical membrane equilibrium potential, but this effect would be too small to influence  $V_{ap}$  measurably. With regard to the delayed basal response, the expected intracellular change is in the wrong direction for the response to be simply the result of a change in basal membrane  $K^+$  equilibrium potential, so at least one other step must be involved in response generation. Also, at this point, no experiments have been done that rule out the possibility of a change in  $[Cl^-]_i$ , rather than  $[K^+]_i$ , as being the important factor.

function can be used before and during hypoxia to predict how the isolated basal response is related to the apical response. This means that the change in the delayed basal hyperpolarization during hypoxia follows directly from the hypoxic effect on  $[K^+]_o$ ; no additional mechanism needs to be postulated.

#### *Hypoxic Changes in Resting $[K^+]_o$ and Membrane Potential*

**STEADY STATE  $[K^+]_o$**  As will be shown below (Fig. 10), the onset of hypoxia in the dark immediately produced changes in both  $V_{ap}$  and  $V_{ba}$ . These changes also were associated with changes in subretinal  $[K^+]_o$ , and can best be analyzed by first describing changes observed in  $[K^+]_o$  when hypoxia began during dark adaptation. In darkness, subretinal  $[K^+]_o$  is taken to be  $\sim 5.5$  mM, 0.5 mM higher than in the vitreous near the retina (Steinberg et al., 1980). Hypoxia led to a rapid increase in dark-adapted subretinal  $[K^+]_o$ , as shown in Fig. 7A. This increase

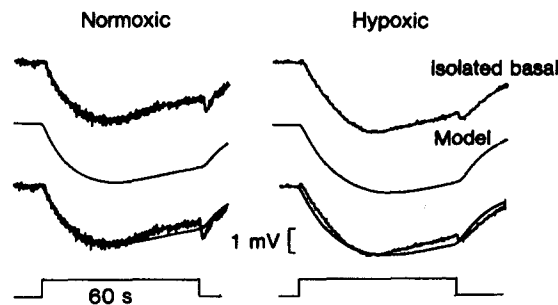


FIGURE 6. Modeling of the delayed basal hyperpolarization before and during hypoxia. The top traces are the isolated basal responses of Fig. 2, A and B. The middle traces are synthesized responses as described in the Appendix. The lower traces are superpositions of the actual and modeled responses. The same parameters of the model were used before and during hypoxia.

began  $\sim 15$  s after changing the gas, and was maximal after another 30–60 s. Since there were several stages of delay between the gas flowmeters and the eye, it is likely that the increase in  $[K^+]_o$  occurred almost as soon as the hypoxic blood reached the eye. In this example,  $P_aO_2$  decreased to 40 mmHg and  $[K^+]_o$  increased by  $\sim 0.7$  mM. Increases in  $[K^+]_o$  of 0.4 mM were observed during the mildest episodes of hypoxia studied ( $P_aO_2$  65 mmHg) and tended to be larger in more severe hypoxia. Overall, the mean increase was 0.5 mM (range, 0.2–0.7 mM; 15 episodes of hypoxia;  $P_aO_2$ 's between 30 and 65 mmHg). The change in the time course of light-evoked responses (Fig. 2) was evident as soon as flashes were presented after the change in dark-adapted  $[K^+]_o$ . At the end of hypoxia,  $[K^+]_o$  recovered, as shown in Fig. 7B, generally with a slightly slower time course than the increase at the onset of hypoxia.

Because of drift in the subretinal  $K^+$  electrode, in order to inquire about the persistence of the hypoxic  $[K^+]_o$  change it was necessary to record in the vitreous, where we could use an electrode with a tip broken to a larger size (50 vs. 2  $\mu\text{m}$ ) that made it more stable and less noisy. Vitreal  $[K^+]_o$  could be used to monitor

subretinal  $[K^+]_o$ , because the subretinal changes are large and  $K^+$  diffuses to or from the vitreous in response to these changes (Steinberg et al., 1980).<sup>4</sup> As shown in Fig. 8, the vitreal response to hypoxia in dark adaptation was as we would have expected for a response originating in the outer retina, slower and slightly smaller than at the source.  $[K^+]_o$  did recover partially during hypoxia, which suggests the presence of some homeostatic capability, but most of the change was maintained. At the end of hypoxia,  $[K^+]_o$  undershot the prehypoxic level before returning to baseline.

During light adaptation, subretinal  $[K^+]_o$  is lower than in dark adaptation by  $\sim 1$  mM (Steinberg et al., 1980), and it was of interest to determine whether

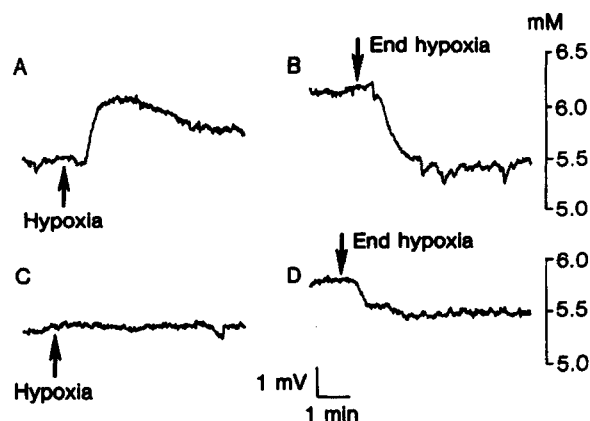


FIGURE 7. Effect of hypoxia on subretinal  $[K^+]_o$  in darkness and during steady illumination. (A) Onset of hypoxia ( $P_aO_2 = 40$  mmHg) during dark adaptation. (B) Recovery from hypoxia in dark adaptation. (C) Onset of hypoxia ( $P_aO_2 = 52$  mmHg) during steady illumination of  $8.3 \log q/\text{deg}^2 \cdot \text{s}^{-1}$ . (D) Recovery from the episode of hypoxia initiated in C, after the retina had been returned to darkness. (Cats 102 and 98.)

changes in  $[K^+]_o$  also occurred when hypoxia was initiated during steady illumination. In these experiments, a diffuse white light at rod saturation was left on 2–4 min prior to the onset of hypoxia. As Fig. 7C shows, there was little or no change in  $[K^+]_o$  when hypoxia began during this light exposure. When the light went off, however,  $[K^+]_o$  increased to a level higher than the previous normoxic dark-adapted value. While this is not shown in Fig. 7, it can be surmised from the decrease in  $[K^+]_o$  in Fig. 7D upon the return to normoxia in dark adaptation.

The striking increase of subretinal  $[K^+]_o$  during dark adaptation and the absence of such a change during adaptation to relatively strong illumination

<sup>4</sup>  $[K^+]_o$  changes in more proximal layers of the retina are unlikely to be a significant source of error. We would expect  $[K^+]_o$  in the inner retina to be relatively unaffected, since autoregulation preserves ganglion cell sensitivity and b-wave amplitude at the levels of hypoxia considered here (e.g., Bos, 1968; Enroth-Cugell et al., 1980; Linsenmeier et al., 1983). Measurements of inner retinal  $[K^+]_o$  responses to light showed no change during hypoxia (see Discussion).

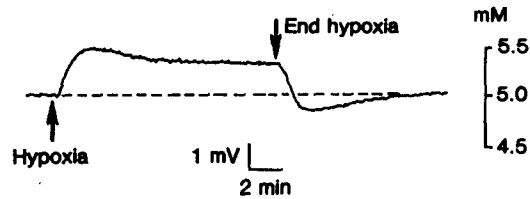


FIGURE 8. Effect of hypoxia on  $[K^+]_o$  measured in the vitreous humor close to the retina during dark adaptation. The  $P_aO_2$  during this episode was 39 mmHg. The calibration assumes a dark-adapted vitreal  $[K^+]_o$  of 5 mM. (Cat 103.)

suggested that the effect of hypoxia on  $[K^+]_o$  might be graded with the level of illumination. This relationship was again studied with vitreal recordings, since it was difficult to perform repeated episodes of hypoxia while holding the electrode at the same location in the subretinal space. Fig. 9 shows that the increase in  $[K^+]_o$  at the onset of hypoxia became smaller as illumination increased. At the high gain used for these recordings, a small  $[K^+]_o$  change (0.2 mM) was apparent at rod saturation (8.3) that could not be detected in the subretinal recordings. The delay between the onset of hypoxia and the change in  $[K^+]_o$  was approximately the same at all illuminations.

**HYPOXIC EFFECTS ON DARK-ADAPTED MEMBRANE POTENTIALS** The increase in  $[K^+]_o$  at the onset of hypoxia should lead to an apical depolarization, just as the decrease of  $[K^+]_o$  at the onset of illumination leads to an apical hyperpolarization. It was not possible to record all of the parameters,  $V_{K^+}$ ,  $V_{ap}$ , and  $V_{ba}$ , during the same episode of hypoxia, but the effects of hypoxia on membrane potentials can be inferred from comparing recordings of  $V_{ba}$  during some episodes of hypoxia with recordings of  $V_{K^+}$  and transepithelial potential (TEP) made

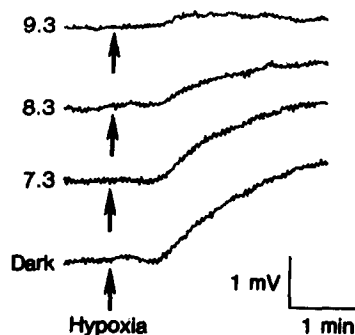


FIGURE 9. Effect of hypoxia on vitreal  $[K^+]_o$  at several levels of steady illumination. The steady illumination in  $\log q/\text{deg}^2 \cdot \text{s}^{-1}$  is given to the left of each trace. Recordings were obtained in the order shown from bottom to top. Between the two highest levels of illumination, however, an additional episode of hypoxia was performed with the retina dark-adapted, and the  $[K^+]_o$  response was nearly identical to that shown in the bottom trace. The  $P_aO_2$  during hypoxia was similar for all episodes (36–39 mmHg). 1 mV is  $\sim 0.25$  mM. (Cat 103.)

during other episodes. The combination of TEP and  $V_{ba}$  gives enough information to allow us to draw qualitative conclusions about events generated at the apical membrane (see Methods). Fig. 10A shows  $V_{ba}$  during the onset of one episode of hypoxia and a TEP recording from the onset of another episode. ( $V_{K^+}$  was obtained simultaneously with the TEP recording.) The increase in  $[K^+]_o$  was accompanied by an initial decrease in TEP and a depolarization of the

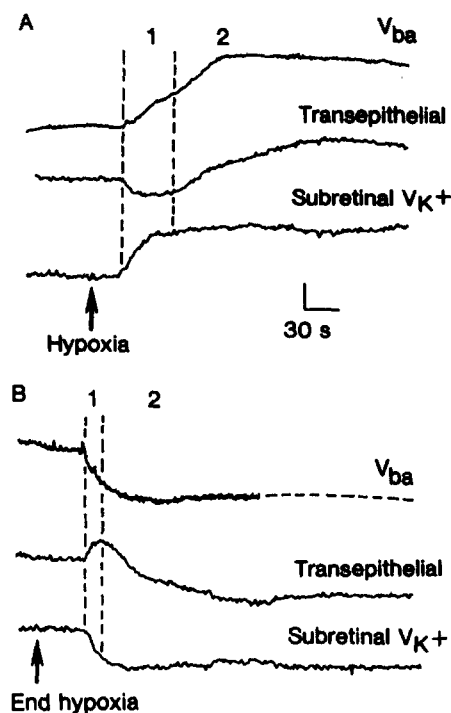


FIGURE 10. Basal membrane potential ( $V_{ba}$ ), transepithelial potential (TEP), and  $[K^+]_o$  at the beginning and end of hypoxia during dark adaptation. (A) Onset of hypoxia. (B) End of hypoxia. Interrupted vertical lines show the two phases of the TEP in both A and B. Only the first phase is related directly to  $V_{K^+}$ . TEP and  $V_{K^+}$  responses were obtained simultaneously, but in different animals than the  $V_{ba}$  responses. The vertical calibration is 2 mV for  $V_{ba}$  and 1 mV for TEP and  $V_{K^+}$ .  $P_aO_2$  was between 23 and 31 for the episodes of hypoxia shown in this figure. ( $V_{ba}$ , cats 100 and 91; TEP, cat 99.)

basal membrane (period 1). Subsequently, TEP increased during a further depolarization of  $V_{ba}$  (period 2). The potential changes in the first part of the record are expected, since the increase in  $[K^+]_o$  should depolarize the apical membrane as the  $[K^+]_o$  equilibrium potential across the apical membrane decreases. Thus, in period 1, the predominant effect was a depolarization that originated at the apical membrane and was partially shunted to the basal membrane, causing the depolarization observed in  $V_{ba}$ . The apical depolarization decreased the difference between apical and basal membrane potentials, resulting

in the decrease in TEP. In the second phase of the response, period 2, the depolarization of  $V_{ba}$  must have originated at the basal membrane, since the TEP increased. The mechanism of this basal response will be considered shortly.

At the end of hypoxia, an opposite set of events occurred, as shown in Fig. 10B. A  $[K^+]_o$  decrease was associated with an initial increase in TEP and a hyperpolarization of  $V_{ba}$  during period 1. This initial hyperpolarization must have resulted from an apical hyperpolarization shunted to the basal membrane. Following the initial increase in TEP, there was a slower decrease associated with a continued hyperpolarization of  $V_{ba}$  (period 2). The later  $V_{ba}$  hyperpolarization, therefore, must have originated at the basal membrane, even though two clearly separate phases of the basal response could not be detected at the end of hypoxia, as they were at the beginning.

**ORIGIN OF THE HYPOXIC BASAL MEMBRANE RESPONSES IN DARK ADAPTATION** Two questions concern us about the mechanism of the basal depolarization at the beginning of hypoxia (Fig. 10A, period 2). First, we would like to know its mechanism. Specifically, does it depend upon the increase in subretinal  $[K^+]_o$  that occurs during period 1 (Fig. 10A) or does it originate from a separate effect of hypoxia on the basal membrane? Second, do changes in basal membrane resistance accompany the change in basal membrane potential in hypoxia? Briefly, the basal depolarization is probably caused by the change in subretinal  $[K^+]_o$ , and it is accompanied by a decrease in resistance.

The reason for asking the first question is that delayed basal responses accompanying illumination are indirect consequences of the light-evoked changes in  $[K^+]_o$  leading to presumed changes in  $[K^+]_i$ . In the same way, the basal depolarization at the onset of hypoxia could result from the increase in  $[K^+]_o$  at the onset of hypoxia. Some evidence suggests that this depolarization is a "delayed" type of  $K^+$ -dependent response. The basal depolarization during hypoxia can be observed in vitreal recordings as an increase in the standing potential (Linsenmeier, R. A., and R. H. Steinberg, manuscript in preparation). During dark adaptation, when  $[K^+]_o$  increases in response to hypoxia, the basal membrane depolarizes as shown here, and the standing potential increases (Linsenmeier et al., 1983). In light adaptation, however, when the  $[K^+]_o$  change is small in response to hypoxia, the standing potential does not change (Linsenmeier, R. A., and R. H. Steinberg, manuscript in preparation), which indicates that the basal depolarization must have been absent. The simplest interpretation of this finding is that the basal depolarization is a delayed type of event that requires the change in  $[K^+]_o$ . This conclusion must remain somewhat tentative, however, since the model relating  $[K^+]_o$  to basal events does not predict the observed time course of the basal depolarization. Also, the recovery of basal membrane potential after hypoxia, observed in the standing potential (Linsenmeier, R. A., and R. H. Steinberg, manuscript in preparation), is more complicated than one would have expected on the basis of the change in  $[K^+]_o$  alone.

**BASAL MEMBRANE RESISTANCE** The second question related to the depolarization in period 2 of Fig. 10A is whether it is accompanied by changes in basal membrane resistance. This question arises because we know that light-evoked basal membrane responses are accompanied by such changes: a decrease

in resistance accompanies the light peak, which is a basal depolarization (Griff and Steinberg, 1982a; Linsenmeier and Steinberg, 1983), and an increase in resistance accompanies the delayed basal hyperpolarization (Griff and Steinberg, 1984).

A resistance change can be sought in two ways. First, the voltage drops can be recorded in response to pulses of injected current, as described in Methods. When these voltage drops are monitored in the subretinal space, we can look for changes in  $R_{sc} + R_t$ , and an example of such measurements, normalized to 100% before hypoxia, is shown in Fig. 11.  $R_t + R_{sc}$  decreased during hypoxia, and, assuming that there was no change in  $R_{sc}$  and that  $R_t$  and  $R_{sc}$  were initially equal (Linsenmeier and Steinberg, 1983), the decrease in  $R_t$  would have been ~13%. This means that at least one of the component resistances in  $R_t$  (i.e.,  $R_{ap}$ ,  $R_{ba}$ , or  $R_s$ ) decreased during hypoxia. We can obtain some information about which resistance changed by using an intracellular electrode to measure voltage drops across the basal membrane and sclera. These  $iR$  drops decreased by an average of 6% during hypoxia (four cells), which suggests that  $R_{ba}$  decreased. A decrease

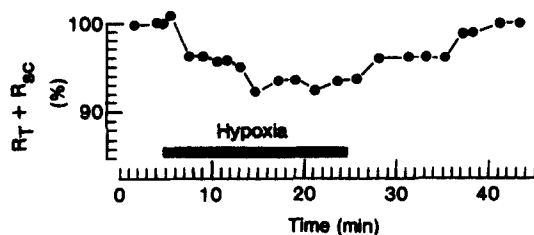


FIGURE 11. Normalized equivalent resistance of the RPE plus sclera ( $R_t + R_{sc}$ ) during hypoxia. Each point represents the average of the voltage responses to four or five current pulses delivered as described in Methods. The duration of hypoxia ( $P_aO_2 = 31$  mmHg) is shown by the dark bar. Short (4 s) flashes were delivered at ~1/min, but otherwise the retina was dark-adapted. (Cat 88.)

in  $R_s$  cannot be ruled out from this measurement alone, however, since this would have allowed more current to flow across  $R_s$ , reducing the  $iR$  drop at the basal membrane by reducing  $i$  rather than  $R$ . A decrease in  $R_{ap}$  alone is not a possible explanation, since this would have allowed more current to flow across the cell, increasing the  $iR$  drop at the basal membrane.

The second way to demonstrate a resistance change is to record the relative sizes of the polarizations at both membranes in response to light. This provides information about resistance, because the sizes of the apical, basal, and TEP responses all depend on the resistances of the RPE circuit. The analysis that follows is described more fully by Linsenmeier and Steinberg (1983). For the  $K^+$ -dependent apical membrane hyperpolarization at the onset of light, the observed apical and basal responses,  $\Delta V_{ap}$  and  $\Delta V_{ba}$  ( $\Delta$  indicates light minus dark), are both functions of the battery ( $\Delta V'_{ap}$ , defined as the potential that would be observed if  $R_s$  were infinite) and the resistances:

$$\Delta V_{ap} = [(R_{ba} + R_s)/(R_{ap} + R_{ba} + R_s)]\Delta V'_{ap}; \quad (2)$$



$$\Delta V_{ba} = [R_s / (R_{ap} + R_{ba} + R_s)] \Delta V'_{ap} \quad (3)$$

These equations assume that all resistances are constant during each flash, but may vary between flashes. The change in transepithelial potential is the difference between  $\Delta V_{ap}$  and  $\Delta V_{ba}$ , or:

$$\Delta \text{TEP} = -[R_s / (R_{ap} + R_{ba} + R_s)] \Delta V'_{ap} \quad (4)$$

The transepithelial event caused by the apical hyperpolarization is an increase in potential called the RPE c-wave.

During hypoxia, any of the factors in the equations above could change, and each would lead to a unique and predictable set of changes in  $\Delta V_{ap}$ ,  $\Delta V_{ba}$ , and  $\Delta \text{TEP}$ . For instance, a decrease in  $R_{ap}$  would lead to an increase in  $\Delta \text{TEP}$ ,  $\Delta V_{ap}$ , and  $\Delta V_{ba}$ . A decrease in  $R_{ba}$  would also lead to an increase in  $\Delta \text{TEP}$ , but to decreases in  $\Delta V_{ap}$  and  $\Delta V_{ba}$ . This latter possibility is the actual set of changes

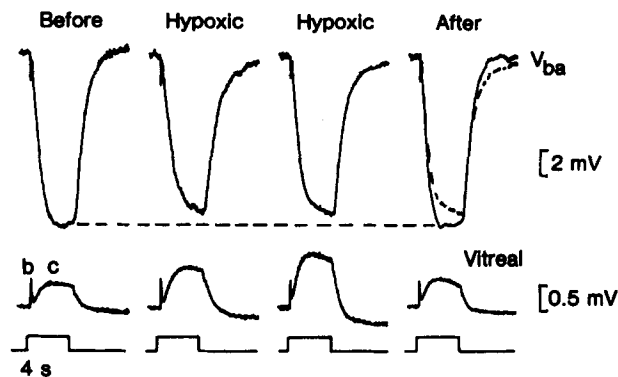


FIGURE 12. Basal membrane potential and the vitreal ERG in response to a 4-s flash at  $8.3 \log q/\text{deg}^2 \cdot \text{s}^{-1}$ . The interrupted line in the fourth record is a tracing of the second hypoxic response. The rapid deflection at the beginning of the traces is the b-wave. The major event seen in the vitreal responses is the c-wave.  $P_a\text{O}_2$  during hypoxia was 51 mmHg. (Cat 87.)

observed during hypoxia, as can be deduced from the set of records presented in Fig. 12.  $\Delta V_{ba}$  decreased during hypoxia and recovered following hypoxia. It was not possible to record TEP simultaneously with the intracellular recordings, but from separate experiments, we know that the RPE c-wave increased during hypoxia by exactly the same amount as the vitreal c-wave (Linsenmeier, R. A., and R. H. Steinberg, manuscript in preparation). Therefore, the vitreal recordings in the lower part of the figure are an adequate substitute for  $\Delta \text{TEP}$ . The increase in the vitreal c-wave indicates that during hypoxia the RPE c-wave, and therefore the difference between  $\Delta V_{ap}$  and  $\Delta V_{ba}$ , increased by 0.3–0.5 mV. Since  $\Delta V_{ba}$  at the peak of the c-wave was 1.6 mV smaller than before hypoxia,  $\Delta V_{ap}$  must have decreased also, by 1.1–1.3 mV. Results similar to those presented in Fig. 12 were obtained in recordings from six other cells in five cats. Thus, during hypoxia the TEP response (c-wave) increased and the hyperpolarizations of both

the apical and basal membranes in response to a short flash decreased. The only mechanism that would cause this set of changes is a decrease in  $R_{ba}$  during hypoxia, as also suggested by the results of current injection. We cannot rule out a small effect of a change in another resistance, but the dominant effect must have been the decrease in  $R_{ba}$ . Interestingly, the resistance change was not always maintained for the entire duration of hypoxia, and occasionally failed to occur at all. This can be deduced from the time course of changes in the c-wave (Linsenmeier, R. A., and R. H. Steinberg, manuscript in preparation).

The records of Fig. 12 may appear to contradict the results presented earlier (Fig. 2), which showed an increase in the amplitude of both the apical hyperpo-

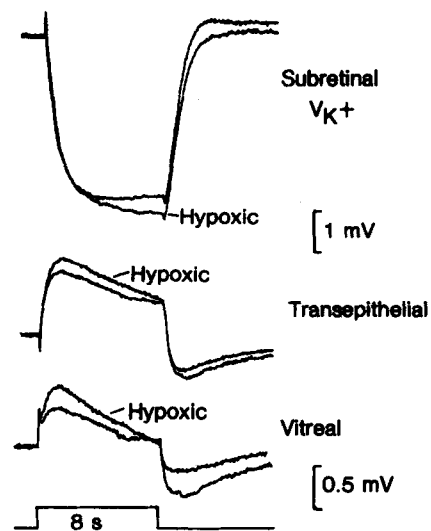


FIGURE 13. Simultaneous recordings of  $V_{K^+}$ , TEP, and the vitreal ERG in response to an 8-s flash at  $8.3 \log q/\text{deg}^2 \cdot \text{s}^{-1}$ . In each case, a hypoxic response is superimposed on a response obtained before hypoxia. This example illustrates the high sensitivity of  $[K^+]_o$  to hypoxia, since the hypoxic  $P_aO_2$  in this case was 68 mmHg. The calibration bar is 1 mV for the  $V_{K^+}$  and TEP recording and 0.5 mV for the vitreal recording. (Cat 102.)

larization and delayed basal hyperpolarization during hypoxia. The results are complicated but not contradictory, since we now see that there can be two separate effects of hypoxia on the light responses. One effect is due to the change in the size and time course of the  $[K^+]_o$  response, and the other to the decrease in  $R_{ba}$ . These have opposite effects on the membrane responses (Eqs. 2 and 3), the first leading to an increase, by increasing  $V'_{ap}$ , and the second to a decrease. These two effects are most important at different times during illumination, as shown by the recordings in Fig. 13, which present simultaneous  $V_{K^+}$ , TEP, and vitreal responses to 8-s flashes before and during hypoxia. At short times, less than  $\sim 2$  s after the onset of light, the increase in the TEP c-wave and the decrease in membrane responses must be a result of the decrease in  $R_{ba}$ , since

the light-evoked  $[K^+]_o$  responses are identical before and during hypoxia. At longer times, greater than  $\sim 4$  s, the light-evoked  $[K^+]_o$  responses are larger during hypoxia, causing the larger membrane responses as in Fig. 2. The resistance change must still tend to reduce the responses after 4 s, so the sizes of  $V_{ap}$  and  $V_{ba}$  depend on both factors. At times between  $\sim 2$  and 4 s, it was often possible to observe that the responses were smaller during hypoxia, as expected from the change in  $R_{ba}$ , but their shape was influenced by the change in the dynamics of the  $[K^+]_o$  response. Comparison of the  $[K^+]_o$  responses of Fig. 13 with the intracellular recordings of Fig. 12 indicates that the change in response time course in Fig. 12 during hypoxia is exactly as expected from the change in the  $[K^+]_o$  response.

#### DISCUSSION

We have shown that the principal effects of systemic hypoxia in the outer portion of the retina (RPE, photoreceptors) are upon the concentration of  $K^+$  in the subretinal space. The effects on subretinal  $[K^+]_o$  can be summarized as follows.

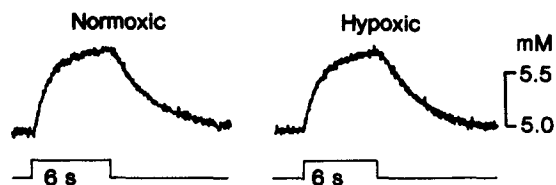


FIGURE 14. Normoxic and hypoxic responses of vitreal  $[K^+]_o$  to 6-s flashes under conditions designed to isolate the contribution of the proximal retina. Illumination of  $7.3 \log q/\text{deg}^2 \cdot \text{s}^{-1}$ .  $P_2O_2$  during hypoxia was 36 mmHg. (Cat 103.)

(a) The onset of hypoxia is accompanied by a rapid elevation of  $[K^+]_o$ . This effect is maximal in the dark and is progressively inhibited as the background illumination is raised to rod saturation. (b) During hypoxia, there is an alteration in the time course and amplitude of the light-evoked subretinal  $[K^+]_o$  decrease.

Since any variation in subretinal  $[K^+]_o$  affects both the apical and basal membrane potentials of the RPE, these hypoxic effects can be recorded at the RPE cell. The effects of hypoxia on  $[K^+]_o$  appear adequate to explain the changes in RPE membrane potential. The change in the time course of the light-evoked  $[K^+]_o$  response accounts for hypoxic changes in both the apical hyperpolarization and the delayed basal hyperpolarization of the RPE. The increase in  $[K^+]_o$  in the dark-adapted retina accounts for the hypoxic depolarization of the apical membrane and probably also for the delayed depolarization of the basal membrane.

The hypoxic  $[K^+]_o$  effects almost certainly originate in the subretinal space, and are not consequences of an altered  $[K^+]_o$  at a more proximal location. Fig. 14 shows vitreal responses to brief, relatively dim stimuli designed to show the light-evoked  $[K^+]_o$  increase that occurs in the inner retina of cat (Steinberg et al., 1980). These responses are nearly identical before and during hypoxia, even at the rather low  $P_2O_2$  used in this experiment. Longer stimuli could not be used here because the  $[K^+]_o$  decrease in the outer retina eventually influences the

inner retina through diffusion or spatial buffering, but 6-s flashes should have been sufficient to show an effect of hypoxia.

We can also conclude that the  $[K^+]_o$  changes were caused by the low  $PO_2$ , and not by an acidosis that might have developed during hypoxia. Even severe acidosis ( $pH_a = 7.1$ ), caused by elevation of inspired  $CO_2$ , failed to change the light-evoked  $[K^+]_o$  responses in a similar way. Hypercapnic responses were slightly smaller, but had the same time course as control responses. Furthermore, in contrast to hypoxia, hypercapnia led to only small, slow increases in dark-adapted  $[K^+]_o$ , and even these were not always observed (Linsenmeier, R. A., and R. H. Steinberg, unpublished observations).

#### *Mechanisms of Hypoxic Effects on $[K^+]_o$*

Further progress in understanding the effects of hypoxia on the photoreceptors and RPE clearly relies on an explanation of the mechanisms by which  $[K^+]_o$  is altered. It is reasonable to begin with a description of  $[K^+]_o$  homeostasis under normoxic conditions. Several lines of evidence indicate that the photoreceptors are of primary importance in causing the light-evoked decrease in  $[K^+]_o$ . For example, the change in  $[K^+]_o$  can be recorded in the neural retina isolated from the RPE, although it is smaller than the change recorded in the intact retina where the RPE acts as a diffusion barrier (Oakley et al., 1979). Second, the decrease in  $[K^+]_o$  is maximal adjacent to the rod inner segments (Oakley and Green, 1976; Oakley et al., 1979). Third, its time course and amplitude are related to the variations in rod receptor potential produced by altering stimulus conditions or retinal temperature (Oakley et al., 1979). Finally, perfusion of the intact retina with barium, while apparently blocking the  $[K^+]_o$  conductances of the Müller cell (as inferred from the loss of slow PIII) (Bolnick et al., 1979; Winkler and Gum, 1981) and the RPE (directly tested in isolated RPE) (Griff, E. R., Y. Shirao, and R. H. Steinberg, manuscript in preparation), leaves the light-evoked  $[K^+]_o$  decrease somewhat smaller but otherwise intact (Oakley, 1983). Thus, while the Müller cell and RPE may contribute to the decrease of  $[K^+]_o$ , the change appears to originate predominantly from the photoreceptors.

A model has been developed to account for the mechanism by which the rods produce the  $[K^+]_o$  decrease (Matsuura et al., 1978; Oakley et al., 1979; Fujimoto and Tomita, 1979). In the dark, the rod is relatively depolarized and there is a continuous  $K^+$  efflux that is balanced by the  $Na^+/K^+$  pump of the inner segment (Stirling and Lee, 1980). Light decreases the  $Na^+$  conductance of the outer segment, hyperpolarizes the rod, and reduces the passive efflux of  $K^+$  as rod membrane potential approaches  $E_{K^+}$ . Since the rate of the  $Na^+/K^+$  pump is initially unchanged, the decreased passive efflux leads to a decrease in  $[K^+]_o$  in the subretinal space. While this model successfully predicted the  $[K^+]_o$  decrease in response to brief flashes ( $<0.3$  s) (Oakley et al., 1979), it did not account for the recovery of  $[K^+]_o$  during maintained illumination (Oakley and Steinberg, 1982).

It had been suggested that diffusion of  $[K^+]_o$  into the subretinal space, spatial buffering by Müller cells, and a decreased active uptake of  $[K^+]_o$  into the cells surrounding the subretinal space alone or together could be responsible for the

reaccumulation of  $K^+$  (Steinberg et al., 1980). It has now been shown in toad that the time course of reaccumulation is similar in the intact retina and in the isolated retina without RPE, which suggests that the RPE has a minimal role in this process (Oakley, 1983). Moreover, changes in the rate of active  $K^+$  uptake have been implicated in reaccumulation by the observation that inhibitors of the pump, including ouabain, 0 mM  $K^+$ , cold, and severe hypoxia, all prevented reaccumulation (Shimazaki and Oakley, 1984). A revised model that takes these observations into account predicts that a steady fall in  $[Na^+]_i$  in the photoreceptors during maintained illumination leads to a gradual slowing of the pump and therefore to a reaccumulation of  $[K^+]_o$  (Shimazaki and Oakley, 1984). It is not possible to rule out a role for pumps in the Müller cell, but the photoreceptor pumps will be affected most directly, since the reason for the decrease in  $[Na^+]_i$  is the light-evoked decrease in photoreceptor  $Na^+$  conductance, in combination with the continued active extrusion of  $Na^+$ .

These findings in toad lead us to hypothesize that graded hypoxia in cat also affects the rate of the rod's  $Na^+/K^+$  pump. This could explain our main findings with respect to  $[K^+]_o$ . First, a slowing of the rate of pumping would reduce the net uptake of  $[K^+]_o$  from the subretinal space in the dark and lead to the rapid increase of  $[K^+]_o$  at the onset of hypoxia. Second, as discussed above, the recovery of light-evoked responses apparently depends on a slowing of the  $Na^+/K^+$  pump. If the pump is already slowed in hypoxia, then illumination may change the pump rate less, slowing or reducing the magnitude of the recovery. This is supported directly by the observation that ouabain and severe hypoxia in toad have similar effects on light-evoked  $[K^+]_o$  responses. Conversely, if the pump is already slowed by steady illumination, then hypoxia would be expected to have a smaller effect. This could explain the dependence of the hypoxic  $[K^+]_o$  change upon illumination (Figs. 7 and 9). Thus, in a sense, light protects the rod from the effects of hypoxia.

The idea that hypoxia affects  $[K^+]_o$  by inhibiting the pump gains support from work on other neural tissue. In rat cerebellar cortex, there is an increase in  $[K^+]_o$  during electrical stimulation and a  $[K^+]_o$  undershoot at the end of stimulation that is blocked by ouabain. The undershoot probably reflects the increased pumping that developed during stimulation in response to the increase in  $[K^+]_o$  or decrease in  $[Na^+]_i$ . Hypoxia (10%  $O_2$  inspired) mimics the effect of ouabain (Ullrich et al., 1982). In frog optic tectum, both ouabain and anoxia elevate resting  $[K^+]_o$  and also slow the decay of  $[K^+]_o$  following its transient elevation by visual stimuli (Sick and Kreisman, 1981). Finally, in cat cuneate nucleus, strophanthidin increases the amplitude of stimulus-evoked increases in  $[K^+]_o$  and slows the decay of  $[K^+]_o$  following stimulation. These same effects are produced by lowering the blood pressure, which may have produced hypoxia (Krnjevic and Morris, 1975). In summary, pump inhibitors may have somewhat different effects on  $[K^+]_o$  in different brain regions, but the effects in a particular region are similar to those produced by hypoxia or anoxia.

In the cat, we found an increase in the amplitude of the light-evoked  $[K^+]_o$  response, in addition to the slowing of recovery. The amplitude change appeared to result mostly from the elevation of the baseline  $[K^+]_o$  rather than a decrease

in the minimum during illumination. In the toad, responses recorded during severe hypoxia were smaller than control responses. The reason for this difference is not clear. We can speculate that the difference might be a result of the absence of the RPE in the toad preparation, or the use of severe as opposed to relatively mild hypoxia, or possibly to a difference in the relative timing of the recovery process in the two species. It is also possible that hypoxia exerts other effects on the rods that contribute to the changes in  $[K^+]_o$ . For instance, there is evidence that hypoxia can increase  $K^+$  conductances (Hansen et al., 1982; Vleugels et al., 1980), and reduce extracellular volume (e.g., Hansen, 1981). Either would lead to an increase in the baseline  $[K^+]_o$ .

#### *Implications for More Proximal Stages of Visual Processing*

As previously pointed out, the  $PO_2$  in the outer retina will fall during even mild hypoxia, because the outer retina depends upon the choroidal circulation (Linsenmeier et al., 1983). Oxygen extraction from the choroid is normally small (Elgin, 1964; Alm and Bill, 1970), and venous oxygen tension is therefore high. During even mild hypoxia, arterial  $PO_2$  will fall below this normally high venous value, so venous  $PO_2$  will fall as well. This would be true even if choroidal oxygen extraction could be reduced by an increase in blood flow, which is not thought to occur (Bill, 1962; Friedman and Chandra, 1970). The point is that the outer retina "sees" small changes in arterial  $PO_2$ .

It appears that the rod's  $Na^+/K^+$  pump, which is maximally stimulated during darkness (Kimble et al., 1980; Zuckerman and Weiter, 1980), is exquisitely sensitive to these small changes in  $PO_2$ . This sensitivity implies that the high  $PO_2$  normally supplied by the choroid is necessary for photoreceptor function. It has been suggested that the major function of the choroid is heat dissipation (Parver et al., 1980), but the present results suggest that oxygenation is at least as important.

The effect of hypoxia on photoreceptors might be expected to propagate to higher levels of visual processing. Hypoxia might affect more proximal neurons through changes in photoreceptor membrane potential, changes in  $[K^+]_o$ , or both. This idea is indirectly supported by human psychophysical work, which shows that absolute sensitivity can be reduced by relatively mild hypoxia (e.g., Hecht et al., 1941; McFarland et al., 1944; Ernest and Krill, 1971). In contrast, physiological experiments have suggested that the ERG a-wave, generated by photoreceptors, is resistant even to anoxia (Granit, 1933; Noell and Chinn, 1951). Rodieck (1972) has pointed out, however, that the a-wave recorded during anoxia after other ERG components have been abolished might be only a small remnant of its initial size. What appeared to be better evidence of the photoreceptor's insensitivity to hypoxia came from experiments showing the absence of any striking effects of hypoxia on more proximal retinal responses. The b-wave amplitude and sensitivity and ganglion cell contrast sensitivity and maintained discharge are all preserved at the levels of hypoxia that lead to changes in  $[K^+]_o$  (Bos, 1968; Enroth-Cugell et al., 1980; Alder and Constable, 1981; Linsenmeier et al., 1983). Thus, at least in the short term, the slowing of the pump does not appear to lead to serious problems for signal transmission in

photoreceptors. Still, there may be some effect on transmission that would occur during the transition from dark to light adaptation, when photoreceptor transmembrane ionic gradients are probably abnormal. A subtle effect on response time course has not yet been looked for in ganglion cell or ERG responses.

#### APPENDIX

The smooth lines in Fig. 6 are predictions of the delayed basal hyperpolarization based on the equation:

$$dV_b/dt = (A/\tau) V_{ap} - (1/\tau) V_b, \quad (\text{A1})$$

where  $V_{ap}$  is the apical hyperpolarization,  $V_b$  is the predicted isolated basal response, and  $A$  and  $\tau$  are constants to be fit in comparing  $V_b$  to actual delayed basal responses. This equation describes a low-pass filter in series with an ideal amplifier of gain  $A$ . The filter consists of a resistor,  $R$ , and a capacitor,  $C$ , with a voltage source,  $V_{in}$ , and an output across the capacitor,  $V_{out}$ . The current through both the resistor and capacitor is:

$$i = C dV_{out}/dt = V_r/R \quad (\text{A2})$$

and

$$V_r = V_{in} - V_{out}. \quad (\text{A3})$$

This leads to:

$$dV_{out}/dt = (1/RC) (V_{in} - V_{out}). \quad (\text{A4})$$

The additional constant  $A$  was introduced because at steady state the apical hyperpolarization is much larger than the delayed basal hyperpolarization, whereas an equation of the form of Eq. A4 would have made them equal.

The curves of Fig. 6 were produced by solving Eq. A1 numerically using a fourth-order Runge-Kutta algorithm (Fox and Mayers, 1968). Constants  $A$  and  $\tau$  giving the best fit of  $V_b$  to the actual data were determined by trial and error. For a sample of 14 normoxic cells,  $A$  was  $0.20 \pm 0.05$  and  $\tau$  was  $19.3 \pm 3.3$  s at illuminations of  $8.3\text{--}9.3 \log q/\text{deg}^2 \cdot \text{s}^{-1}$ . One set of parameters could describe data over a range of illuminations, but generally at low illuminations a longer time constant was required. For seven responses in five cells studied at  $7.3$  and  $7.8 \log q/\text{deg}^2 \cdot \text{s}^{-1}$ ,  $A$  was the same ( $0.20 \pm 0.05$ ), but  $\tau$  was much longer ( $37 \pm 13$  s). In general, the fits were good, often better than the ones in Fig. 6. The most common deviation of the modeled response was its inability to recover after the peak as much as the actual response, without being seriously distorted at earlier times. This discrepancy may have several explanations, but since the isolation of the actual delayed basal hyperpolarization is subject to several assumptions and approximations (Linsenmeier and Steinberg, 1984), it may be the "actual" response rather than the model that is in error.

We thank Drs. E. R. Griff and P. A. Sieving for assistance with experiments. This work was supported by National Institutes of Health grant EY01429 to R.H.S.

*Original version received 23 April 1984 and accepted version received 7 August 1984.*

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