Mechanisms of Hypoxic Effects on the Cat DC Electroretinogram

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Mild hypoxia elevates the standing potential and alters three slow components of the DC electroretinogram in the cat: the c-wave, the fast-oscillation trough, and the light peak. This paper considers the cellular mechanisms of these effects. Elevation of the standing potential results from a depolarization of the basal membrane of retinal pigment epithelial (RPE) cells. The depolarization is indirectly initiated by an elevation of \([K^+]_o\) in the subretinal space during hypoxia, and is accompanied by a decrease in basal membrane resistance that leads to an increase in the c-wave. There is also some evidence that hypoxia may alter the standing potential by directly affecting the basal membrane of the RPE. The fast-oscillation trough, which follows the c-wave when illumination is maintained, deepens during hypoxia. This is caused primarily by an increase in the amplitude of the delayed hyperpolarization of the RPE basal membrane that results from a slowing of the rate of recovery of light-evoked \([K^+]_o\) during hypoxia. The changes in \([K^+]_o\) probably result, in turn, from a decrease in the rate of the photoreceptors' Na⁺/K⁺ pump. The light peak's amplitude is reduced during hypoxia and its time-to-peak is lengthened, and this may be related to a change in photoreceptor metabolism that is distinct from the effect on the Na⁺/K⁺ pump. Knowledge of these mechanisms may eventually enhance the clinical usefulness of the standing potential and the c-wave, fast-oscillation, and light peak. Invest Ophthalmol Vis Sci 27:1385-1394, 1986

The DC electroretinogram (DC ERG) of higher vertebrates comprises a complex series of waves that continue for many minutes after the onset of illumination.\(^1\)\(^2\) The first potentials are the rapid a- and b-waves, and these are followed by a series of slower events: the corneal-positive c-wave, a trough named the fast oscillation or fast-oscillation trough, and the light peak. In the cat, the times-to-peak are 2 sec for the c-wave,\(^3\)^\(^4\)^\(^5\) 20 sec for the fast-oscillation trough,\(^6\) and about 5 min for the light peak.\(^7\) Although the fast oscillation and light peak can be recorded by DC electroretinography in humans,\(^8\) they are more conveniently recorded by electrooculographic (EOG) techniques.\(^9\)^\(^10\)^\(^11\)

In the cat eye, the three slower components are each quite sensitive to hypoxia, and begin to change at an arterial oxygen tension (P\(_{O_2}\)) of 60-80 mm Hg.\(^12\) In contrast, effects of hypoxia on b-wave amplitude do not begin until P\(_{O_2}\) is below about 40 mm Hg.\(^12\)^\(^13\) During hypoxia c-wave amplitude increases,\(^12\)^\(^14\)^\(^15\) the fast-oscillation trough deepens, becoming more negative, and light-peak amplitude decreases.\(^16\) These effects are illustrated in Figure 1 by the responses to 8 min of illumination before and during hypoxia. In addition, the onset of hypoxia elevates the dark-adapted standing potential (SP).\(^12\)^\(^15\)^\(^18\)

The c-wave, fast-oscillation trough, and light peak result largely from three light-evoked changes in retinal pigment epithelial (RPE) cell membrane potentials.\(^3\)^\(^6\)^\(^7\)^\(^19\)^\(^21\) The c-wave and fast-oscillation trough are modified, however, by potentials that are generated simultaneously in the neural retina. Since the previous work on hypoxic changes in the DC ERG relied on recordings from the vitreous humor or cornea, it was only possible to speculate on whether RPE or neural retinal contributions to the ERG potentials were affected. It is necessary, therefore, to demonstrate the degree to which hypoxia affects RPE or neural retinal contributions to each component. Furthermore, even if the RPE potentials were affected by hypoxia, we have to remember that they each originate ultimately from light-dependent processes at the photoreceptors, so that the actual site of the hypoxic effects could be either the photoreceptors or RPE.

The purpose of this paper is to explain how hypoxia alters each of the components, first by demonstrating the degree to which hypoxia affects its RPE or neural retinal portion. Then, using recently obtained evidence on the mechanisms of hypoxic effects on RPE cells and \([K^+]_o\) homeostasis,\(^22\) we can understand the cellular mechanisms underlying changes in most of the slow DC ERG components.
Light-peak
C-wave
Hypoxic
FOT
Normoxic
\[ 1 \text{ mV} \]
\[ 9 \text{ sec} \]

Fig. 1. Effects of hypoxia on the amplitude and time-course of the DC ERG. The components that change during hypoxia are the c-wave (inset), fast-oscillation trough (FOT), and light peak. These are vitreal recordings in response to 8 min of illumination at rod saturation. The hypoxic response was recorded 2 hr after the onset of hypoxia, when \( P_{a\text{O}_2} \) was 30 mm Hg. Vitreal positive signals are shown with an upward polarity in all figures. (cat 100)

Materials and Methods

The methods of animal preparation, stimulation, and recording from the intact cat eye have been described previously.\textsuperscript{3-7,12,22,23} We have adhered to the principles of the ARVO Resolution on the Use of Animals in Research. Experiments were done on urethane anesthetized or decerebrate cats paralyzed with pancuronium bromide (Pavulon; Organon, West Orange, NJ) and artificially respirated. Ordinarily the animals breathed air, or air plus enough oxygen to maintain the arterial oxygen tension (\( P_{a\text{O}_2} \)) above 90 mm Hg. Hypoxia was induced by adding nitrogen to the inspired gas. The effects of changes in inspired gases were observed rapidly, within 30 sec. Stimuli were periods of diffuse white light, having an illumination at about rod saturation in most cases, presented to an initially dark-adapted retina. The ERG was measured between a chlorided silver wire in the vitreous and a chlorided silver plate behind the eye. The contribution of the RPE to the DC ERG was assessed by recording the transepithelial potential (TEP) between a microelectrode in the subretinal space and the reference electrode. The neural retinal contribution was obtained by subtracting the ERG from the TEP. This is equivalent to referencing the microelectrode to the vitreous. This transretinal recording is inverted in the figures to show the polarity with which the two components add to give the vitreal ERG.

The term standing potential refers to the potential difference across the eye during darkness or after the potential stabilizes during illumination. In our recordings, it was measured between a chlorided silver wire in the vitreous and a reference behind the eye, but a corneal electrode would have yielded the same results. The several light-evoked responses (Fig. 1) are changes in the SP comprising the DC ERG.

Results

Standing Potential

To understand changes in the light-evoked responses in hypoxia, it was first helpful to understand how and why the standing potential was altered. The standing potential recorded during dark adaptation increased at the onset of hypoxia as shown in Figure 2A (and Figs. 3A and 7). Following its elevation, SP often partially recovered during hypoxia (Figs. 2A, 7), sometimes nearly to the prehypoxic baseline. The magnitude of the maximum change was variable and not closely related to \( P_{a\text{O}_2} \) (Fig. 2C). At the end of hypoxia, a long-lasting series of events was consistently observed: a prominent decrease, in which the potential often went below the prehypoxic level, followed by an overshoot and finally a return to baseline (Fig. 2B). This oscillation after hypoxia generally took more than 25 min. Often a brief increase in potential, seen more clearly in Figure 3B (vitreal), preceded the decrease at the end of hypoxia. This series of events occurred following hypoxic exposures from 10–100 min, the entire range studied.

Fig. 2. Vitreal recording of the dark-adapted standing potential at the beginning and end of hypoxia. A, Onset of hypoxia. \( P_{a\text{O}_2} \) during hypoxia was 39 mm Hg; duration 13 min. B, End of hypoxia. \( P_{a\text{O}_2} \) = 44 mm Hg; duration 19 min. (cats 103, 102) C, Maximum change in SP as a function of the \( P_{a\text{O}_2} \) during hypoxia (19 episodes of hypoxia; 15 cats).
Normally the SP originates almost exclusively from the transepithelial potential (TEP) across the RPE. Changes in SP during hypoxia, however, could have been caused by changes in the transretinal potential, since any potential change recorded in the vitreous may theoretically have both RPE and neural retinal components. As shown in Figure 3, there were changes in both components. The initial effects at the beginning and end of hypoxia were small changes in the TEP and transretinal potentials that were of opposite polarity and of similar time-course. At the onset of hypoxia, these effects often cancelled, although a small decrease in vitreal SP was sometimes observed (Fig. 4A). At the end of hypoxia, a small initial increase in SP occurred because the TEP effect was larger than the transretinal one. The initial simultaneous effects on the TEP and transretinal potentials were followed both at the beginning and end of hypoxia by major changes in only the TEP: an increase at the beginning (Fig. 3A) and a decrease at the end (Fig. 3B). Since the transretinal potential was nearly flat during the time the TEP was changing, the TEP response was observed essentially intact in the vitreal SP recording. The partial recovery of SP during hypoxia and the oscillation after hypoxia (Fig. 2) are not shown in this figure, but also resulted from changes in TEP alone.

The increase in TEP at the onset of hypoxia is a basal membrane depolarization that we have suggested is caused indirectly by a hypoxic increase in extracellular potassium in the subretinal space ([K⁺]₀) (see Discussion). The hypoxic elevation of [K⁺]₀ was largest under dark-adapted conditions and was small or absent in steady light. Therefore, if the standing potential change depends on the change in [K⁺]₀, it should also be smaller under light-adapted conditions. In experiments such as the one illustrated in Figure 4, the effect of hypoxia was first studied in dark adaptation, then during illumination, and finally in dark adaptation again. During illumination, the animal was made hypoxic after the light-evoked changes in SP had ended. In all five experiments, the hypoxic standing potential changes during light-adaptation were much smaller, as illustrated in Figure 4.

**C-wave**

The c-wave originates from two events, one from the RPE and the other from the neural retina. A decrease in [K⁺]₀ in the subretinal space during illu-
Fig. 5. Changes in c-wave amplitude during hypoxia. Four second flashes at 8.2 log quanta-deg²·sec⁻¹ (rod saturation) were given each minute, and transepithelial, transretinal, and vitreal c-waves were recorded. The small decrease over time in the transretinal c-wave in this example may have been due to electrode movement during hypoxia, but there is no change in this component related to the change in the vitreal response. The duration of hypoxia is shown by the dark bar. Pao2 during hypoxia was 59 mm Hg. (cat 54)

Fig. 6. Responses to 4-sec flashes before, during, and after hypoxia. The left, middle, and right sets of traces correspond to the first, seventh, and twenty-first data points in Figure 7.

Fig. 7. C-wave amplitude and the standing potential during one episode of hypoxia. The initial value of standing potential was arbitrarily taken to be 0 mV. Standing potential was recorded continuously, and the points shown are the values measured just before each flash. Flashes were 4 sec in duration, 1 min apart except where no points are shown, and at rod saturation. (cat 69)

minimization hyperpolarizes the RPE apical membrane, because the equilibrium potential for [K⁺]o increases. This leads to an increase in TEP that we have called the RPE component of the c-wave. The decrease in [K⁺]o also produces slow PHI, a decrease in potential of similar time-course across the neural retina, probably resulting from a hyperpolarization of the distal portion of Müller cell membranes. In the dark-adapted cat retina, the RPE component is about 20% larger than slow PHI, so the c-wave, which is the sum of the two components, is positive-going.

Previous recordings showed that the c-wave increased during hypoxia. The first question in the present work was whether this increase was caused by a larger RPE component, a smaller retinal (slow PHI) component, or both. Figures 5 and 6 show results of an experiment in which 4-sec flashes were delivered every 60 sec, and the amplitudes of the vitreal c-wave, TEP c-wave, and slow PHI were followed as a function of time. The increase in the vitreal c-wave resulted entirely from the increase in the RPE component (TEP). The increment in vitreal c-wave amplitude was the same as the increment in the RPE component, but the percentage increase in the vitreal response was larger, because a large constant signal, slow PHI, subtracted from the RPE component.

Changes in the amplitude of the c-wave and standing potential were closely related. Within an episode of hypoxia, c-wave amplitude mimicked the changes in SP as illustrated in Figure 7. As the SP increased and partially recovered during hypoxia, c-wave amplitude followed. At the end of hypoxia, the slow oscillation of SP was accompanied by a corresponding oscillation of c-wave amplitude. Figure 8 shows the correlation.
between c-wave amplitude and SP across many episodes of hypoxia. The maximum c-wave change during an episode is shown by open circles, the steady state change in the c-wave is shown by filled circles, and the decrease of c-wave amplitude following hypoxia is shown by squares. These changes are plotted against the changes in SP (relative to the prehypoxic baseline) at the corresponding times. The change in c-wave amplitude was quite variable, but was closely related to the change in SP (correlation coefficient = 0.89). A similar covariation between c-wave amplitude and SP changes during hypoxia has been found in the perfused cat eye by Kreienbuhl and Niemeyer.

Fast-Oscillation Trough

Following the c-wave, the potential declines to a minimum at 20 sec, which may be at or just below the dark-adapted baseline (Figs. 1, 9—normoxic vitreal trace), and then begins to rise again. We have termed this wave the fast-oscillation trough, because of its relation to the fast oscillation observed in humans and animals in response to repetitive stimuli having equal light and dark phases of about 1 min each. While the fast-oscillation trough is the response only to the onset of illumination, the complete fast oscillation consists of a response to the onset of illumination followed by a response to the offset of illumination.

An increase in amplitude, or “deepening,” of the fast-oscillation trough is one of the most sensitive and reliable indicators of hypoxia in the cat. The example in Figure 9 demonstrates that the trough can increase even though there is no change in c-wave amplitude. In these experiments, the trough, measured from the dark-adapted baseline to the minimum potential, increased by 0.2-0.8 mV during hypoxia (average = 0.5 mV; n = 19), and the time-to-peak increased from 22 to 28 sec in Figure 9. The amplitude change was larger than the change in the c-wave in about 85% of the experiments, although there was no correlation (r = 0.019; n = 19) between the change in the fast-oscillation trough and the change in the c-wave.

The transepithelial and transretinal recordings of Figure 9 suggest that a slowing of the recovery of the transretinal response from its peak is primarily responsible for the deepening of the fast-oscillation trough.
trough. At this level of analysis, such a conclusion would be correct, but it overlooks the known complexity of the TEP response. While the transretinal response probably results from a single membrane potential change in Müller cells, the TEP response during this time period results from two events, both of which are modified by hypoxia. One event is the recovery of the apical membrane potential from its maximal hyperpolarization during light. This should have a similar time-course to the recovery of the transretinal potential, since both depend directly on \([K^+]_o\). The recovery of the apical response slows during hypoxia, and this would, by itself, slow the recovery of TEP. The second RPE event is a delayed hyperpolarization of the basal membrane that becomes larger during hypoxia. By itself, this would make the recovery of TEP faster during hypoxia. In order to estimate the relative effects of changes in apical and basal events on the TEP, we constructed an artificial TEP in which only the apical event changes. In order to do this, we assumed that, in the absence of a basal hyperpolarization, the TEP would recover from its peak (c-wave) with about the same time-course as the recovery of slow PIII (Fig. 10A). We measured peak amplitudes of the TEP response and slow PIII during nine episodes of hypoxia, and determined the percentage recovery of slow PIII after 25 sec, approximately the minimum of the fast-oscillation trough. This percentage recovery was then assumed to apply to the TEP as well, since both depend on \([K^+]_o\). In Figure 10, these points (the peak and 25 sec values) are connected by straight lines for simplicity. The vitreal response is the sum of the TEP and transretinal responses, and shows that, if only the apical and slow PIII responses changed, then the fast-oscillation trough would be shallower, not deeper, during hypoxia (Fig. 10A). By comparison, Figure 10B shows the actual average value for the TEP recovery, and thus includes the effect of the delayed basal hyperpolarization. Since this is the only difference between Figures 10A and B, we can conclude that the change in the delayed basal hyperpolarization is essential in causing the larger fast-oscillation trough during hypoxia.*

**Light Peak**

The final component of the DC ERG that is very sensitive to hypoxia is the light peak. This is the corneal positive signal that follows the fast-oscillation trough, and it takes much longer to develop, peaking in 5 min in the cat. The light peak arises in the RPE as a basal membrane depolarization. Figures 1 and 11 illustrate the severe reduction in the light peak during hypoxia, and Figure 1 also shows an increase in the time-to-peak. Extracellular measurements showed that no new potential change developed across the neural retina during hypoxia, and that the change in the light peak occurred because the transepithelial response decreased (Fig. 11). We were unable to obtain adequate intracellular recordings of the light peak during hypoxia, but it is likely that the smaller TEP light peak implies a smaller depolarization at the basal membrane.

The rapidity with which retinal oxygenation can be altered, in contrast with the slow time-course of the light peak, allowed us to attempt to interfere with the light peak at different times after it had already started. If an episode of hypoxia, begun during the latter part of a 5-min period of illumination, were to reduce the light peak, then it would be reasonable to suggest that hypoxia acted at a late stage in its generation, and probably directly at the RPE cell, where the voltage change occurs. If hypoxia were to reduce the light peak only if induced during the first or second minute of hypoxia, then it would be more likely that hypoxia affected an earlier stage of response generation, either in the RPE or neural retina. Surprisingly, hypoxia had no effect on the light peak when it was initiated at any time during illumination, but only if initiated at least 2.5 min prior to the beginning of illumination. This

* The basal resistance change that leads to the small increase in TEP c-wave amplitude (see Discussion) has been neglected here. Inclusion of this effect would make the vitreal fast-oscillation trough in Figure 10A still shallower.
might suggest that an early stage in the generation of the response was affected, or that, in order to affect the light peak, a relatively long period of hypoxia is necessary. Alternatively, this may be a light-dependent effect instead of a time-dependent one (see Discussion).

**Discussion**

In this work, we have used intraretinal recordings to investigate the contribution of retinal and RPE potentials to hypoxic changes in components of the DC ERG. A more complete picture of the mechanisms involved can be provided by relating these findings to previous work on RPE cell membrane potentials and [K⁺]o. Figure 12 is a summary of all of the work that can provide a framework for this discussion.

**Mechanisms of Hypoxic Effects**

Hypoxia appears to exert its major effect on the RPE-photoreceptor complex. One effect is an increase in [K⁺]o in the subretinal space during dark adaptation (Fig. 12, left). Reasoning from work by Shimazaki and Oakley on [K⁺]o in the toad retina, and from other work on the similarity of the effects of hypoxia and pump inhibitors, we have suggested elsewhere that the hypoxic increase in [K⁺]o results from a slowing of the Na⁺/K⁺ pump in the inner segments of rods, although the pump in RPE or Müller cells or a rod K⁺ conductance could also be affected. In addition to the increase in dark-adapted [K⁺]o, the slowing of the

**Fig. 11. Effect of hypoxia on the transepithelial and transretinal potentials during 5 min of illumination at rod saturation. Hypoxic PaO₂ was 49 mm Hg; duration 60 min. (cat 46)**

**Fig. 12. Summary of the effects of hypoxia on slow components of the DC ERG.** Two major effects of hypoxia are on [K⁺]o. The left side shows effects resulting from the elevation of dark-adapted [K⁺]o and the right shows the effects of the change in time-course of the light-evoked [K⁺]o response. An upward arrow means an increase in potential or response amplitude, and a downward arrow a decrease. The brackets show steps for which evidence is incomplete. The increase in dark-adapted [K⁺]o affects both the Müller cell and the RPE apical membrane, but leads to little effect on standing potential. A slower effect at the RPE basal membrane, which may consist of both a [K⁺]o-mediated and a more direct component, lead to the increase in standing potential. A basal membrane resistance change leads to the increase in c-wave. On the right, a slower recovery of light-evoked [K⁺]o has effects on the recovery of slow PIII, the apical membrane potential, and the size of the delayed basal hyperpolarization, and the latter dominates. Effects on the light peak are shown separately at the bottom, since these changes are probably not a result of the change in [K⁺]o shown at the top.

**Effects of Hypoxia on SP and Slow ERG Components**

- **Photoreceptors**
  - Decrease in light-peak depolarization
  - Slower recovery of light-evoked [K⁺]o response
  - Slower recovery of apical hyperpolariz.

- **RPE apical depolarization**
  - Slower recovery of [K⁺]o response

- **Dark adapted subretinal [K⁺]o**
  - Little or no decrease in standing potential

- **RPE [K⁺]o**
  - Slower recovery of [K⁺]o response

- **Trans-retinal**
  - Fast oscillation

- **Standing potential**
  - TEPC-wave

- **TEP C-wave**
  - Decrease in light-peak depolarization

- **HYPOXIA**
  - Photoreceptor effect

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pump also changes the time-course of the light-evoked \([K^+]_0\) response in the subretinal space (Fig. 12, right). These changes in \([K^+]_0\) affect the RPE cell and result in changes of the standing potential, c-wave, and fast oscillation, as shown in Figure 12. Simultaneously, changes occur in the transretinal potential, presumably because Müller cells are also sensitive to the same changes in \([K^+]_0\).

The effects on the RPE cell arise at both the apical and basal membranes. As far as vitreal potentials are concerned, however, the effects on the apical membrane are much less significant. This is because the direct effect of the hypoxic change in subretinal \([K^+]_0\) on the apical membrane potential, and, therefore, on the TEP, tends to be cancelled by nearly equal and opposite changes of the transretinal potential. Such a cancellation occurs in the consideration of the dark-adapted standing potential, where the decrease in TEP (Fig. 3) is produced by an apical hyperpolarization, and the increase in transretinal potential is produced by a corresponding hyperpolarization of Müller cells. This leaves the later increase in TEP, which is caused by a basal membrane depolarization, and as the main cause of the standing potential increase.

The hypoxic depolarization of the basal membrane may occur by two mechanisms: an “indirect” effect, related to the change in \([K^+]_0\), and a direct effect that is not mediated by \([K^+]_0\). The indirect effect is considered to be analogous to that hypothesized for the delayed light-evoked responses of the basal membrane. By this mechanism, changes in subretinal \([K^+]_0\) produced by any means lead to changes in \([K^+]_0\) (or \([Cl^-]_0\)) that affect the basal membrane. While a change in intracellular concentration is believed to be important, subsequent steps must actually cause the membrane potential to change, since the expected changes in intracellular concentration are in the wrong direction to explain the potential changes on the basis of a change in \(E_K^*\) at the basal membrane.

There may also be a direct effect of hypoxia on the basal membrane that would contribute to the increase in SP (Fig. 12, bracketed “hypoxia”). This is suggested by several factors. First, SP increases more slowly at the beginning of hypoxia than is expected from the change in \([K^+]_0\) and our current understanding of delayed basal responses. Second, the oscillations of SP following the end of hypoxia are more complex than we would predict based on the monotonic decrease of \([K^+]_0\) and our model, although it is possible that the oscillatory behavior is a property of the basal membrane that is simply triggered by the decrease in \([K^+]_0\). Finally, preliminary results in gecko indicate that the basal membrane can depolarize during hypoxia in an isolated RPE preparation, although, in such a preparation, the retina has been removed so that the hypoxic change in \([K^+]_0\) is not a factor (E. R. Griff, personal communication). Arguing against a third effect of hypoxia in cat are the observations that: 1) SP changes are reduced during steady illumination as are the \([K^+]_0\) changes, and 2) the SP changes are in the direction we would expect, and are about the size we would expect, if they were completely dependent on the changes in dark-adapted \([K^+]_0\).

We have shown that the increase in c-wave amplitude is due to an increase in the RPE component with no change in slow PHI (Figs. 5, 6). This suggested that a change in the light-evoked \([K^+]_0\) response could not be responsible for the effect, since changes in \([K^+]_0\) should affect both components. \([K^+]_0\) measurements showed that, for the first 2–4 sec of illumination (to the peak of the c-wave), hypoxia had no effect on the magnitude of the light-evoked \([K^+]_0\) response. Other possible mechanisms for changes in the RPE component, alone, involve changes in the resistances of the apical or basal membranes or paracellular pathway. Intracellular recordings and resistance measurements indicated that the resistance of the basal membrane decreased during hypoxia, and that changes in current flow in the RPE led to the increase in the c-wave.

The present observations on the covariation of the standing potential and c-wave suggest that changes in basal membrane resistance, as reflected in the increase in c-wave, and changes in basal membrane potential, as reflected in the SP increase, must be related in a nearly linear way. Furthermore, the hypoxic changes in c-wave amplitude are exactly like those previously observed during the light peak. In both cases, they are caused by a decrease in basal membrane resistance accompanying a depolarization, evoked in one case by hypoxia and in the other case by light. The slope of the relation between changes in the SP and the c-wave is similar in the two cases, indicating that the relationship between depolarization and resistance change is fixed. We assume that the depolarization causes the resistance change, but there is no direct evidence of this yet. In any event, the change in the c-wave can be used as an index of the resistance change of the basal membrane during hypoxia. Since c-wave amplitude followed the SP even during the oscillations following the end of hypoxia, all of these changes in SP must have been due to changes in basal membrane potential, a point that was impractical to demonstrate with intracellular recordings.

There is a large variation in the size of the change in standing potential (Fig. 2C) or c-wave as a function of \(P_{O_2}\). The source of this variability is unknown, but it probably points to the presence of another parameter or factor that can aggravate or ameliorate the effect of hypoxia, or saturate the mechanism on which hypoxia acts. We can speculate that the circulation may be
compromised in some preparations, so that some ocular hypoxia, or hypercapnia, is present despite our best efforts to maintain a normal physiological state. Alternatively, a circulating or retinal agent, over which we have no control, could be present that affects the RPE or photoreceptors. In the worst case, this might be altered slowly as a function of the number of episodes or total duration of hypoxia, or of the previous light history of the animal. Isolating the contribution of such a factor may be difficult, but this does not negate the primary findings here, which relate to the correlation between c-wave and standing potential effects, and to the mechanism of action of hypoxia.

The change in the fast-oscillation trough is complex, since three events influence the potential over this time, but, as for the SP change, a basal event dominates. Apical membrane potential recoveries from its peak more slowly during hypoxia, and the recovery of slow PIII is altered similarly, so these effects tend to cancel. The basal response that dominates is the delayed basal hyperpolarization evoked by light, and its increase during hypoxia can be accounted for entirely by a slowing of the recovery of light-evoked $[K^+]_o$. The increase in the fast-oscillation trough, therefore, is the only indication in the DC ERG of the hypoxic change in the light-evoked $[K^+]_o$ response (Fig. 12, right).

As shown at the bottom of Figure 12, we conclude that the mechanism for changes in the light peak differs from that of the c-wave, standing potential, or fast-oscillation trough. First, hypoxic effects on the light peak were much more reliable than those on the c-wave. Second, in the present series of experiments, the light peak decreased substantially in two episodes of hypoxia at times when the standing potential had not changed. Third, the change in basal membrane resistance is in the wrong direction to explain the change in the light peak, and no change in resistance (for instance in apical membrane resistance) would explain the change in time-to-peak from 5 to 7 min during hypoxia. Also, it is unlikely that changes in $[K^+]_o$ underlie changes in the light peak, since there is normally no relation between the time-course of the light peak and that of light-evoked $[K^+]_o$. Therefore, the slowing of the photoreceptor pump, which causes the changes in $[K^+]_o$, appears not to be the cause of the change in the light peak. This does not rule out the possibility that some other metabolic effect in the photoreceptors leads to the change in the light peak. This is supported by the observation that the light peak was affected only if hypoxia was initiated in the dark, when $[K^+]_o$ recordings indicated that the photoreceptors are most sensitive to hypoxia. If the light peak itself is a manifestation of a decrease in some aspect of photoreceptor (or RPE) metabolism during illumination, then hypoxia might not be able to produce a further reduction.

A more complete understanding of the effects of hypoxia on the light peak will probably not be possible until more is understood about the origin of this potential.

**Comparison With the Electrooculogram**

The standing potential, conventionally recorded in the electrooculogram (EOG), is a measure of the dipole across the eye, of which the TEP is the principal source. For a variety of reasons, neither the DC ERG nor the EOG provides an absolute measure of SP, but changes in SP evoked by light and hypoxia can be observed equally well by both techniques. Several human studies have reported a hypoxic elevation of standing potential during darkness or dim illumination (also Linsenmeier RA, Smith VC, and Pokorny J, unpublished observations), but less effect has been found in studies done under light-adapted conditions. This correlates well with our observations in cat (Fig. 4), and suggests that the mechanisms of changes may be quite similar in cat and human. Further evidence of mechanistic similarities might be obtainable in humans by recording the c-wave and standing potential simultaneously, since the c-wave can give an indication of basal membrane resistance changes. Simultaneous recordings of the c-wave and standing potential may also be useful in evaluating the effects of other agents that alter the standing potential, such as hypercapnia and hyperosmolarity. If changes in the standing potential and c-wave are in the same direction, then the basal membrane potential should be considered as a candidate for the site of the effects. The relation between basal membrane potential and resistance also suggests that, if changes in SP and the c-wave are in opposite directions, the basal membrane must not be the primary site of the effects. A recording of the standing potential alone during mild hypoxia may be useful as a test of the integrity of RPE-photoreceptor interactions.

The light peak/dark trough ratio of the EOG is also reduced in humans during mild hypoxia (Linsenmeier RA, Smith VC, and Pokorny J, unpublished observations). The unique origin of the light peak compared to the other DC ERG or EOG measures, and the special nature of hypoxic effects on this potential, suggest that it will provide information that is different from that obtained by recording the SP. We have suggested elsewhere some types of pathology in which a reduced light peak may be a sign of ocular hypoxia.

It should be clear by now that all of the slow DC ERG (or EOG) responses result from interactions between the photoreceptors and the RPE. They do not, therefore, reflect the activity of one or the other cell type, and this is true, as well, for the effects of hypoxia. This is not a disadvantage if the mechanisms and site
of hypoxic changes are known. A further understanding of these potentials should encourage the clinical use of the EOG (or DC ERG) in combination with ERG potentials that are more purely retinal in origin in diagnosing pathophysiological events in the photoreceptor-RPE relationship.

Key words: hypoxia, oxygen, retina, retinal pigment epithelium, electroretinogram, c-wave, standing potential

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