Effects of Hypoxia and Hypercapnia on the Light Peak and Electroretinogram of the Cat

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The effects of systemic hypoxia and hypercapnia on the standing potential, light peak, and electroretinogram (ERG) of the intact cat eye were studied. DC recordings were made in the vitreous humor. The amplitude and waveform of the light peak were surprisingly sensitive to hypoxia. The light peak began to decrease at an arterial oxygen tension (P_AO2) of 60 to 80 mmHg, and was reduced to 25 to 60% of the control amplitude at a P_AO2 of 40 mmHg. Increases in c-wave amplitude were also observed during hypoxia, beginning at about the same P_AO2 as decreases in the light peak. In contrast, the b-wave and ERG threshold were generally unchanged when the P_AO2 was above 40 mmHg. The light peak and c-wave were also more sensitive than the b-wave and ERG threshold to hypercapnia. Decreases in light peak amplitude and increases in c-wave amplitude began at an arterial pH of about 7.3. The b-wave was reduced, and ERG threshold was elevated, beginning at a pH of about 7.2. The standing potential of the eye, recorded in darkness, generally increased in both hypoxia and hypercapnia. A common factor in the generation of the light peak and c-wave is that both involve changes in membrane potential of retinal pigment epithelial (RPE) cells. These events were affected much more by hypoxia than were the b-wave and ERG threshold, suggesting that the RPE is unusually sensitive to hypoxia. Similarly, the earliest effect of retinal hypercapnia appears to be on the RPE rather than on the neural retina. Furthermore, hypoxia and hypercapnia may have acted on the RPE through similar mechanisms, because their effects on the light peak, c-wave, and standing potential were in the same direction. Invest Ophthalmol Vis Sci 24:37-46, 1983

The light peak is a slow light-evoked increase in the DC potential across the eye (standing potential; corneo-retinal potential), that is usually recorded in humans as the light peak of the electro-oculogram. Recently it has been shown, with DC recordings from gecko, cat, and monkey, that the light peak is generated across the retinal pigment epithelium (RPE); no component of the voltage arises across the neural retina. RPE cells have two distinct membranes: an apical membrane facing the photoreceptors and a basal membrane facing the choroid, and a potential could arise at either site. The c-wave of the corneal or vitreal ERG is generated in part by a hyperpolarization of the apical membrane in response to a decrease in retinal potassium concentration. The light peak, however, arises from a depolarization of the basal membrane that is not due to a change in potassium concentration. Presumably some signal from the photoreceptors initiates a series of events that culminates in the basal depolarization, but this sequence has not yet been defined.

In the course of our experiments on the origin of the light peak in the cat eye, we noted that a rather small decrease in respiratory minute volume led to a decrease in the amplitude of the light peak and an increase in the standing potential (SP). It seemed that pursuing these findings might provide further information on the mechanisms underlying the light peak. Furthermore, a description of the effects of respiratory parameters on the light peak could be valuable clinically, because reductions in the light peak in ocular diseases are not well understood.

Changes in respiratory minute volume could have exerted an effect on the light peak and standing potential through hypoxia, hypercapnia, or both. The effect of hypercapnia on these phenomena has not been studied previously, and the effects of hypoxia have received rather little attention. Fenn et al were the first to show increases in the SP of some human subjects in hypoxia, and Kolder found that these increases began when arterial blood was still 70 to 75% saturated. Drummond and Rebuck have recently found a small reduction in light peak amplitude in humans whose arterial oxygen saturation was reduced to 80%, but they were unable to replicate the earlier findings on the SP.
Some work has also been done on the effects of ischemia. The SP decreases after acute experimental elevation of intraocular pressure, following about the same time course as the decrease in b- and c-wave amplitudes. The light peak is also abolished under these conditions, showing that it depends critically on the blood supply. In the present experiments, graded hypoxia and hypercapnia were produced separately to avoid the multiple changes that may occur in ischemia. The major goal was to study changes in the light peak and SP, but, for comparison, the b- and c-waves of the ERG and ERG threshold were studied also. In general, the c-wave and light peak were more sensitive to both hypoxia and hypercapnia than events originating in the inner retina. The effect of hypoxia on the light peak was particularly striking. Light peak amplitude decreased at arterial oxygen tensions as high as 80 mmHg, while the b-wave was unchanged at arterial oxygen tensions above 40 mmHg.

Materials and Methods

The animal preparation, stimulation, and recording are described in detail elsewhere. Briefly, adult cats were anesthetized with urethane (200 mg/kg loading dose followed by 20–30 mg·kg⁻¹·hr⁻¹) and were paralyzed with gallamine triethiodide (Flaxedil) throughout the experiments. In rabbits large doses of urethane can depress the light peak. Urethane may have affected light peak amplitude to some extent in the cat, but results in the isolated eye, and our own work on decerebrate cats (Linsenmeier and Steinberg, unpublished observations) has shown that the time course and maximum amplitude of the light peak are similar with or without urethane. A chlorided silver wire was introduced into the intact right eye through a hypodermic needle and positioned in the vitreous humor. DC recordings were all made between this vitreal electrode and a chlorided silver plate behind the eye. Surgery was performed under dim illumination, and at least one hour of dark adaptation was allowed before recording began. Light peaks were elicited with 5-min stimuli of diffuse white light about a log unit above rod saturation (9.3 log quanta·(507)⁻¹·deg⁻²·sec⁻¹). At least 25 min were allowed for dark adaptation after each light peak. Potentials were amplified with a high input resistance amplifier, and were displayed on a storage oscilloscope (Tektronix 5111) and chart recorder (Brush 220, Gould Instrument Co.). Data was stored on an FM tape recorder (Racal Store 4DS), and later digitized and plotted using a PDP 11/03 computer (Digital Equipment Corp.) and digital plotter (Tektronix 4662).

Determinations of arterial oxygen tension (PₐO₂), carbon dioxide tension (PₐCO₂) and arterial pH were made intermittently with a Radiometer or Corning blood gas analyzer. Under normal conditions the inspired gas was air, supplemented with enough pure oxygen to insure an arterial oxygen tension of at least 90 mmHg. Under control conditions arterial pH was maintained between 7.35 and 7.45. Systemic hypoxia and hypercapnia were produced by changing the mixture of inspired gas to one containing more N₂ or CO₂. Systemic hypoxia (unaccompanied by significant hyperoxia) was produced by increasing the rate of respiration with the control gas. Hypoxic episodes lasted an average of 76 min and hypercapnic ones 83 min. Hypoxia and hypercapnia were both accompanied by increases in mean blood pressure of 0 to 30 mmHg.

During hypoxia changes in arterial pH were small enough that arterial pH remained in the normal range of 7.35 to 7.45. The values of arterial pH that resulted from the changes in inspired CO₂ are shown in Figure 1. Figure 1 also shows that at normal values of arterial pH the arterial CO₂ tension in these cats was about 30 mmHg, as previously reported for awake cats. Cat arterial carbon dioxide tension (28–32 mmHg) and bicarbonate concentration (16–21 meq/l) are both lower than the corresponding values in humans (40 mmHg and 24 meq/l). In some cases, only pH and not arterial carbon dioxide tension was measured during hypercapnia, so in the remainder of the paper changes in retinal potentials will be plotted against arterial pH.

Fig. 1. Values of arterial pH and carbon dioxide tension (PₐCO₂) resulting from variations in inspired CO₂ (pH < 7.35) and from hyperventilation (pH > 7.45) for 13 cats. The line is a least squares fit of pH vs log PCO₂.
Results

Hypoxia

Light peak: Figure 2 shows DC electoretinograms (DC ERGs) recorded from two animals at decreasing levels of arterial oxygen tension. On the left are normoxic responses that, on this time scale, consist primarily of a c-wave peaking a few seconds after the onset of light, followed by a large slow change, the light peak, that reaches its maximum in about 5 min, regardless of the duration of illumination. With 5-min stimuli the potential returns to the baseline 10 to 12 min after the onset of illumination, but recovery is prolonged with longer periods of illumination. The b-wave is not seen in the recordings in Figure 2 because it merges with the c-wave. Between the c-wave and the light peak are (1) a dip to or below the baseline, called here the fast oscillation, and (2) a shoulder on the rising phase of the light peak, called the second c-wave. At the offset of illumination a fast negative deflection, the off c-wave, occurs, followed by the descent of the potential to the baseline. The descent is often interrupted by a shoulder with about the same time course as the second c-wave.

Fig. 2. DC ERGs before and during hypoxia for two cats. Arterial oxygen tension in mmHg is given above each stimulus trace. The stimulus was a diffuse white light about one log unit above rod saturation (9.3 log quanta-deg^-2-sec^-1), five minutes in duration. Recordings were made between an electrode in the vitreous humor and a reference behind the eye. Positive is up in all recordings in this and subsequent figures.

Responses obtained during hypoxia are shown in the second, third, and fourth traces in each row of Figure 2. Hypoxia led to changes in the waveform and amplitude of the response that began at a surprisingly high arterial oxygen tension, about 80 mmHg. Here the fast oscillation became more negative, and the second c-wave and light peak became smaller. At progressively lower oxygen tensions, changes in both waveform and amplitude were more pronounced. It may appear from Figure 2 that a possible explanation for the change in amplitude of the light peak is a slowing of the rate of rise of the potential, so that measurements of responses to 5-min stimuli do not accurately represent the change in amplitude. Responses to longer stimuli showed that the time to peak did increase slightly during hypoxia, to about 7 min. The rate of rise between 5 and 7 min was slow enough, however, that responses to 5-min stimuli gave a good indication of actual changes in light peak amplitude.

The effect of hypoxia on the amplitude of the light peak (measured from the dark-adapted baseline to the maximum voltage reached) is shown in Figure 3. There was a graded change of amplitude with oxygen tension beginning at 60 to 80 mmHg. When plotted
in terms of relative amplitude, as in Figure 3, the effect of hypoxia was similar from cat to cat, although the amplitude of the normoxic light peak varied from 2.5 to 5.5 mV in these animals. Blood pressure changes accompanying hypoxia cannot account for the decrease in light peak amplitude, since the light peak was reduced even when the blood pressure did not change.

**ERG:** Each stimulus used to evoke a light peak also produced an ERG, consisting of scotopically saturated b- and c-waves, and the effect of hypoxia on these responses is shown in Figure 4. In striking contrast to the light peak, the b-wave was nearly unchanged at arterial oxygen tensions above 40 mmHg, in good agreement with the findings of others on the b-wave, and on ganglion cell contrast sensitivity and mean firing rate. The c-wave, on the other hand, often increased during hypoxia, beginning at about the same level of arterial oxygen tension as decreases in the light peak. The c-wave changes, however, were more variable.

In the data presented so far and in many previous studies of the effect of hypoxia on the ERG, the stimulus used has been of saturating intensity for the b-wave. It seemed possible that the ERG threshold might be a more sensitive indicator of retinal function during hypoxia. To test this, the intensity required to evoke a small vitreal ERG was measured. A diffuse 4- to 6-sec flash was used to evoke ERGs (Fig. 5) consisting of only a d.c. component. The d.c. component could be elicited with about one log unit less light than the b-wave. The log illumination required for a 100 μV response was taken to be threshold, and the change in threshold during hypoxia is shown in Figure 5. This measure of retinal function behaved much like the b-wave, changing little over the range of arterial oxygen tensions studied. Thus,
the increase in c-wave amplitude and the decrease in light peak amplitude were by far the most sensitive measures of retinal hypoxia.

**Time course:** Light peak amplitude changed relatively rapidly at the beginning of hypoxia. Two 5-min stimuli were often given at each hypoxic level, the first about 15 min and the second about 45 min after the onset of hypoxia, and the responses to these stimuli were similar. Recovery was also relatively rapid. The first light peak after the return to normoxia had recovered most of the way to the prehypoxic level and was occasionally larger than the prehypoxic response.

The time course of changes in the c-wave, particularly after hypoxia, was sometimes slower than the recovery of the light peak. The true changes in c-wave amplitude may, in fact, be underestimated by the points in Figure 4, because recovery of the c-wave was not always complete before another episode of hypoxia or hypercapnia was begun. This is probably not sufficient, however, to account for the more variable effect of hypoxia on the c-wave than on the light peak.

**Hypercapnia**

**Light peak:** Hypercapnia also led to dramatic alterations in the waveform and amplitude of the light peak, as shown in Figure 6. The waveform changed differently than in hypoxia, since it became flatter at progressively lower pH. That is, the light peak was apparently affected more than the second c-wave by hypercapnia. The relative amplitude of the light peak as a function of arterial pH is shown in Figure 7. As with hypoxia, there was a graded reduction in amplitude, beginning at an arterial pH of about 7.3. An increase in light peak amplitude was sometimes found during hypocapnia.

The data of Figure 7 summarize the results from 10 of 12 animals. In two cats the light peak was not reduced during hypercapnia, and in one there was a large reduction in the first three-hour episode of hypercapnia (open circles in Fig. 7), but no reduction in a subsequent episode of the same duration. These latter animals were otherwise typical, in that b- and c-wave changes were observed during hypercapnia, and the light peak was reduced during hypoxia. The resistance to hypercapnia observed in these few cases suggests that an unknown factor can sometimes protect the light peak.

**ERG:** Hypercapnia was not as selective as hypoxia, because the b-wave began to decrease at a pH of about 7.2, only slightly lower than that which caused a reduction in the light peak (Fig. 8). As in hypoxia, the c-wave increased, beginning at a pH of about 7.3, but
again the changes in the c-wave were more variable than those in the light peak. The threshold for a 100 μV ERG (Fig. 9) was generally elevated at an arterial pH of 7.2 or less, as one might expect from the decrease of b-wave amplitude. Thus, all aspects of retinal function studied were rather sensitive to decreases in arterial pH induced by hypercapnia, but the light peak and c-wave appeared to be affected with less severe acidosis. Also, maximal reductions in the light peak (to 40% of normal) were larger than the maximal reductions in the b-wave (generally to 70% of normal).

**Time course:** Changes in light peak amplitude occurred somewhat more slowly in hypercapnia than in hypoxia. The second light peak during hypercapnia, generally recorded about 45 min after the gas change, was often smaller than the first, which was recorded at about 15 min, but on the occasions when more than two responses were obtained there was no further reduction in amplitude after the second. (Second responses were plotted in Fig. 7.) At the end of severe acidosis (arterial pH of 7.1), recovery of the light peak sometimes took up to an hour. The time course of c-wave changes was similar.

**Standing Potential**

Hypoxia and hypercapnia altered the standing potential (SP) recorded in the dark, as well as the light peak. But unlike the changes in the light peak, SP changes were quite inconsistent in magnitude and time course.

Hypoxia led to increases in SP of 0.5 to 2.0 mV when arterial oxygen tension was reduced to less than 70 mmHg (Fig. 10A). Most of this change occurred over the first 5 min. Measurements of the standing potential were usually interrupted by light stimuli about 15 min after gas changes, so we have only limited information on the longer term changes in standing potential. During hypoxia, however, it was sometimes possible to observe that the potential slowly began to fall back toward the prehypoxic level. The return to normoxia gave the most reproducible changes in SP: a brief increase, followed by a decrease, followed in turn by a slow increase (Fig. 10B). After this sequence, the potential was often at least temporarily above the level observed at the end of hypoxia.

Hypercapnia led to increases in SP of about the same magnitude as hypoxia, but often the SP decreased transiently first (Fig. 11A). These changes were slower than those observed in hypoxia and may simply reflect the longer time needed to reach a new steady state retinal pH (8 to 10 min) compared to a new steady state retinal oxygen tension (about 2 min).
Following hypercapnia the SP usually decreased (Fig. 11B). In two cases this decrease following hypercapnia was remarkably large, about 4 mV in one animal and about 12 in the other and occurred at a rate of 0.1 mV/min. Corresponding increases had not occurred at the onset of hypercapnia, but it is unlikely that the large decreases were due to electrode drift because they were arrested by subsequent episodes of hypercapnia.

Hypocapnia led to changes opposite in direction to those seen in hypercapnia, that is, a decrease of SP at the beginning and an increase upon return to normocapnia. These are opposite to those reported for humans by Fenn et al.10

Discussion

Hypoxia

The most striking finding of this work is that the light peak and c-wave are exquisitely sensitive to hypoxia, changing at arterial oxygen tensions at which blood is nearly saturated, and well above oxygen tensions that lead to changes in inner retinal function.23-25 (also Figs. 4, 5). Such sensitivity to hypoxia has not been reported before for the light peak or the c-wave, although Fujino and Hamasaki30 showed transient increases in the monkey c-wave in ischemia, and Niemeyer, Nagahara, and Demant31 found increases in the cat c-wave at arterial oxygen tensions of 20 to 30 mmHg. The trout c-wave also increases in hypoxia.32 This has been suggested to result from the closer contact between the rods and retinal pigment epithelium during hypoxia.33 It seems unlikely that this could explain the c-wave changes in cat or monkey because movements of the RPE and photoreceptors are not known to occur in mammals.

The sensitivity of the light peak and c-wave to hypoxia is an indication of their dependence on the choroidal circulation. Inner retinal function can be preserved until arterial hypoxia becomes severe, since the retinal circulation keeps inner retinal PO2 nearly normal.23,35 This is because hemoglobin buffers retinal venous PO2 effectively, and because retinal blood

![Figure 9](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933338/)

**Fig. 9.** Changes in ERG threshold during hypercapnia and hypocapnia. Other details as in Figure 5.

flow increases,34 allowing the oxygen extraction per unit volume of blood to decrease. Outer retinal oxygenation, however, depends upon the choroidal circulation, which has little capacity for regulating tissue oxygen tension. Oxygen extraction from the choroid is normally small,36,37 and venous oxygen tension is high. During even mild hypoxia, arterial oxygen tension will fall below this normally high venous value, so venous oxygen tension must drop considerably 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.38,39 The change in arterial and venous oxygen tensions must lead, in turn, to a relatively large decrease in outer retinal

![Figure 10](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933338/)

**Fig. 10.** Examples of changes in the standing potential with: A, hypoxia and B, recovery from hypoxia. Gas changes occurred at the arrows, resulting in the arterial oxygen tensions given above each record.

![Figure 11](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933338/)

**Fig. 11.** Examples of changes in the standing potential with A, hypercapnia and B, recovery from hypercapnia. Gas changes occurred at the arrows, resulting in the values of arterial pH above each record.
oxygen tension. Consequently, decreases in arterial oxygen tension will be felt directly by the photoreceptors and RPE.

Changes in the light peak and c-wave in hypoxia could then be the result of an effect on the RPE itself or an effect on the outer retina. An effect of hypoxia at either site would be expected to alter both the light peak and c-wave. The RPE is responsible for the electrical events of the light peak and for the RPE component of the vitreal c-wave. The photoreceptors are responsible for the ionic change underlying the c-wave, and they probably also provide a chemical event resulting in the light peak. It should be pointed out that a change in the vitreal c-wave could also be mediated by an effect on slow PIH, the retinal potential with about the same time course as the RPE component of the c-wave. Preliminary evidence, however, obtained with microelectrodes in the sub-retinal space, suggests that the increase in the vitreal c-wave is due to a change in the RPE component of the c-wave, rather than a change in slow PIH. With regard to both the c-wave and light peak, it seems most likely that hypoxia affects some function of the RPE itself rather than affecting the retina. This is partially because the a-wave, which reflects one aspect of photoreceptor function, is resistant to hypoxia and partially because an effect on the retina might be expected to alter some later stage of retinal processing, such as the b-wave or ganglion cell sensitivity. It is now well documented that hypoxia affects the inner retina only at much lower levels of oxygen tension. It is not possible to say what the effects on the RPE are, but it is conceivable that the increase in the c-wave and SP and the decrease in the light peak all stem from a single change.

Hypercapnia

The effects of hypercapnia on the retina have been studied very little. Decreases in the b-wave and increases in the c-wave have been described, but the dependence of these functions on arterial pH has never been defined before. While the retina was clearly affected by hypercapnia, decreases in light peak amplitude and increases in the c-wave were observed at an arterial pH at which the b-wave and ERG threshold were normal, suggesting that the site of the earliest changes was the RPE. It is possible that hypercapnia and hypoxia act through similar mechanisms, because their effects on the c-wave, standing potential, and light peak are all in the same direction. For example, hypoxia could cause a fall in intracellular pH in the RPE. It seems unlikely that their mechanisms are completely identical, however, because hypoxia and hypercapnia lead to somewhat different changes in the waveform of the light peak.

A question that arises from the studies of hypercapnia is whether intracellular or extracellular pH is the important variable. Hypercapnia leads to changes in both, but extracellular pH can be altered without altering intracellular pH (in the short-term) by injections of HCl or NaHCO3 to cause metabolic acidosis or alkalosis. These metabolic changes were investigated in three animals, and while the data are relatively limited, it was clear that the standing potential changes were larger and more rapid with metabolic than with respiratory changes. Over the same time course, and at the same level of arterial pH, however, changes in the light peak appeared to be smaller. This subject is treated more fully in a study on the perfused cat eye.

Conclusions

The observation that triggered this study was that the light peak and standing potential were sensitive to decreases in respiratory minute volume. It seems now that both hypercapnia and hypoxia probably contributed to this finding. Clinically, one might expect relatively mild disturbances in acid base balance or systemic oxygenation to affect the light peak and c-wave before other measures of retinal function. Local changes in choroidal circulation could also have dramatic effects. For instance, circulatory changes could be the cause of the reduced light peaks in patients with choroidal malignant melanomas.

The b-wave in these patients may be normal. Another disease, acute posterior multifocal placoid pigment epitheliopathy, may also result from decreased choroidal perfusion. The limited available evidence shows that the electro-oculogram is abnormal during the acute phase, but usually normal following the resolution of this disease.

This study also shows that RPE membrane potential changes are very sensitive to oxygen and carbon dioxide. The sensitivity to oxygen is intriguing, since
the tissue oxygen tension at which hypoxic effects begin is 40 to 60 mmHg higher than that around most cortical and inner retinal cells under normoxic conditions. Conceivably this unusual sensitivity to hypoxia could be reflected in other aspects of RPE function.

Key words: light peak, hypoxia, hypercapnia, electro-oculogram, retinal pigment epithelium, acidosis, electroretinogram, ERG.

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References