Intraretinal Study of Cat Electroretinogram during Retinal Ischemia–Reperfusion With Extracellular K⁺ Concentration Microelectrodes

Kano Hiroi, Fumiaki Yamamoto, and Yoshihito Honda

**Purpose.** Intraretinal electroretinograms (ERG) during retinal ischemia–reperfusion were analyzed to clarify which cells are affected in the cat dark-adapted eye.

**Methods.** Ischemia was induced by raising the intraocular pressure above arterial systolic pressure in vivo. Double-barreled microelectrodes were used to record the intraretinal ERGs and the light-evoked [K⁺]₀ decrease in the subretinal space. Vitreal ERGs, the standing potential, and the transepithelial resistance were also recorded.

**Results.** After 10 minutes of ischemia, vitreal ERG b- and c-waves had recovered fully in 2 hours, and after 30 minutes of ischemia, recovered approximately 85% and 77% of their control amplitudes in 4 hours, respectively. At the early period of reperfusion after 10 minutes of ischemia we observed the supernormal c-wave (124% of its control amplitude, n = 10) with an elevated standing potential. The transepithelial potential (TEP) c-wave increased without changes of slow PI and transepithelial resistance when the supernormal amplitudes occurred. The light-evoked [K⁺]₀ decrease in the subretinal space (90% retinal depth) in the area centralis recovered to about 90% of the control amplitude in 2 hours, and after 30 minutes of ischemia recovered to about 70% of the control amplitudes in 4 hours. The recovery courses of the slow PI and TEP c-wave were almost the same as that of the light-evoked [K⁺]₀ decrease.

**Conclusions.** The temporary supernormal amplitude of the ERG c-wave was originated from the retinal pigment epithelium (RPE) during the early period of reperfusion after 10 minutes of ischemia. The photoreceptors, Müller cell and RPE are responsible for the changes in the light-evoked [K⁺]₀ decrease, and the transepithelial c-wave, respectively. Intraretinal recordings suggested that after ischemia of 10 to 30 minutes duration, the responses of photoreceptors, Müller cells and the RPE recovered with a similar time course. These findings suggest that in all cases, primarily photoreceptors were damaged. Invest Ophthalmol Vis Sci. 1994;35:656-663.

Experimental retinal ischemia has been studied physiologically, histologically, and biochemically over the past few decades. Early electrophysiologic studies dealt with a mass response recorded from the whole retina by corneal electrodes, and did not record the intraretinal components of the electroretinogram (ERG) that could reflect the function of individual retinal cells. On the other hand, it is known that ischemic tissue undergoes severe injury induced by posts ischemic products; for example, glutamate and oxygen-derived free radicals. Glutamate receptor antagonists, free radical scavengers, NMDA receptor antagonists and calcium overload blockers have protective effects against posts ischemic damage. We used intraretinal recordings to clarify which cells are affected in the dark-adapted retina during ischemia–reperfusion and to elucidate the action sites of these drugs on cellular level. In these recordings the light-evoked [K⁺]₀ decrease, the slow PI and the transepithelial potential (TEP) c-wave reflect the functions of photoreceptors, Müller cells and retinal pigment epithelium, respectively.

**METHODS**

Preparation, recording, and visual stimulation were performed as previously described. These experiments adhered to the ARVO Statement for the Use of...
Animals in Ophthalmic and Vision Research and were performed on 30 adult cats, which were initially anesthetized with an intramuscular injection of ketamine hydrochloride. Before surgery, anesthesia was induced with a loading dose of carbamic acid ethyl ester (200 mg/kg, iv) and then maintained at 20 mg·kg⁻¹·h⁻¹ during the experiment. Lidocaine hydrochloride (2%) was locally supplemented during surgery. The animals were given atropine sulfate (0.05 mg/kg, subcutaneous) and penicillin G (20,000 U/kg, intramuscular) before surgery. Animals were paralyzed with pancuronium bromide (0.2 mg·kg⁻¹·h⁻¹, iv) and artificially ventilated. Arterial blood pressure was monitored continuously and arterial blood pH, PCO₂ tension, and PO₂ tension were measured intermittently. Arterial pH was maintained in the range of 7.35 to 7.45, and the arterial O₂ tension was kept above 90 mm Hg by means of adjusting the ventilator rate and stroke volume. The body temperature was maintained between 38°C and 39°C using a water-filled heat-ing pad.

A 15-gauge hypodermic needle was used to penetrate the sclera 10 mm from the limbus, and a micro-electrode was advanced into the eye through the lumen of this guide needle. The junction between the micro-electrode and the needle was tightly sealed by means of a silicone boot so that the intraocular pressure (IOP) was maintained. All of the recordings were made from dark-adapted retinas in the region of the area centralis. Intraretinal recordings were obtained with double-barreled, K⁺-selective microelectrodes (ISM). One of the barrels (active barrel) was siliconized by dimethyl-dichloro-silane and the K⁺ionophore (No. 60398, Fluka Chemical Co., Ronkonkoma, NY) was injected into the tip. All ISMs were tested at room temperature before and after each experiment by measuring their response to a change from 1 to 10 mM KCl. The responses before the experiment were 43 to 57 mV (50.3 ± 4.1, n = 19). During the experiment, ISM voltages were recorded using a DC microelectrode amplifier as shown in Figure 1. Both barrels of the ISM recorded the local field potentials, and VK⁺ was monitored as the difference in signals between the active barrel and the reference barrel. The vitreal ERG was recorded between the vitreal electrode (Ag–AgCl wire) in the vitreous humor, and a reference electrode (Ag–AgCl plate) behind the eye using another DC microelectrode amplifier. The depth of the ISM in the retina was expressed in terms of percent retinal depth between the retinal surface and the RPE, where 0% is the vitreoretinal interface and 100% is the apical side of the RPE. With the tip of the ISM placed in the subretinal space (SRS), the transretinal potential was recorded between the reference barrel of the ISM and the vitreal wire, and the TEP was recorded between the reference barrel of the ISM and the reference electrode behind the eye. Potentials were amplified by a four-channel storage oscilloscope having differential amplifiers with a bandpass of 0 to 300 Hz. The ERG a-wave was recorded at a bandpass of 0 to 1000 Hz.

The light stimuli were provided by a tungsten-fila-ment lamp and delivered to the eye using an optic fiber...
bundle. The pupil was fully dilated with 5% phenylephrine hydrochloride and 1% atropine sulfate eye drops. The intensity of the light stimulus was usually 8.3 log quanta (509 degree·second⁻¹, hereafter abbreviated log q (rod saturation occurs at about 8.2 log q)²³). The ERG b- and c-waves were obtained by 4-second flashes with an intensity of 8.3 log q, and the a-wave was recorded by a 20-millisecond flash with 12.3 log q. Animals were dark-adapted at least 1.5 hours before data collection.

IOP was regulated with a sterile intraocular irrigating balanced salt solution perfused into the eye through a needle placed in the anterior chamber. Mean arterial pressure was continuously monitored through a femoral artery catheter. IOP was measured with a strain gauge connected to a monitor and regulated by changing the height of the irrigating solution. Ischemia was produced by raising the IOP beyond the arterial systolic pressure (Fig. 1). The passage of this system was confirmed through experiments.

To measure changes in RPE resistance, the microelectrode was placed in the SRS. Current pulses (500 milliseconds in duration, 50 μA) were generated by a stimulator (SEN3301, Nihon Kohden) connected to a stimulus isolation unit (SS302J, Nihon Kohden). Current was passed between another vitreal electrode and the retrobulbar electrode. The voltage changes in response to the current pulses were recorded between the microelectrode and the retrobulbar reference electrode.

**RESULTS**

**Standing Potential and Vitreal ERG**

Figure 2 shows the change of standing potentials (SP) during retinal ischemia (10, 20, and 30 minutes) and reperfusion. At the onset (left arrows) of ischemia SPs temporarily increased for a short time, by 0.86 ± 0.68 mV (mean ± standard deviation, n = 23) for 10 minutes, 0.37 ± 0.59 mV (n = 3) for 20 minutes and 0.83 ± 0.50 mV (n = 10) for 30 minutes of ischemia. After the initial increase, the SPs gradually decreased until the end of ischemia, by 2.80 ± 1.76 mV for 10 minutes, 4.17 ± 2.23 mV for 20 minutes and 4.19 ± 1.80 mV for 30 minutes of ischemia. During the SP increase at the ischemia onset, the vitreal ERG b-wave was abolished and the c-wave became negative. This negative wave, which Fujino and Hamasaki²⁶ reported to be slow PHI, was observed even 10 minutes after the onset of ischemia. However, in 30 minutes it was abolished in all cats. During the early period of reperfusion after 10 minutes of ischemia, the SP showed a particular oscillation: a rapid decrease just after the reperfusion start (right arrow) followed by a large increase with a notch, where the supernormal ERG c-waves (described later) were observed. The SP returned to the preischemic value slowly (Fig. 2, top trace). When 20 or 30 minutes of ischemia was induced in the retina, the similar large increase of the SP were observed as shown after 10 minutes of ischemia.

Figure 3 shows changes in the b- and c-wave am-

![FIGURE 2. Effect of ischemia–reperfusion on the SP recorded by the vitreal electrode and retrobulbar reference. Top trace, 10 minutes of ischemia; middle trace, 20 minutes of ischemia; bottom trace, 30 minutes of ischemia. Arrows, the onset and the offset of ischemia; asterisks, postischemic increase of SP.](image-url)
Intraretinal Electroretinogram During Reperfusion

After 30 minutes of ischemia, the c-wave also rapidly increased (recovery) in amplitude similar to the recovery after 10 minutes of ischemia, and its amplitude recovered to $49 \pm 29\%$ ($n = 10$) of the preischemic state in 5 minutes. This recovery was slower than that after 10 minutes of ischemia, and was $82 \pm 15.5\%$ of the control amplitude ($n = 6$) 6 hours after reperfusion. ERG A-waves were observed in two cats after 30 minutes of ischemia in order to examine the effect of ischemia−reperfusion on the photoreceptors. The recovery of the A-wave after reperfusion seemed to be more rapid than that of the b-wave, but we did not examine A-waves in detail because stronger stimulus was required for its elicitation and a much longer dark-adaptation period between flashes was needed.

**Intraretinal Recording**

During reperfusion after 10, 30, and 60 minutes of ischemia, the depth profiles of intraretinal potentials were recorded. The slow PI, TEP c-wave and light-evoked $[K^+]_o$ decrease at 95% retinal depth were plotted in Figure 4. The potentials were further reduced in amplitude and recovered more slowly, as the duration of ischemia increased. However, there was no remarkable difference in the recovery course among the slow PI, the TEP c-wave and the light-evoked $[K^+]_o$ decrease. In most cases the recovery time courses of the slow PI and the TEP c-wave were comparable to that of the vitreal ERG c-wave as shown in Figure 5. At 160 minutes after ischemia the amplitudes of the vitreal ERG b- and c-waves were mostly recovered, whereas the slow PI, the TEP c-wave, and the light-evoked $[K^+]_o$ decrease in the SRS (90% depth) of the area centralis were still reduced remarkably as compared with the preischemic control. The V-log I curves of slow PI, TEP c-wave, and vitreal ERG c-wave were compared before and after ischemia in two cats. After the amplitude of vitreal ERG c-wave had recovered at any intensity, that of slow PI or TEP c-wave was still reduced at dimmer illumination. Thus, both the slow PI and the TEP c-wave might be more sensitive in detecting retinal damage than the vitreal ERG c-wave.

**Origin of the Supernormal ERG c-Wave**

Intraretinal potentials were recorded after 10 minutes of ischemia in order to examine the origin of the postischemic supernormal c-wave. Figures 6 and 7 show the changes in the SP, vitreal ERG, intraretinal potentials and the light-evoked $[K^+]_o$ decrease before, during and after 10 minutes of ischemia. While the ischemia was being induced, the ISM was placed in the vitreous to prevent damage to the retina. Immediately after the offset (Fig. 6, right arrow) of ischemia, the ISM was then advanced into the SRS promptly and the intraretinal potentials were recorded. When the SP increased after the offset (Fig. 6, right arrow) of isch-
In intraretinal potentials in the SRS during reperfusion. Potentials were obtained at the same retinal depth (95% depth from the vitreoretinal surface). Amplitudes are expressed as a percentage of the preischemic control value (normalized amplitude). Top, slow PHI; middle, TEP c-wave; bottom, light-evoked $[K^+]_o$ decrease. Filled symbols, 10 minutes of ischemia ($n = 5$); open symbols, 30 minutes ($n = 4$); plus signs, 60 minutes ($n = 1$).

After ischemia, the TEP increased with the increase of ERG c-wave amplitudes, while the transretinal potential did not change. Figure 7, which was obtained from the data in Figure 6, showed the time course of slow PHI and TEP c-wave (upper plots), light-evoked $[K^+]_o$ decrease (middle plot) and the vitreal ERG b- and c-waves (lower plots) before, during and after reperfusion. When the vitreal ERG c-wave was maximum in amplitude at the peak of the SP (6 minutes after reperfusion), the TEP c-wave was much larger than slow PHI, while the light-evoked $[K^+]_o$ decrease was still small. The TEP c-wave increased transiently around the SP peak, while the slow PHI and the light-evoked $[K^+]_o$ decrease recovered gradually after reperfusion. Therefore the temporary supernormal amplitude of the ERG c-wave could be caused by a change in the RPE that generated the TEP c-wave.

**Transepithelial Resistance Measurement**

One of possible mechanisms for altering the TEP c-wave is a change in the resistance of the apical or basal membranes of the RPE (Rap or Rba) or of the paracellular shunt (Rs) across the tight junctions between RPE cells. The TEP c-wave after ischemia could be increased in three ways: Rs could increase, or either Rap or Rba could decrease. It is possible to calculate

**FIGURE 5.** Intraretinal recordings at the SRS (90% depth) before and 160 minutes after 10 minutes of ischemia with the K$^+$-selective microelectrode. Top trace, vitreal ERG; top middle trace, transretinal potential; bottom middle trace, TEP; bottom trace, light-evoked $[K^+]_o$ decrease induced by 4 seconds of illumination (8.3 log q).

**FIGURE 6.** Changes in the responses after 10 minutes of ischemia. Spikes on each trace during reperfusion are responses (top, slow PHI; top middle, TEP c-wave; bottom middle, light-evoked $[K^+]_o$ decrease; bottom, vitreal ERG) to 4 seconds of illumination (8.3 log q). During ischemia, a microelectrode (ISM) was placed in the vitreous humor. Just after the reperfusion, a microelectrode was advanced into the SRS and then placed at the same position as before ischemia. Asterisk indicates the time ISM reached to the SRS.
Intraretinal Electroretinogram During Reperfusion

1 - 0.5 O 0

m —»

•

TEP c-wave

slow PHI

light-evoked [K+]o decrease

c-wave

b-wave

-20

20 40

60

80

Time (min)

FIGURE 7. Changes in the vitreal ERG and intraretinal potentials in the SRS during reperfusion after 10 minutes of ischemia. These plots were made from the data of Figure 6. Top, slow PHI (open circles) and TEP c-wave (closed squares); middle, light-evoked [K+]o decrease; bottom, vitreal ERG c-(closed diamonds) and b-(open circles) waves.

Rt, the equivalent resistance of the whole RPE. Rt is determined as follows:

\[ Rt = \frac{R_s(R_{ap} + R_{ba})}{R_s + R_{ap} + R_{ba}} \]

Rt + Rsc, where Rsc is scleral resistance, is measured by passing constant current pulses from the vitreous to the outside of the eye and recording the voltage drop between a microelectrode in the SRS and the retrobar reference electrode. Five minutes after reperfusion, Rt + Rsc had increased little (Fig. 8). Because it is unlikely that Rsc can change during reperfusion, Rt may be changed very slightly. However, the change in the RPE resistance was too small to explain the increase of the TEP c-wave. Therefore, the increase of the RPE resistance was little responsible for the TEP c-wave increase after ischemia.

DISCUSSION

Standing Potential

ERGs, which used to be recorded with corneal electrodes, have been observed during ischemia and reperfusion. However, the SP was not observed because it was very difficult to record DC potentials from eyes in vivo using corneal electrodes. We used vitreal Ag–AgCl electrodes to obtain stable DC recordings from the dark-adapted cat eye. Just after the onset of ischemia, SP temporarily increased, a change that seemed similar to the SP change during systemic hypoxia. Hypoxia, which could be induced by retinal ischemia, might cause the depolarization of the RPE basal membrane by two possible mechanisms; one is a direct effect on the basal membrane, and the other is an indirect effect related to the change of [K+]o in the SRS. Although the vitreal ERG c-wave increases during systemic hypoxia by the depolarization of the basal membrane, c-waves diminished even during the SP increase after the onset of ischemia. These results suggested that the ischemia might cause not only hypoxia in the retina but also damage to the retinal cells, for example, photoreceptors. After the initial elevation, SP decreased continuously until the end of ischemia. The SP is thought to be generated from the RPE; the difference between the apical and basal membrane potentials. The long-lasting decrease of SP during ischemia might result from a dysfunction of the RPE, but it might be possible that the neural retina also contributes to the SP change during ischemia. In the current study, a few recordings with intraretinal microelectrodes were obtained during ischemia and showed the possibility of a contribution by both the RPE and the neural retina. But because the major objective of this study was to examine the intraretinal ERG during reperfusion, we did not refer to the intraretinal recording of SP during ischemia.

During reperfusion after ischemia, SP remarkably increased during the early period, and the intraretinal recording in the SRS showed that the SP change was induced by the change of transepithelial potential (TEP). The light-evoked [K+]o decrease and [K+]o in the SRS recovered gradually during the period of the

FIGURE 8. Changes in the transepithelial resistance (Rt + Rsc) and ERG b- and c-wave amplitudes before ischemia and during reperfusion after 10 minutes of ischemia.
SP increase and did not fluctuate like the TEP. Therefore, the increased SP during reperfusion was mainly generated from the RPE itself, which produced the TEP. The elevation of the TEP could have originated from hyperpolarization of the apical membrane or depolarization of the basal membrane in the RPE or from both. However, it was impossible to determine which membrane contributed to the TEP change during reperfusion because it was very difficult to obtain intracellular recordings from the cat eye in vivo. Furthermore, although the change of RPE resistance may be related to the increased SP, the transepithelial resistance during reperfusion did not change remarkably.

**Intraretinal ERGs During Reperfusion**

In previous studies the retinal ischemia of more than 30 to 120 minutes caused an irreversible decrease of ERG b-wave.1-8 The variations of reversible ischemic duration may be induced by the differences of anesthetic condition,4 body temperature,5 and the method of inducing ischemia (for example, retinal or retinal and choroidal, by IOP elevation or ligation or other methods). We monitored and maintained body temperature and blood pressure during experiments to avoid these influences. And we induced short-term ischemia in order to examine the recovery phase during reperfusion. After 10 minutes of ischemia the vitreal ERG showed almost complete recovery, and after 30 minutes of ischemia it was reduced for several hours. This finding was consistent with previous histologic findings.5,9 We investigated these changes in ERG thoroughly by recording intraretinal ERG.

After 10 minutes of ischemia, the vitreal ERG c-wave showed a temporary supernormal amplitude, and then fully recovered. Ulrich and Reimann8 also demonstrated a postischemic supernormality of the ERG c-wave after 10 minutes of ischemia, but they did not refer to it. When the vitreal ERG c-wave changes, there must be some change in slow PIII or TEP c-wave, because the ERG c-wave is the summation of the RPE (TEP c-wave) and Muller cell components (slow PIII).20 Intraretinal recording suggested that the vitreal ERG c-wave of the supernormal amplitude was caused by change in the TEP c-wave originating from the RPE.

It is believed that the TEP c-wave is generated by the hyperpolarization of the apical membrane of the RPE in response to the light-evoked decrease of [K+]o in the SRS, and the slow PIII is generated by the hyperpolarization of Muller cells in response to the same [K+]o decrease.17,18 Therefore, by observing the slow PIII and the TEP c-wave, it might be possible to evaluate ischemia-induced damage in the Muller cell and the RPE, respectively. Furthermore, because the light-evoked [K+]o decrease originates from the photoreceptor,17,32 and if its Na+-K+ pump is modulated by stress, the light-evoked [K+]o response would be affected. By recording the light-evoked [K+]o decrease, photoreceptor function can be monitored. After 10 minutes of ischemia, the slow PIII, TEP c-wave and light-evoked [K+]o decrease that were recorded in the SRS were still remarkably reduced, as compared with the preischemic control, although the vitreal ERGs were mostly recovered (Fig. 5). This inconsistency suggests two events. Firstly, because the vitreal ERG c-wave is the sum of the slow PIII and the TEP c-wave, the reduction of both the slow PIII and the TEP c-wave did not induce the reduction of the vitreal ERG c-wave (false normal). Secondly, the whole retina was almost recovered except for the localized site where the intraretinal recording was performed. In either case the intraretinal recordings could indicate a dysfunction of photoreceptors that could not be detected by the vitreal ERGs. However, it was impossible to clarify dysfunctions of the Muller cell and the RPE because the light-evoked [K+]o response itself was also affected. If the RPE was damaged more severely than photoreceptors, the TEP c-wave would have been reduced more than the light-evoked [K+]o decrease.

Earlier investigators,5,17,33 who observed the recovery of ERG a-wave after ischemia, reported that photoreceptors were more resistant to ischemic stress than b-wave. We also observed the ERG a-wave after a 30 minutes of ischemia in two cats and found that the a-waves recovered more rapidly than the b-wave (recovered fully 30 to 50 minutes after the start of reperfusion). The a-wave can reflect part of the photoreceptor activity.34 However, as shown in Figure 4, the light-evoked [K+]o decrease did not recover as quickly as the a-wave after 30 minutes of ischemia. Although there is a difference in the generating mechanism between the a-wave and the light-evoked [K+]o response, the light-evoked [K+]o response is more sensitive than the a-wave for detecting damage in photoreceptors. To evaluate photoreceptor damage, it will be useful to record the light-evoked [K+]o response using the intraretinal microelectrode.

**Key Words**

electroretinogram, ischemia, reperfusion, potassium ion, c-wave

**Acknowledgments**

The authors thank S. Kashii, MD, and A. Akaike, MD, for statistical analysis.

**References**