Effects of Intravenous Iodoacetate and Iodate on pH Outside Rod Photoreceptors in the Cat Retina

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**Purpose.** Effects of intravenous iodoacetate (a glycolysis inhibitor) and iodate (a metabolism inhibitor selective to retinal pigment epithelium) on light-evoked alkalinizations and hypoxia-induced acidifications were studied in the dark-adapted cat retina, in vivo, to learn about pH regulation.

**Methods.** pH was recorded in the extracellular space surrounding rod photoreceptors with double-barreled H⁺-selective microelectrodes.

**Results.** Intravenous infusion of 5 mg/kg iodoacetate-induced alkalinizations in the outer nuclear layer and suppressed both light-evoked and hypoxia-induced pH responses immediately. Iodate injection (30 mg/kg) produced acidifications in the subretinal space and affected light-evoked alkalinizations gradually but not hypoxia-induced acidifications.

**Conclusions.** These results suggest that rods glycolysis plays an important role in both light-evoked and hypoxia-induced pH responses. And the retinal pigment epithelium may have little concern with light-evoked alkalinizations except that it plays an important role in regenerating the rhodopsin to be needed for the light responses of photoreceptors. Furthermore, the finding of the intravenous-iodoacetate-induced alkalinization in the outer nuclear layer supports that acid production by rods in the dark is originated from glycolysis to support the dark current. The iodate-induced acidification in the subretinal space indicates that the retinal pigment epithelium might actively transport acids from the subretinal space to the choroid.


The retinal pH changes induced by light, darkness, hypoxia, hyperoxia, acetazolamide, intraocular pressure, and blood pressure were recently studied using double-barreled H⁺-selective microelectrodes placed intraretinally in the living cat eye to learn about pH regulation. Acid production by rod photoreceptors is highest in the dark, reflecting a high rate of energy metabolism, and it is suggested that glycolysis is required to support the dark current. Illumination alkalinizes the extracellular space surrounding rod photoreceptors by suppressing both glycolysis and respiration. Hypoxia in the dark further acidified the extracellular space-surrounding rods. This suggests that the energy metabolism of dark-adapted rods is exquisitely sensitive to systemic hypoxia so that any small decrease in PaO₂ increases rod glycolysis. Thus it is possible that the pH changes induced by light and hypoxia are closely related to metabolism, especially glycolysis in rods. Then we wished to further confirm the relationship between pH responses (to light and hypoxia) and rods glycolysis and how the retinal pigment epithelium (RPE) could contribute to the pH regulation and these pH responses.

There are two potent visual poisons that have been known commonly since their toxicity to retina was reported in detail by Noell: iodoacetate (IAA, consid-
pleted primarily to block metabolic activity in the photoreceptors) and sodium iodate (SI, considered primarily to affect the RPE). IAA is an inhibitor of glycolysis that inactivates the enzyme glyceraldehyde-
3-phosphate dehydrogenase and thereby decreases the production of pyruvate and lactate. This study examined the effects of intravenous administration of IAA on both light-evoked and hypoxia-induced pH responses, and clarified the contribution of glycolysis to these responses. Furthermore, it investigated the role of the RPE on these responses by means of an intravenous injection of SI, which could damage the RPE rapidly.

MATERIALS AND METHODS

Preparation

These experiments adhered to the ARVO Resolution on the Use of Animals in Research and were performed on the intact left eye of 21 adults cats. Cats were initially anesthetized with an intramuscular injection of ketamine hydrochloride (20 mg/kg; Ketalar 50, Sankyo, Tokyo, Japan). During surgery anesthesia was maintained with a loading dose of carbamic acid ethyl ester (200 mg/kg intravenously; Urethane, Tokyo Kasei, Tokyo, Japan) and was locally supplemented with 2% lidocaine hydrochloride (Xylocaine, Fujisawa, Tokyo, Japan). Urethane was then administered at 20 mg/kg/hr to maintain anesthesia during the experiment. The animals were also given atropine sulfate 0.05 mg/kg subcutaneously (Tanabe, Tokyo, Japan) and penicillin G 20,000 U/kg, intramuscularly (Meiji, Tokyo, Japan) before surgery.

Animals were paralyzed with pancuronium bromide 0.2 mg/kg/hr, intravenously (Moban, Sankyo) and respired as dictated by arterial blood pH, PaCO2 and PaO2 values, measured intermittently either with a blood gas analyzer (model 175, Corning, Medfield, MA) or another one (STAT profile 5, Nova biomedicai, Waltham, MA). Arterial pH was maintained in the range of 7.35–7.50, and arterial oxygen tension was kept above 90 mm Hg by means of adjusting ventilatory rate, stroke volume, and composition of inspired gas, which was generally room air supplemented with oxygen as needed. Hypoxia was induced by adding nitrogen to the inspired mixture. When the same hypoxic episode was consecutively repeated, for instance when responses were studied at different retinal depths, blood gases were monitored only for individual episodes occurring near the beginning, middle, and end of a series. Mild hypoxia (between 40 and 70 mm Hg in PaO2) was induced in cats in the present study. Rectal temperature was maintained between 38 and 39°C by using a water-filled heating pad.

Recording and Electrodes

The technique of recording from the intact cat eye was essentially the same as previously described although some different materials were used. Briefly, a 15-gauge hypodermic needle was used to penetrate the sclera 10 mm from the limbus, and a microelectrode was advanced into the eye through the lumen of this needle. The junction between the microelectrode and the needle was tightly sealed by means of a silicone boot so that the normal intraocular pressure was maintained. The boot provided free penetration and withdrawal of the microelectrode, but prevented leakage of intraocular fluid.

All of the recordings were obtained from dark-adapted retinas in the region of the area centralis. Double-barrelled, H+-selective microelectrodes (ISM) were used to measure [H+]o. These ISM were constructed from double-barrel glass capillary tubing (thick theta septum, TST150-6, World Precision Instruments, Sarasota, FL). The back ends of the two barrels were separated by 8 mm to prevent electrical contact between them. The tubing was pulled into two microelectrodes 8 cm long with a horizontal microelectrode puller (Model PD-5, Narishige, Tokyo, Japan). They were then placed in an oven at 130°C for 1 hour to remove any condensed water. One of the barrels was silanized by dimethyl-dichloro-silane (Sigma Chemical Co., St. Louis, MO) at room temperature for 20–40 sec. The pH ionophore (No. 95297, Fluka Chemical Corp., Ronkonkoma, NY) was injected into the silanized barrel and formed a column approximately 0.5 cm long. The injected microelectrodes were bevelled (Narishige beveller, Model EG-7) and had tip diameters of 2–3.5 μm.

The ionophore-containing barrel (active barrel; AB) was back-filled with a solution that 40 mM KH2PO4, 23 mM NaOH, and 15 mM NaCl and that was titrated to pH 7.0. The reference barrel (RB) was filled with 145 mM NaCl and 5 mM KCl. The resistance of the RB was 30–60 MΩ. The resistance of the AB was estimated to be 1000 times larger than that of the RB. A chlorided silver wire was placed in each barrel, and all ISMs were tested at room temperature before and after each experiment by measuring their response to a solution change from pH 7.0 to pH 8.0 of 1/15 M phosphate buffer solutions (Nacalai tesque, Kyoto, Japan). Slopes before the experiment were 45 ± 6 μV (n = 21) and data were accepted only from ISMs that had final slopes within 10% of their original slopes after the experiment.

During the experiment, ISM voltages were recorded using a DC microelectrode amplifier (MEZ-8300, Nihon Kohden, Tokyo, Japan). Both barrels of the ISM recorded the local field potentials, and VH+ was the difference signal between the AB and the RB. In many experiments, the difference signal contained
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a transient caused by imperfect cancellation of the common mode field potential. This occurred because the AB had a slower time constant than the RB. This problem was mitigated by a feature of the amplifier called “transient cancellation” or more accurately, “cross capacitance compensation.” 14 However, field-potential differencing artifacts that could not be eliminated by the amplifier contaminated the recordings (Fig. 1).

The vitreal electroretinogram (ERG) was recorded using another DC microelectrode amplifier (MEZ-7200, Nihon Kohden) between the vitreal electrode (Ag-AgCl wire) in the vitreous humor, and a reference electrode behind the eye, which was a chlorided silver plate (0.3 X 1 cm). The vitreal electrode was sealed into a 19-gauge hypodermic needle that was inserted through the wall of the eye. The local ERG recordings were made between the RB of the ISM and the vitreal wire. It was extremely useful to know the distinct characteristics of the local ERG recordings at different retinal depths to locate the depth of the microelectrode. 15

Usually the depth of the ISM in the retina was estimated in terms of percentage of retinal depth between the retinal surface and the RPE, where 0% is the vitreal–retinal interface and 100% is the basal side of the RPE. These sites were recognized by the voltage shifts that occurred when each was encountered. Another field potential was also observed between the RB of the ISM and the reference electrode behind the eye, which was named as Vx (transepithelial potential when the tip of the ISM was placed in the subretinal space).

Potentials were amplified by a four-channel storage oscilloscope with differential amplifiers (VC-11, Nihon Kohden) with a band pass of 0–300 Hz. In case of recording the ERG a-wave, a band pass of 0–1000 Hz was used. Responses were recorded on an eight-channel chart recorder (RTA-1200, Nihon Kohden), digitized, and stored on computer (PC-9800EX and VM, NEC, Tokyo, Japan) for later analysis using a data sampling software (Labtech Notebook, Contec, Osaka, Japan). Data were plotted on a laser printer (Laserjet IIP, Hewlett Packard, Palo Alto, CA) using

![Figure 1](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933399/ on 11/18/2018)
an IBM computer (PS/2 55SX, IBM, Racine, WI) and a spreadsheet program (123/G, Lotus, Cambridge, NJ).

Visual Stimulation

Stimuli were provided by a tungsten-filament lamp (KP-12, Kondo Sylvania, Tokyo, Japan) and led to the eye using an optic fiber bundle (diameter 1 cm). Neutral density, color and heat filters, and an electromagnetic shutter were interposed in the beam between the source and the fiber bundle. The pupil was fully dilated with 5% phenylephrine hydrochloride and 1% atropine sulfate eye drops. When the opening of the fiber bundle was located at 3 cm in front of the eye, maximum intensity of a light stimulus was 350 mWatt/cm² (509 nm) just in front of the cornea. When the retinal illumination was calculated using the following values: pupil area (1.2 cm²), visual angle of the fiber bundle opening (20° in diameter), this intensity was estimated to be 12.3 log quanta (509) deg⁻²sec⁻¹, hereafter abbreviated to log q. The intensity of light stimulus used in this study was usually 8.3 log q (rods saturation occurs at about 8.2 log q¹⁶). The ERG b- and c-waves were obtained by 4 sec flashes with the intensity of 8.3 log q and a-wave was recorded by a 20 msec flash with 12.3 log q. The light-evoked pH responses were produced by 3-min flashes with 8.3 log q. Animals were dark-adapted at least 1.5 hours before data collection.

Calculation of Changes in the Strong Ion Difference

To obtain the strong ion difference (SID, i.e., the equivalent addition of strong base) underlying a given pH change,¹⁷ the following equation was used¹⁸:

\[
dSID = (S')\left(\frac{1}{1 + \frac{P(CO_2)}{P(K)}}\right)(e^{2.3pK} - e^{2.3pH})
\]

where \( S' \) is the solubility coefficient of CO₂ in water (\( S' = 0.032 \) mM/l/mm Hg and \( pKa = 6.13 \)). \( P(CO_2) \) in cat retina was not measured, and we used the value for cat venous \( P(CO_2) \) of 40 mm Hg.¹⁹ \( pH1 \) was obtained from the continuous dark-adapted depth profile in the previous article.¹ The amplitudes of both light-evoked and hypoxia-induced pH responses were evaluated by calculating the SID.

Administration of Iodoacetate and Iodate

Iodoacetic acid, sodium salt (IAA, Aldrich Chemical Co., Milwaukee, WI) in saline (5 mg/ml) was injected usually in a dose of 5 mg/kg of body weight (additional injection was done in a few cats) and for two cats in a dose of 10 mg/kg of body weight. Sodium iodate in water 30 mg/ml (SI, Wako Pure Chemical, Osaka, Japan) was injected in a dose of 30 mg/kg of body weight and for only one cat in a dose of 10 mg/kg of body weight. These solutions were administered slowly by the intravenous route during a more than 60 sec pe-

![FIGURE 2. Effect of iodoacetate (IAA) on the intraretinal pH in outer nuclear layer and the standing potential (DC recording) of the dark-adapted retina. (Top trace) transepithelial potential. (Second trace) transepithelial potential. (Third trace) pH recording. (Bottom trace) standing potential. A downward movement of the pH trace means alkalinization (pH shift by 0.15 pH unit) after the administration of IAA. Horizontal bar below the bottom trace indicates the intravenous infusion of IAA (5 mg/kg) for 60 sec. Left longitudinal scale is for calibration (div). The abscissa is time scale. (C041)
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The pH of injected solutions was 6.2 in IAA and 6.25 in SI. The osmotic pressure of them was 322 mosm/kg in IAA and 250 mosm/kg in SI. Arterial blood pH, PaO₂ and PaCO₂ were measured before and after the administration of these drugs.

RESULTS

Retinal pH Changes after IAA and SI IV

Intravenous infusion of IAA produced slow and prominent intraretinal alkalinizations as illustrated as Figure 2. This effect was largest in amplitude when the ISM was positioned in the distal portion of the outer nuclear layer (ONL). The alkalinizations were observed in all eleven cats to which IAA was administered. There were no significant changes in blood pH, PaO₂, PaCO₂, and blood pressure before and after administration of IAA. The standing potentials increased after the IAA injection but the changes were not remarkable.

SI injection induced rapid and remarkable intraretinal acidifications in ten cats as shown in Figure 3. This effect was largest in amplitude when the ISM was put in the subretinal space as close as possible to the apical surface of RPE. In contrast with IAA administrations, the standing potentials went down remarkably after the injection of SI, originating from the change of membrane potential in RPE (see transepithelial recording in Fig. 3). The SI administrations induced a mild increase of blood pressure by approximately 20 mm Hg but there were no significant changes in blood pH, PaO₂, and PaCO₂.

Effects of IAA and SI on Light-evoked Alkalinization

Intravenous infusion of IAA caused immediate suppression on light-evoked alkalinizations in ONL of dark-adapted retina, as shown in Figure 4. Light-evoked alkalinizations were suppressed completely by IAA in most cats, but sometimes IAA of 5 mg/kg was not enough to suppress light-evoked responses completely. ERG a-, b-, and c-waves were also affected by IAA injection immediately as described in Noell's paper. After light-evoked alkalinizations, ERG a-, b-, and c-waves were abolished completely by the IAA injection (5 mg/kg), there was an incomplete recovery of these responses at 12 hours after the infusion (Fig. 4).

SI administration produced the decreases in an amplitude of light-evoked alkalinizations in ONL, as shown in Figure 5. The SI effect was not so rapid that it took about 1 hour for the light-evoked alkalinizations to diminish by a half size of control responses in SID as illustrated in Fig. 6, although the c-wave of ERG became negative within 30 min as reported previously. ERG b-wave also reduced its amplitude gradually by about 30 % one hour after SI injection as reported by Kiryu et al. The SI injection of reduced dose (10 mg/kg) gave the reduced effects on both light-evoked and ERG responses.

**FIGURE 3.** Effect of iodate (SI) on subretinal pH and the standing potential (DC recording) of the dark-adapted retina. (Top trace) transretinal potential. (Second trace) transepithelial potential. (Third trace) pH recording. (Bottom trace) standing potential. Acidification (pH shift by 0.25 pH unit) produces a upward movement of the pH trace. Horizontal bar below the bottom trace indicates the intravenous infusion of SI (30 mg/kg) for 60 sec. (C004)

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Effects of IAA and SI on Hypoxia-induced Acidification

Hypoxia-induced acidifications in ONL were abolished completely and immediately after intravenous injection of IAA in the dark as illustrated in Figure 7. IAA suppressed hypoxia-induced acidifications more rapidly than light-evoked alkalinizations. Intravenous IAA of 5 mg/kg was enough to abolish hypoxia-induced acidifications completely. In three cats SI administration had little effect on hypoxia-induced acidifications in ONL as shown in Figure 8, although light-evoked pH responses were reduced as shown in Figure 6.

DISCUSSION

It has been known that electron microscopy of both light- and dark-adapted animals showed a severe and irreversible involvement of the visual cells, starting about 3 hr after intravenous administration of IAA and that the rhodopsin content of the degenerating retina remained at an almost normal level for a long time (30–40 hrs.) after the appearance of the first ultrastructural changes. Evidence of morphologic changes in the photoreceptors, even at an ultrastructural level, seems to follow slowly the loss of function of the cell resulting from IAA treatment. In the current study such intraretinal pH responses as light-evoked alkalinization and hypoxia-induced acidification were suppressed by intravenous infusion of IAA.
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FIGURE 6. Change of the light-evoked alkalinization as a function of time passage after iodate (SI) injection (30 mg/kg). In each retina of seven cats pH responses were recorded in outer nuclear layer by 3-min illuminations at 8.3 log q. Data at "0 min" after SI injection mean control data at 20 min before SI. Each response is shown as normalized data (% response; control data before SI injection were defined as 100%) after calculating strong ion difference. (C4,9,11,14,20,25,28)

quickly, whereas ERG extinction, which indicates loss of function, was induced a few minutes after injection of the poison.6

IAA selectively inhibits in vivo the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase8 and induces the morphologic changes of mitochondria in the inner segment of visual cells initially21. Furthermore IAA selectively damages of the photoreceptors, especially rods, but does not affect the other retinal layers.21,22 Our results, that IAA injection suppressed both light-evoked and hypoxia-induced pH responses, coincide with the hypothesis that their pH responses relate to rods glycolysis closely; that is, the light-evoked alkalinizations are caused by the suppression of acid production by glycolysis which supports the dark current in dark-adapted rods, and the hypoxia-induced acidifications are provoked by the acceleration of anaerobic glycolytic rate to maintain energy production under hypoxia. Although hypoxia-induced acidifications were suppressed completely by IAA, there were two cats that had the incomplete suppression of light-evoked alkalinizations by IAA of 5 mg/kg. And the additional injection of IAA (10 mg/kg) resulted in the complete suppression of light-evoked pH responses (Fig. 4).

Alkalinizations in ONL were observed immediately after the intravenous IAA administration in the dark. In the dark-adapted retina a considerable portion of glycolysis has been shown to occur in rod photoreceptors and to be required by the activity of the Na+-K+ pump in support of the dark current.23,24 Therefore the IAA-induced alkalinizations might be attributable to a decrease of lactic acid by the suppression of rods glycolysis because of the pharmacologic effects of the intravenous IAA. It took several minutes for the saturation of IAA-induced alkalinization to be observed (Fig. 2) and for IAA to affect photoreceptors so fully that hypoxia-induced pH responses were suppressed completely. Although the hypoxia-induced

FIGURE 7. Effect of iodoacetate (IAA) on hypoxia-induced acidifications in the outer nuclear layer of dark-adapted retina. Each trace was obtained with 90 sec periods of systemic mild hypoxia (bar below bottom trace; from PaO2 92 mm Hg to 46 mm Hg). The control response was obtained 10 min before the onset of IAA infusion (5 mg/kg), and the post-IAA response was recorded at 10 min after the IAA injection. (C039)
Acidifications have never been recorded within 9 minutes after IAA injection, we postulate that both the IAA-induced alkalinizations and the suppression of hypoxia-induced pH responses were caused by glycolysis inhibition because the IAA is a glycolysis inhibitor.

Although the intravenous infusion of SI can affect retinal cells, it damages the RPE with relative selectivity. SI initially affects the RPE, where profound ultrastructural changes can be seen 100-150 min after the SI injection. It is well known that the ERG c-wave, which originates from RPE, is affected by SI injection immediately. Furthermore, such RPE functions as retinal adhesion, barrier and regeneration of rhodopsin also are damaged early by SI. Thus the RPE is the initial and main target for SI toxicity. Although it has been recently reported that the SI kills the RPE by allowing the build-up of toxic levels of unesterified vitamin A in the cells, the immediate effects of SI would seem unlikely to result from accumulating vitamin A. The intravenous injection of SI affected the light-evoked alkalinizations but not the hypoxia-induced acidifications. If SI could suppress rod glycolysis as well as IAA, the hypoxia-induced pH changes must have been affected. It is probable that SI is not an inhibitor of enzymes in the glycolytic process. The mechanism of light-evoked pH responses seems to be more complex than that of hypoxia-induced acidifications because it contains many more steps from light perception to metabolic change.

Remarkable acidifications were obtained in the subretinal space immediately after the intravenous infusion of SI. There are at least two possible explanations for this. Acids might come from the damaged RPE itself and/or acids might accumulate in the subretinal space. For example, lactic acid originating from rod photoreceptors might accumulate in the subretinal space because SI affects uptake of lactic acid at the apical membrane of RPE. It was reported recently that the RPE could transport hydrogen ion and lactic acids from the subretinal space to the choroid. Our data can support the presence of such an acid transport system in RPE. If such an acidified environment in the retina as observed in this study can be maintained for a long period after SI administration then acidification may be one of the factors affecting the function of retinal cells.

Key Words
intraretinal pH, glycolysis, rod photoreceptor, iodoacetic acid, sodium iodate

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