ELSEVIER

Retinal Pigment Epithelial Origin of Bicarbonate Response

Yasunori Segawa, Yutaka Shirao and Kazuo Kawasaki

Department of Ophthalmology, Kanazawa University School of Medicine, Kanazawa, Japan

Abstract: The mechanisms of the bicarbonate-induced decrease in the ocular standing potential (bicarbonate response) were investigated in the cat. An intravenous infusion of 1.4% sodium bicarbonate solution caused a decrease in the standing potential. A high-bicarbonate solution decreased or increased the potential across the retinal pigment epithelium-choroid tissue of the excised cat eye when applied basally or apically, respectively, but did not affect the potential across the anterior portion of the excised eye or across the isolated neural retina. A high-bicarbonate solution principally depolarized the apical membrane of the retinal pigment epithelium (RPE) when applied basally, and hyperpolarized it when applied apically. These results suggest that the bicarbonate response in the cat is primarily due to the effects of an increase in bicarbonate concentration on the basal membrane of the RPE. **Jpn J Ophthalmol 1997;41:231–234** © 1997 Japanese Ophthalmological Society

Key Words: Bicarbonate, electro-oculogram, membrane potential, ocular standing potential, retinal pigment epithelium.

Introduction

The human ocular standing potential is lowered by an intravenous infusion of 7% sodium bicarbonate,[1] a treatment widely used for vertigo or to correct metabolic acidosis. This response, called the bicarbonate response, is sensitively suppressed in retinal diseases such as retinitis pigmentosa, rhegmatogenous retinal detachment, Grönblad-Strandberg syndrome, and Vogt–Koyanagi–Harada disease.[2] Because the ocular standing potential is generated mainly by the retinal pigment epithelial (RPE) layer, a reduction in the bicarbonate response might reflect a functional disorder of the RPE. However, the cellular origin of the response is still unknown. The present study explored the effects of a sodium bicarbonate infusion on the ocular standing potential and on the membrane potential of the RPE, and demonstrated the RPE origin of the bicarbonate response.

Received: March 4, 1996

Address correspondence and reprint requests to: Kazuo KAWASAKI MD, Department of Ophthalmology, Kanazawa University School of Medicine, 13-1 Takara-machi, Kanazawa, Ishikawa 920, Japan

Materials and Methods

In Vivo Experiments

Cats were anesthetized by intramuscular injection of ketamine hydrochloride (10–20 mg/kg; Ketalar®, Sankyo, Tokyo) and immobilized with intravenous tubocurarine chloride (0.8 mg/kg per hour; Amerizol®, Takeda Pharmaceutical, Osaka). All measurements were made with adequate artificial ventilation. The active electrode (a silver chloride wire in a 0.3 mm-diameter glass tube) was inserted into the vitreous; the reference electrode (a silver chloride 5.0 mm-diameter disc) was placed in the orbit behind the eyeball. Potentials between the intravitreal and retrobulbar electrodes were fed into a unity-gain pre-amplifier with a DC bandpass to 10 Hz (MEZ-7101, Nihon Kohden, Tokyo), and then DC amplified (AD-621G, Nihon Kohden). Maximum voltage drift was 1 mV/h. Responses were stored on an instrumentation tape recorder (NFR-3000, Sony, Tokyo) and traced on a chart recorder (SP-G6P, Riken Denshi, Tokyo). After a minimum of 40-minute dark adaptation, three solutions were infused in sequence: first, lactated Ringer's (solutes/mM: NaCl, 102.0; KCl, 4.0; sodium lactate, 28.0; $CaCl_2$, 1.5; pH 6.0–8.5; 300 mOsm/kg); then, 1.4% sodium bicarbonate (300

Jpn J Ophthalmol 41, 231–234 (1997)
© 1997 Japanese Ophthalmological Society
Published by Elsevier Science Inc.

0021-5155/97/$17.00
PII S0021-5155(97)00049-X

Jpn J Ophthalmol
Vol 41: 231–234, 1997

mOsm/kg); and, finally, lactated Ringer's again. The infusion rate was controlled at a constant 0.56 mL/kg per minute with an infusion pump (STC-503, Terumo, Tokyo). The dose of 1.4% sodium bicarbonate delivered over 5–8 minutes was 5.0–15.0 mL.

In Vitro Experiments

Under general anesthesia with ketamine hydrochloride (Ketalar®, 50 mg/kg, intramuscularly) and retrobulbar anesthesia (1.0 mL 2% lidocaine [Xylocaine, Fujisawa Pharmaceutical, Osaka]), the cat's eyeball was enucleated and hemisected into an anterior portion (cornea, lens, ciliary body, and part of the sclera) and a posterior portion (neural retina, RPE, choroid, and sclera).

The anterior portion was mounted between two 40-mL chambers, with an opening at each side of the corneal and ciliary surfaces. The chambers were screwed together to ensure watertightness and each was perfused at a flow rate of 25 mL/min. Silver chloride plate electrodes were connected to the baths immersing the corneal and ciliary surfaces via junction pots and salt agar bridges. The potential between the electrodes was amplified, stored, and recorded as in the in vivo experiments.

The posterior portion was dissected into the neural retina, the RPE-choroid tissue, and the sclera. The neural retina was carefully stripped from the RPE, placed on a nylon mesh, and inserted into a Ussing-type chamber consisting of two 3.0 mL baths separated by the mounted tissue.[3] Both sides of the tissue were bathed by separate perfusions at a flow rate of 10 mL/min. The potential between the vitreal and receptoral baths was measured as during the anterior portion perfusion described above.

The RPE-choroid tissue, obtained by careful removal of the neural retina and the sclera from the posterior portion, was also placed on a nylon mesh and inserted into a Ussing's chamber. The potential across the RPE-choroid tissue, composed almost entirely of the transepithelial potential (TEP),[3] was measured.

The membrane potentials of the RPE cell were also measured. A glass microelectrode filled with 3.0 mol/L KCl, with an impedance of 0–50 MΩ, was inserted into the RPE cell soma through the apical membrane. The potentials were fed to unity-gain amplifiers (MEZ-7101 or MWZ-8201, Nihon Kohden) and DC amplified (AVH-10 or RDU-5, Nihon Kohden). Responses were stored and recorded as before. The microelectrode gave the apical (V_{ap}) and basal (V_{ba}) membrane potentials in reference to the silver chloride plate electrode connected to the apical and basal baths via junction pots.

To measure tissue resistance, extrinsic transtissue current pulses (1.0 μA; 3.0 seconds) were supplied by a constant current source (SS-101J, Nihon Kohden) via another pair of silver chloride electrodes.

The control perfusion solution for the in vitro experiments contained the following solutes/mmol/L NaCl, 110.0; KCl, 3.6; $CaCl_2$, 0.5; $MgCl_2$, 0.5; $NaHCO_3$, 15.0; CH_3COONa, 23.0; glucose 20.0. The high-bicarbonate test solution contained: NaCl, 110.0; KCl, 3.6; $CaCl_2$, 0.5; $MgCl_2$, 0.5; $NaHCO_3$, 35.0; NaH_2PO_4, 3.0; glucose, 20.0. The pH of each solution was adjusted to 7.43 ± 0.02 by adding NaH_2PO_4, CH_3COOH, or NaOH. The perfusion solutions were maintained in airtight containers at 35°C.

Results

In Vivo Study

No changes in standing potential were observed in response to an infusion of lactated Ringer's solution (Figure 1, left hollow bar). Within 2 minutes after changing to the 1.4% sodium bicarbonate solution (Figure 1, filled bar), the standing potential began to decrease. At 11 minutes, it was 0.38 mV below the steady-state level prior to sodium bicarbonate exposure. When the infusion was changed back to the lactated Ringer's (Figure 1, right hollow bar), the potential returned to the pre-sodium bicarbonate level. The bicarbonate concentration and the arterial blood pH had increased from 17.0 mEq/L and 7.42, to 22.0 mEq/L and 7.50 at the end of the sodium bicarbonate perfusion. The experiment was repeated five times with identical results.

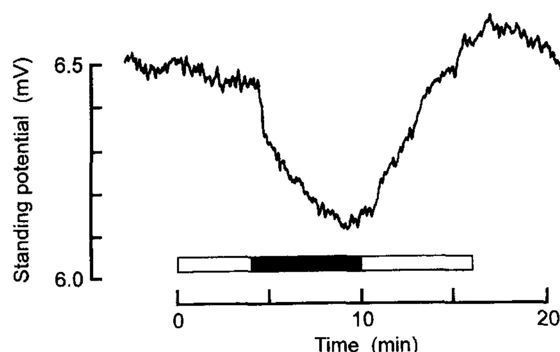

Figure 1. Decrease in cat ocular standing potential in response to intravenous administration of 1.4% sodium bicarbonate solution. Lactated Ringer's solution was infused (hollow horizontal bars) before and after 1.4% sodium bicarbonate solution (filled horizontal bar). Flow rate: 0.56 mL/min per kg.

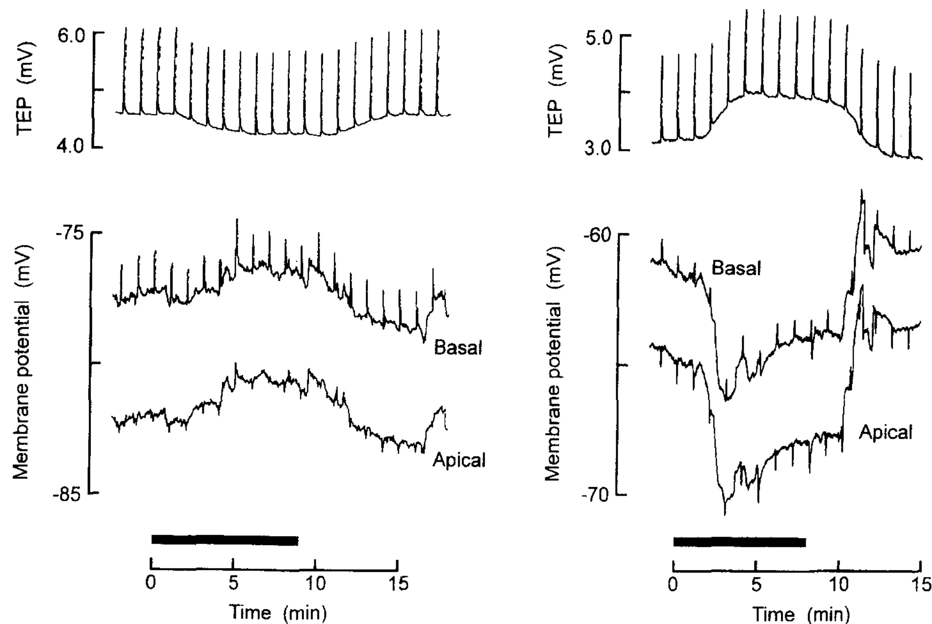

Figure 2. Changes in TEP and membrane potentials of RPE cell induced by raising bicarbonate concentration in basal bath (left) and apical bath (right). Isolated RPE-choroid tissue of the cat was used. Basal and apical bicarbonate concentration was raised from 15.0 mmol/L to 35.0 mmol/L during elapsed time shown by horizontal bar. Basal membrane potential was superimposed on apical membrane potential by amplitude of TEP. Spikey deflections were produced by extrinsic transtissue current pulses (1.0 μA, 3.0 seconds) to measure electrical resistance of tissue.

In Vitro Study

The potential across the anterior portion of the excised cat eye was unchanged when the bicarbonate concentration in the vitreal perfusion solution was increased (not shown). The potential across the neural retina was also unchanged by an increase in bicarbonate concentration of either (receptoral or vitreal) bath (not shown).

The TEP decreased or increased, respectively, in response to a high-bicarbonate solution in the basal or apical bath (Figure 2). These effects were confirmed in three additional RPE-choroid tissue experiments (not shown).

In four sessions, the TEP and the membrane potentials of the RPE were continuously monitored during solution exchanges. An increase in the bicarbonate concentration of the basal bath depolarized V_{ap} by 2.0 mV and V_{ba} by 1.6 mV (Figure 2), resulting in a decrease of 0.4 mV in the TEP (Figure 2). In contrast, an increase in the bicarbonate concentration of the apical bath hyperpolarized V_{ap} by 4.9 mV and V_{ba} by 4.1 mV (Figure 2), resulting in an increase of 0.8 mV in the TEP (Figure 2). The transtissue resistance was slightly decreased (1.54 kΩ–1.41 kΩ) by increasing the bicarbonate concentration in the basal bath, but an elevation of apical bicarbonate concentration did not significantly affect the transtissue resistance (1.49 kΩ) (Figure 2, spikes on upper tracings). Three repeat experiments produced similar results (not shown).

Discussion

In the in vivo study of the cat, the standing potential was decreased by the intravenous infusion of the 1.4% sodium bicarbonate solution (Figure 1). Because the sodium ion (Na^+) concentration in the solution (148 mmol/L) is almost equal to the Na^+ of the cat serum, we would expect the serum Na^+ concentration to be unaffected by the infusion, and that the sodium ion is not implicated in the decrease of the standing potential. However, because the arterial bicarbonate concentration (HCO_3^-) was markedly elevated after the infusion, an increase in HCO_3^-, or changes related to this, may be responsible for the decreased standing potential. Bicarbonate-related changes in both ocular standing potential and RPE membrane potential have been reported in cats with well-controlled pCO_2 and pH.[4–6]

The standing potential change may come from either the anterior portion, posterior portion, or both. However, because the in vitro experiments demonstrated that the potential across the anterior portion

Jpn J Ophthalmol
Vol 41: 231–234, 1997

did not respond to a high-bicarbonate solution on the ciliary side, it is clear that the anterior portion does not participate in a bicarbonate-induced decrease of standing potential.

The RPE-choroid tissue did respond to the high-bicarbonate solution (Figure 2, upper tracings). The polarity of the potential change was identical in a clinical bicarbonate response to intravenous bicarbonate administration,[1,2] our in vivo experiment in the cat (Figure 1), and the basal high-bicarbonate application to the in vitro RPE-choroid tissue of the cat (Figure 2, left). The most plausible explanation for these is that, in the cat as well as in the human eye, the bicarbonate concentration increases chiefly on the basal (choroidal) side of the RPE following intravenous infusion of high-bicarbonate solution.

Changes in the RPE membrane potentials in response to an apical high-bicarbonate application are easily explained by a change in the HCO_3^- equilibrium potential. In the frog, and other species, the apical membrane of the RPE permits passage of HCO_3^-; the HCO_3^- equilibrium potential is shifted negatively by an increase in extracellular HCO_3^- causing hyperpolarization of V_{ap}, concomitant passive hyperpolarization of V_{ba}, and a resultant increase in TEP.[3]

The mechanism of the change in RPE membrane potential in response to basal bicarbonate application needs further investigation. If the increased HCO_3^- acts directly on the bicarbonate conductance, if any, of the basal membrane, it should first hyperpolarize V_{ba}. We found just the opposite: A basal high-bicarbonate application depolarized V_{ba} (Figure 2, upper left tracing) and, to a greater extent, V_{ap} (Figure 2, lower left tracing). According to Steinberg and Miller,[3] if the membrane potential of one (apical or basal) side of the RPE is altered via some change in membrane conductance or a modulation of an electrogenic pump, the membrane potential of the other side is also altered in the same polarity via paracellular shunt resistance, but always to a lesser degree. If the paracellular shunt resistance is affected, the two membrane potentials must move in opposite directions. This did not occur in the basal high-bicarbonate application in the present study (Figure 2). Therefore, we conclude that the elevated bicarbonate concentration on the basal side acts chiefly on the apical membrane of the RPE. The underlying mechanism by which V_{ap} responds to a change of basal extracellular HCO_3^- is not clear; Keller et al[7] reported that bovine RPE has a pH-sensitive potassium conductance. The basal high-bicarbonate application might alter the intracellular pH of the RPE, resulting in a modification of some pH-sensitive conductance that may exist on the apical membrane.

References

1. Tanabe J, Segawa Y, Shirao Y, Kawasaki K, Fujii S. Bicarbonate-induced response from the retinal pigment epithelium as a clinical test. I: Normative data. Clin Vision Sci 1992;7:449–54.
2. Segawa Y. Electrical response of the retinal pigment epithelium to sodium bicarbonate. II: Clinical use for electrophysiological evaluation of the retinal pigment epithelium activity. J Juzen Med Soc 1987;96:1022–41.
3. Steinberg RH, Miller SS. Transport and membrane properties of the retinal pigment epithelium. In: Zinn KM, Marmor MF, eds. The retinal pigment epithelium. Cambridge: Harvard University Press, 1979:205–25.
4. Linsenmeier RA, Steinberg RH. Effects of hypoxia on potassium homeostasis and pigment epithelial cells in the cat retina. J Gen Physiol 1984;84:945–70.
5. Miller SS, Steinberg RH. Passive ionic properties of frog retinal pigment epithelium. J Membr Biol 1977;36:337–72.
6. Niemeyer G, Steinberg RH. Differential effects of pO_2 and pH on the ERG and light peak of the perfused cat eye. Vision Res 1984;24:275–80.
7. Keller SK, Jentsch TJ, Koch M, Wiederholt M. Interactions of pH and K^+ conductance in cultured bovine retinal pigment epithelial cells. Am J Physiol 1986;250:C124–37.