Bicarbonate Modulates Photoreceptor Guanylate Cyclase (ROS-GC) Catalytic Activity*

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Background: ROS-GCs generate cGMP and control phototransduction in rods and cones.

Results: Through a unique [Ca2+]i-independent mechanism, bicarbonate stimulates ROS-GC activity to increase circulating current, quicken flash responses, and reduce relative sensitivity.

Conclusion: Bicarbonate is a novel modulator of the photoreceptor ROS-GC.

Significance: Vision and certain forms of retinal diseases may be affected by the metabolic states of retinal cells.

By generating the second messenger cGMP in retinal rods and cones, ROS-GC plays a central role in visual transduction. Guanylate cyclase-activating proteins (GCAPs) link cGMP synthesis to the light-induced fall in [Ca2+]i, to help set absolute sensitivity and assure prompt recovery of the response to light. The present report discloses a surprising feature of this system: ROS-GC is a sensor of bicarbonate. Recombinant ROS-GCs synthesized cGMP from GTP at faster rates in the presence of bicarbonate with an ED₅₀ of 27 μM for ROS-GC1 and 39 μM for ROS-GC2. The effect required neither Ca²⁺ nor use of the GCAPs domains; however, stimulation of ROS-GC1 was more powerful in the presence of GCAP1 or GCAP2 at low [Ca²⁺]. When applied to retinal photoreceptors, bicarbonate enhanced the circulating current, decreased sensitivity to flashes, and accelerated flash response kinetics. Bicarbonate was effective when applied either to the outer or inner segment of red-sensitive cones. In contrast, bicarbonate exerted an effect when applied to the inner segment of rods but had little efficacy when applied to the outer segment. The findings define a new regulatory mechanism of the ROS-GC system that affects visual transduction and is likely to affect the course of retinal diseases caused by cGMP toxicity.

Retinal rods and cones begin the process of vision by converting light into an electrical signal, the biochemical process termed phototransduction. Within a highly specialized cilium known as the outer segment, a photon absorbed by a rhodopsin molecule turns on a transducin G protein that activates a cGMP phosphodiesterase. In darkness, cGMP holds open cyclic nucleotide-gated (CNG)³ ion channels through which cations enter the cell. By causing cGMP hydrolysis, light closes the CNG channels and the cell hyperpolarizes. The change in membrane potential spreads through the soma (inner segment) to the synaptic terminal, where it alters synaptic transmission.

The enzyme responsible for maintaining a basal level of cGMP and for restoring its level to end the photon response is the photoreceptor guanylate cyclase ROS-GC (reviewed in Refs. 1 and 2). Mammals express two isoforms, ROS-GC1 and ROS-GC2, whereas three forms are present in fish. Unlike hormone receptor membrane guanylate cyclases, ROS-GCs do not respond to extracellular ligands and contain an additional structural tail at the C terminus (see Figs. 1 and 7). ROS-GCs do, however, respond to changes in intracellular [Ca²⁺] with a neuronal calcium sensing subunit, guanylate cyclase activating protein (GCAP). Most vertebrates express two isoforms, GCAP1 and GCAP2, but some fish may express as many as eight (3). With Ca²⁺ bound, GCAPs slightly suppress ROS-GC activity. Following a light-induced decline in [Ca²⁺]i, GCAPs stimulate ROS-GC activity as part of a negative feedback loop that limits the size of the single photon response and quickens the recovery phase of the response.

Bicarbonate has been shown to increase the circulating current in rods and cones in the isolated amphibian retina (4, 5). Faster flash responses and lower sensitivity in rods of toad and monkey are also associated with bicarbonate (6–8). These changes could be produced by direct stimulation of cGMP synthesis, but the effect of bicarbonate on ROS-GC activity is controversial (9–11). Here, we systematically analyzed the effect of bicarbonate on ROS-GC1 and ROS-GC2 activities and tested for an interaction with GCAPs at low [Ca²⁺]. In addition, we studied the effect of bicarbonate on the flash responses of intact rods and cones. Some of the results have appeared previously in abstract form (70).

EXPERIMENTAL PROCEDURES

Measurement of Recombinant ROS-GC Activity—COS-7 cells were transfected with cDNA for bovine ROS-GC1, ROS-GC2, and GCAP3. Bicarbonate was effective when applied either to the outer or inner segment of red-sensitive cones. In contrast, bicarbonate exerted an effect when applied to the inner segment of rods but had little efficacy when applied to the outer segment. The findings define a new regulatory mechanism of the ROS-GC system that affects visual transduction and is likely to affect the course of retinal diseases caused by cGMP toxicity.
Mechanically dissociated retinas were loaded into an experimental chamber and perfused continuously with Ringer’s solution (58 mM NaCl, 2.5 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, 0.02 mM EDTA, 10 mM glucose, 5 mM HEPES, 5 or 10 mM MOPS, 55 mM sodium MOPS, pH 7.6, and 0.05 mg ml⁻¹ bovine serum albumin; Fraction V; Sigma). When desired, perfusion was switched to a solution containing 50 mM NaHCO₃ in place of an equimolar amount of sodium MOPS. In some experiments, the rod outer segment was perfused with a low Cl⁻ solution (108 mM NaCH₃SO₄, 2.5 mM KCH₃SO₄, 1 mM MgCl₂, 1.5 mM CaCl₂, 0.02 EDTA, 10 mM glucose, 10 mM HEPES, pH 7.5, and 0.05 mg ml⁻¹ bovine serum albumin). For these experiments, bicarbonate was introduced by substituting 30 mM NaHCO₃ for equimolar NaCH₃SO₄. The effects of elevated pH were evaluated by perfusing the bath with 108 mM NaCl, 2.5 mM KCl, 1 mM MgCl₂, 1.5 mM CaCl₂, 0.02 mM EDTA, 10 mM glucose, 10 mM HEPES, pH 7.6 or 8.1, and 0.05 mg ml⁻¹ bovine serum albumin. TAPS replaced HEPES for experiments at pH 8.5, and CHES was used for experiments at pH 8.8. The outer or inner segment of a photoreceptor was pulled into a polished, silanized glass pipette that was filled with the HEPES-buffered Ringer’s solution, pH 7.6, without albumin. These recording configurations will be referred to as OS-in and IS-in, respectively. The position of the cell in the electrode was monitored closely with a closed circuit, infrared television system because movement into or out of the electrode would change the measured circulating current (17).

Light from an electronically shuttered xenon source passing through a six-cavity interference filter (Omega Optical, Brattleboro, VT) was used to stimulate the cells. The light was calibrated with a digital photometer (UDT 350; Graseby). Signals were recorded at room temperature (21–23 °C) with a current to voltage converter (Axopatch 200B; Axon Instruments), low pass filtered with an 8-pole Bessel filter at 30 Hz ( –3 dB; Frequency Devices, Haverhill, MA), and digitized at 400 Hz. Cell type was identified by visual inspection and by the responses to flashes: at 512 and 618 nm; at 378, 434, and 599 nm; or at 434, 500, and 540 nm.

**RESULTS**

**Bicarbonate as a Signal for Catalytic Activation of ROS-GC**—We first verified the original observation that bicarbonate stimulates the catalytic activity of ROS-GC1 (11). COS cells were induced to transiently express bovine ROS-GC1 or ROS-GC2, and their membranes were assayed for cGMP synthesis. There was indeed a dose-dependent increase in activity of up to 4.7-fold with bicarbonate (Fig. 2A). For ROS-GC1, the $ED_{50}$ was 27 mM with a Hill coefficient of 2.8, similar to results reported previously (11). ROS-GC2 activity was stimulated 4.1-fold, although it was somewhat less sensitive, with an $ED_{50}$ of 39 mM and a Hill coefficient of 2.3.

Bicarbonate solutions left open to the air become alkaline over time because of the formation and release of CO₂. To ensure that the increases in ROS-GC activity were not due to a change in pH, two control experiments were carried out. First, the pH of assay mixture samples containing final concentrations of 0–100 mM bicarbonate in 20 mM increments was monitored at 37 °C. The pH remained constant at 7.5 ± 0.02 after an hour, a duration that was substantially longer than the 10-min
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**FIGURE 2. Bicarbonate boosted ROS-GC activity.** Lines show fits with a Hill function: ROS-GC activity = minimal activity + (maximal activity-minimal activity)\((\text{[HCO}_3^-]\text{)}^{n}/(\text{[HCO}_3^-]^{n} + \text{ED}_{50}^{n})\), A, ROS-GC1 activity increased 4.7-fold with bicarbonate, with a Hill coefficient of 2.8 and an ED$_{50}$ of 27 mM, whereas ROS-GC2 activity increased 4.1-fold with a Hill coefficient of 2.3 and an ED$_{50}$ of 39 mM. All assays were done in triplicate. ROS-GC1 was tested three times, and the results shown are the means ± S.E. ROS-GC2 was tested once. B, ROS-GC activity was independent over the pH range 7–9. ROS-GC1 (circles) and ROS-GC2 (diamonds) were each assayed in triplicate, using membranes from two independent cell transfections (open and filled symbols, respectively). C, similar bicarbonate-dependent activities of ROS-GC1 mutants. Activity was plotted relative to that in the absence of bicarbonate for each mutant. For the mutant lacking ExtD, ED$_{50}$ was 41 mM, the Hill coefficient was 2.0, and maximal activity rose 5.9-fold. For the mutant lacking JmD and KHD, ED$_{50}$ was 38 mM, the Hill coefficient was 2.1, and maximal activity increased 5.4-fold. Specific activities of mutants in the absence of bicarbonate were 93 pmol cGMP min$^{-1}$ (mg prot)$^{-1}$ for the mutant lacking ExtD, for the mutant lacking JmD and KHD, and for the mutant lacking ExtD, JmD, and KHD, respectively (n = 3 for each mutant).

incubation period in our assays. Second, the activities of recombinant ROS-GC1 and ROS-GC2 in COS membranes were determined at pH 7, 7.5, 8, 8.5, and 9 (Fig. 2B). There were no changes, consistent with the insensitivity of ROS-GCs solubilized from bovine retina to changes in pH from 7 to 9 that was reported previously (18).

**Localization of ROS-GC1 Domain Involved in Bicarbonate Signaling**—ROS-GC is a single transmembrane-spanning protein, composed of modular blocks: extracellular domain (ExtD), transmembrane domain, juxtamembrane domain (JmD), kinase homology domain (KHD), signaling helix domain, core catalytic domain (CCD), and a C-terminal extension. For other membrane guanylate cyclases, the ExtD is known to bind ligands such as natriuretic peptides (ANF (atrial natriuretic factor), BNP (B-type natriuretic peptide), and CNP (C-type natriuretic peptide)) or enterotoxin (reviewed in Ref. 1). To test whether bicarbonate binds the ExtD or neighboring domains, abridged versions of ROS-GC1 lacking the ExtD, JmD, and KHD or all three domains were expressed in COS cells for testing (Fig. 1). All three mutants retained sensitivity to bicarbonate with ED$_{50}$ values of ~30–40 mM, Hill coefficients of 2 and a maximal stimulation of nearly 6-fold (Fig. 2C). This bicarbonate does not bind to and signal from the ExtD, JmD, nor the KHD of ROS-GC1. Moreover, none of these domains is required for stimulation of activity by bicarbonate. By exclusion, the ROS-GC1 domain(s) required for bicarbonate signaling reside between Leu$^{770}$ and Lys$^{1054}$, where the numbering corresponds to mature bovine ROS-GC1 (19).

**Influence of GCAPs on Bicarbonate Signaling**—Because GCAPs and bicarbonate both modulate ROS-GC activity, we asked whether they act independently. To find out, membranes of COS cells expressing ROS-GC1 or ROS-GC2 were incubated with 50 mM bicarbonate and increasing concentrations of either GCAP1 or GCAP2 at low [Ca$^{2+}$] in assays for cGMP synthesis. The presence of GCAP1 enhanced the effect of bicarbonate on GC activity (Fig. 3A), indicating that the two factors operated synergistically rather than additively. GCAP2 had an even greater impact (Fig. 3B). In control experiments carried out in the absence of GCAPs, ROS-GC1 activity was indifferent to the addition of 1 mM EGTA to essentially reduce [Ca$^{2+}$] to 0 or to 100 μM Ca$^{2+}$ (Fig. 3C).

Other groups failed to observe bicarbonate stimulation of recombinant ROS-GCs (9, 10). Because different expression systems were used, their enzymes may have been subject to different post-translational modifications or may have existed in complexes with alternative protein binding partners. It was therefore important to test native ROS-GC from retinal photoreceptors. WT mouse outer segment membranes express a mixture of ROS-GC1 and ROS-GC2 complexed with GCAP1 and GCAP2. Nrl$^{-/-}$ mouse outer segments restrict expression to ROS-GC1 and GCAP1 (20–21). The results confirmed bicarbonate sensitivity of native ROS-GCs (Fig. 4). For Nrl$^{-/-}$, the ED$_{50}$ was 47 mM, whereas for WT, the ED$_{50}$ was somewhat higher, consistent with the involvement of a second ROS-GC that was less sensitive to bicarbonate. Overall maximal stimulation of GC activity at high bicarbonate concentration determined from three separate experiments was 8.6 ± 0.6-fold (means ± S.E.) for Nrl$^{-/-}$ and 10.3 ± 0.8-fold for WT.
We infer that the greater maximal stimulation of the ROS-GC mixture from WT mouse compared with that of ROS-GC1 from Nrl/H11002/H11002 (Fig. 4) was related to GCAP2 expression. First, bicarbonate stimulation of recombinant bovine ROS-GC1 was similar to that of ROS-GC2 (Fig. 2A). Second, bicarbonate exerted a more powerful effect on ROS-GC with GCAP2 (Fig. 3), and WT rods express comparable levels of GCAP1 and 2, whereas Nrl/H11002/H11002 photoreceptors express little, if any, GCAP2.

Impact of Bicarbonate on the Circulating Current of Rods and Cones—In photoreceptors, a higher basal rate of ROS-GC activity raises intracellular [cGMP] and opens more CNG channels to increase the circulating current. So when probed with a bright flash, the maximal, saturating response amplitude is larger. In our initial recordings from salamander photoreceptors attached to pieces of retina with outer segment inside the pipette (OS-in), switching the inner segment perfusion from Ringer’s solution buffered with 30 mM phosphate to one containing 30 mM bicarbonate increased the response to a bright, saturating flash in all five cones tested by 52% and in all three rods tested by 18%. Maximal response amplitude increased by 59% in two of two rods exposed to 50 mM bicarbonate. However, high phosphate can precipitate Ca2+ in the Ringer’s solution (22–23). Exposure of the outer segment to low extracellular [Ca2+] would in turn reduce intracellular levels, accelerate ROS-GC activity, and raise the affinity of the CNG channel for cGMP. Thus the use of phosphate as a “control” might artificially marginalize the effects of bicarbonate. Fortuitously, when the outer segment is sequestered inside the pipette during OS-in recording, exposure of the inner segment to low [Ca2+] increases the circulating current by only a few percent (24). Another issue was that the bicarbonate solution was slightly unstable, and pH sometimes rose as high as 8.3 during experiments that lasted many hours. As will be shown below, circulating current rose with pH, but the effects were not as great as those produced by bicarbonate.

Therefore, despite the less than ideal conditions imposed by using phosphate-buffered Ringer’s solution, there was an increased maximal response amplitude in bicarbonate that was at least partially attributable to a direct action of bicarbonate on the ROS-GC activity. Nevertheless, both complications were circumvented by replacing phosphate with the “Good” buffer, MOPS (23), for all subsequent experiments on salamander photoreceptors.

With OS-in recordings, the maximal, saturating response was enlarged by 50 mM bicarbonate in every retina-attached rod and cone (Fig. 5, A and D). On average, the maximal response became 12% larger in rods. The average increase in seven retina-attached and in three isolated red-sensitive cones was 12% larger in rods.
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was 26 ± 6%. This robust effect on rods and cones was reversed after washing with Ringer’s solution and was reinstated in one rod and in three cones when bicarbonate was applied a second time.

Regional Uptake of Bicarbonate in Rods but Not Cones—When isolated salamander cones were recorded with the inner segment inside the pipette (IS-in), perfusion of their outer segments with 50 mM bicarbonate invariably increased circulating current by an average of 31 ± 10% (n = 4) (Fig. 6A). In contrast, maximal response did not increase with bicarbonate in IS-in recordings from four isolated rods (3 ± 3%, Fig. 6C), consistent with previous reports (4, 25). However, circulating current in three of these rods increased 22 ± 2% with 30 mM bicarbonate applied to their outer segments when external Cl⁻ was lowered to reverse the direction of operation of the HCO₃⁻/Cl⁻ exchanger (Fig. 6D), similar to the findings of Ref. 26. Flash response kinetics in rods were unchanged or may have slowed slightly in low Cl⁻.

Dynamic Action of Bicarbonate during the Flash Response—Because the time course of the photon response depends on restoration rate of cGMP levels, the powerful bicarbonate stimulation of ROS-GC activity with GCAPs present at low [Ca²⁺] could accelerate flash response recovery. The expectation was upheld; in salamander rods recorded with OS-in, bicarbonate reduced the integration time of the dim flash response, given as the response integral divided by peak amplitude, by ~29 ± 10% (n = 5), indicating faster recovery. Time to peak may have shortened by ~9 ± 3% (Fig. 5B). Washing the cell with Ringer’s solution to remove the bicarbonate slowed response recovery. In red-sensitive cones recorded with OS-in or with IS-in, changes in flash response kinetics were less consistent. Response recovery was faster in six cells (Fig. 6B) but was slower in three others.

The response to a dim flash in rods and cones did not generally increase in proportion to the saturating flash response with bicarbonate. In many cases, the dim flash response became smaller. Thus for most cells, relative sensitivity was lower (Fig. 5, C and E). On average, the flash strength that produced a half-maximal response, i₀.₅, was 1.4 ± 0.2-fold higher in five rods and 1.8 ± 0.2-fold higher in eight cones.

Dependence of Circulating Current on pH—Previously, it was proposed that the bicarbonate-induced increase in circulating current arises from intracellular alkalinization (4, 27). Here we show that elevating pH does not reproduce all of the physiological effects of bicarbonate on rods described above.

In experiments on five rods with OS-in, raising extracellular pH around the inner segment from 7.6 to 8.1 increased the circulating current by 9 ± 3%. Circulating current was 10 ± 3% (n = 4) higher at pH 8.5 (Fig. 7, A and C). These results are broadly consistent with those of Liebman et al. (25), who reported a ~10% increase in circulating current in rods per-
In contrast to the decrease in relative sensitivity to flashes observed with bicarbonate treatment, elevated pH produced a very slight increase in relative sensitivity (Fig. 7E) in nine of ten rods. The $i_{0.5}$ was 1.17 ± 0.05-fold lower at pH 8.1 ($n$ = 3), 1.12 ± 0.07-fold lower at pH 8.5 ($n$ = 3), and 1.33 ± 0.04-fold lower at pH 8.8 ($n$ = 4). These effects on sensitivity differ from the ~2.5-fold loss in sensitivity at pH 10.5 that was reported by Liebman et al. (25). Moreover, the accelerated dim flash response recovery observed by Liebman et al. (25) at pH 10.5 was not observed over the more limited pH range in our study.

DISCUSSION

Although bicarbonate stimulates cGMP synthesis by the odorant uroguanylin receptor guanylate cyclase ONE-GC (also referred to as GC-D) in some olfactory neurons (11, 29), there were conflicting reports about its effects on ROS-GCs. The cGMP content of physiologically active photoreceptors in amphibian retina increased upon exposure to 6 to 24 mM bicarbonate (4, 30, 31). Bicarbonate enhanced cGMP synthetic activity in membranes of COS cells transiently induced to express ROS-GC1 with an ED$_{50}$ of 30 mM (11); yet 24 mM bicarbonate did not raise the ROS-GC activity of isolated toad rod outer segments (31), and 40 mM bicarbonate had no effect on cGMP synthesis in CHO cells permanently expressing ROS-GC1 or ROS-GC2 (9). Fifty mM bicarbonate actually inhibited cGMP synthesis by ROS-GC1 and by ROS-GC2 when they were expressed separately in HEK-293T cells (10).

The present study confirms bicarbonate stimulation of ROS-GC1 activity and extends the observations to ROS-GC2 (Fig. 2A). It further demonstrates bicarbonate stimulation of native ROS-GCs that form complexes with neuronal Ca$^{2+}$-sensing subunits, GCAP1 or GCAP2. In mammalian photoreceptors ROS-GC1 is the predominant guanylate cyclase, constituting ~80% in WT mouse rod outer segments (32) and more than 90% in those of bovine (33). In the all-cone retina of mutant Nrl$^{-/-}$ mouse, ROS-GC1 is expressed almost exclusively and is bound to GCAP1 (20) and another Ca$^{2+}$ sensor, S100B (15). The shift in ED$_{50}$ to higher bicarbonate concentrations in ROS-GC activity from WT mouse compared with that from Nrl$^{-/-}$ mouse (Fig. 4) was evidence that bicarbonate stimulated both ROS-GC1 and ROS-GC2, the latter being less sensitive to bicarbonate. In that regard, native mouse ROS-GC1 and ROS-GC2 were either less sensitive to bicarbonate than the recombinant bovine counterparts or the presence of GCAP raised the ED$_{50}$ of ROS-GCs for bicarbonate. The contradictory results reported by others may have arisen from differences in post-translational processing, alternative protein binding partners in the ROS-GC complex, or difficulties in bicarbonate accessing the appropriate binding domain in ROS-GC in their respective systems.

ROS-GCs possess the hallmark features of the membrane guanylate cyclase family; they are composed of modular blocks arranged into a single transmembrane-spanning polypeptide chain that forms a homodimer (Fig. 8). Mutagenesis experiments on ROS-GC1 indicated that bicarbonate does not bind the ExtD, nor does it bind to neighboring domains (Fig. 2C). Through an analysis of deletion constructs, bicarbonate binding in ONE-GC was mapped to a cytoplasmic site (9, 10), spe-
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Specifically, to the cytoplasmic CCD in a region that does not overlap the \textit{LSPEI} \textit{913} motif critical for neurocalcin \textit{\delta} regulation (11). Because this part of the ONE-GC CCD shares more than 86% sequence identity with the corresponding Tyr\textit{858}–\textit{Pro}\textit{864} segment of ROS-GC1 and Tyr\textit{913}–\textit{Pro}\textit{1093} segment of ROS-GC2, we predict that bicarbonate also stimulates ROS-GCs at their CCDs (Fig. 8). The presence of a single bicarbonate binding site per monomer is consistent with the Hill coefficients near 2 in the dose-response relations for ROS-GC1 and ROS-GC2 (Figs. 2, A and C; 3C; and 4).

All cells generate CO\textsubscript{2}, some of which will convert spontaneously to bicarbonate. The reaction is greatly accelerated by carbonic anhydrases, which are among the most powerful enzymes in the body. In retina, carbonic anhydrases are expressed heavily in Müller cells and retinal pigment epithelium. There is also lesser expression in a subset of cones, as well as in horizontal cells and amacrine cells of some species (34–37). Bicarbonate is charged, so unlike CO\textsubscript{2}, it does not easily traverse the plasma membrane. Instead, its movement into or out of cells relies on anion exchangers, Na\textsuperscript{+} coupled co-transporters, certain metal transporters, anion channels, and gap junctions.

The lack of effect of bicarbonate applied to the outer segments of rods (Fig. 6C) suggests that uptake occurs at their inner segments, through the action of sodium bicarbonate cotransporters (38, 39) and through gap junctions and Cl\textsuperscript{−} channels (40) located at the synapse (41, 42). Bicarbonate then diffuses to the outer segment, where it is extruded by an HCO\textsubscript{3}/Cl\textsuperscript{−} exchanger (26). Muller cells and pigment epithelial cells remove the bicarbonate from the retina. Less is known about cones. Interestingly, red- and green-sensitive cones but not blue-sensitive cones express carbonic anhydrase in their outer segments (43). The susceptibility of red-sensitive cones to bicarbonate applied to their outer segments in this study is unclear. It could arise from CO\textsubscript{2} in equilibrium with bicarbonate diffusing across the membrane and being converted back to bicarbonate.

The magnified circulating current with bicarbonate arises from the combination of an indirect effect of raising intracellular pH and a direct effect of stimulating ROS-GC activity that ultimately results in a larger, saturating response amplitude (Figs. 5, A and D, and 6A). These effects may form the basis for the larger photoreceptor response in the ERG of the isolated retina treated with bicarbonate (4, 5). Lowered endogenous levels of bicarbonate explain the reduced photoreceptor response in the ERGs of mutant mice deficient for carbonic anhydrase (47) and of human subjects and animal models treated with carbonic anhydrase inhibitors (48–50).

The synergistic action on ROS-GCs of bicarbonate with GCAPs when intracellular [Ca\textsuperscript{2+}] is low (44–46).
Inherited defects in ROS-GC1, GCAP1, PDE6, and AIPL1 that abnormally raise cGMP lead to blinding retinal degenerations (reviewed in Refs. 58 – 61). Excessive levels of cGMP open too many CNG channels, and the elevated influx of Ca\(^{2+}\) triggers apoptotic cell death. Mutations that render the CNG channel hypersensitive to normal levels of cGMP also cause degeneration (62, 63). It is now evident that elevated cGMP is toxic on its own, even without channel involvement (21). Extreme physiological fluctuations in bicarbonate levels, e.g. during under-water diving, may raise cGMP production in photoreceptors and place them at risk in normal persons. Furthermore, high bicarbonate levels would exacerbate pathology in the aforementioned inherited retinal degenerations. Comorbidity of disturbances in bicarbonate transport in the retina may be especially harmful. Inhibition of bicarbonate production, e.g. by carbonic anhydrase inhibitors, may be therapeutic in attenuating the disease process. A note of caution is that carbonic anhydrase inhibitors may be detrimental to some patients and carriers of recessive retinal disease in which there is insufficient production of cGMP (64).

In summary, this study marks the initial biochemical and physiological characterization of a novel phototransduction-linked ROS-GC signal transduction pathway. In contrast to all other ROS-GC signal transductions, the bicarbonate pathway is Ca\(^{2+}\)-independent. However, via its extraordinary mode of regulation, it introduces an external influence on the conventional rod and cone vision-linked systems. The future task will be to expand upon the basic molecular principles of this pathway and to explore clinical applications.

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REFERENCES

1. Sharma, R. K., and Duda, T. (2014) Membrane guanylate cyclease, a multimodal transduction machine: history, present, and future directions. Front. Mol. Neurosci. 7, 56
2. Wen, X.-H., Dizhoor, A. M., and Makino, C. L. (2014) Membrane guanylyl cyclease complexes shape the photoresponses of retinal rods and cones. Front. Mol. Neurosci. 7, 45
3. Imanishi, Y., Yang, L., Sokal, I., Filipek, S., Palczewski, K., and Baehr, W. (2004) Diversity of guanylate cyclease-activating proteins (GCAPs) in teleost fish: characterization of three novel GCAPs (GCAP4, GCAP5, GCAP7) from zebrafish (Danio rerio) and prediction of eight GCAPs (GCAP1–8) in pufferfish (Fugu rubripes). J. Mol. Evol. 59, 204–217
4. Donner, K., Hemilä, S., Kalamkarov, G., Koskelainen, A., and Shevchenko, T. (1990) Rod phototransduction modulated by bicarbonate in the frog retina: roles of carbonic anhydrase and bicarbonate exchange. J. Physiol. 426, 297–316
5. Koskelainen, A., Donner, K., Lerber, T., and Hemilä, S. (1993) pH regulation in frog cones studied by mass receptor photoresponses from the isolated retina. Vision Res. 33, 2181–2188
6. Lamb, T. D., McNaughton, P. A., and Yau, K.-W. (1981) Spatial spread of activation and background desensitization in toad rod outer segments. J. Physiol. 319, 463–496
7. Lamb, T. D. (1984) Effects of temperature changes on toad rod photocurrents. J. Physiol. 346, 557–578
8. Baylor, D. A., Nunn, B. J., and Schnapf, J. L. (1984) The photocurrent, noise and spectral sensitivity of rods of the monkey, Macaca fascicularis. J. Physiol. 357, 575–607
9. Guo, D., Zhang, J. J., and Huang, X.-Y. (2009) Stimulation of guanylyl cyclase-D by bicarbonate. Biochemistry 48, 4417–4422
10. Sun, L., Wang, H., Hu, J., Han, J., Matsunami, H., and Luo, M. (2009) Guanylyl cyclase-D in the olfactory CO\(_2\) neurons is activated by bicarbonate. Proc. Natl. Acad. Sci. U.S.A. 106, 2041–2046
11. Duda, T., and Sharma, R. K. (2010) Distinct ONE-GC transduction modes and motifs of the odorants: uroguanin and CO\(_2\). Biochem. Biophys. Res. Commun. 391, 1379–1384
12. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., pp. 161–161, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
13. Duda, T., Pertzev, A., and Sharma, R. K. (2011) Distinctive bicarbonate-binding motifs of the photoreceptor ROS-GC1: a general phototransduction switch. Biochem. Biophys. Res. Commun. 408, 236–241
14. Nambi, P., Aiyar, N. V., and Sharma, R. K. (1982) Adrenocorticotropic hormone-dependent particulate guanylate cyclase in rat adrenal and adrenocortical carcinoma: comparison of its properties with soluble guanylate cyclase and its relationship with ACTH-induced steroidogenesis. Arch. Biochem. Biophys. 217, 638–646
15. Fen, X.-H., Duda, T., Pertzev, A., Venkataraman, V., Makino, C. L., and Sharma, R. K. (2012) S100B serves as a Ca\(^{2+}\) sensor for ROS-GC guanylate cyclase in cones but not in rods of the murine retina. Cell. Physiol. Biochem. 29, 417–430
16. Makino, C. L., Groesbeek, M., Lugtenburg, J., and Baylor, D. A. (1999) Spectral tuning in salamander visual pigments studied with dihydroretinylidene chromophores. Biophys. J. 77, 1024–1035
17. Baylor, D. A., Lamb, T. D., and Yau, K.-W. (1979) The membrane current of single rod outer segments. J. Physiol. 288, 589–611
18. Fleischmann, D., and Denisovich, M. (1979) Guanylate cyclase of isolated bovine retinal rod axonemes. Biochemistry 18, 5060–5066
19. Goraczniak, R. M., Duda, T., Sitaramayya, A., and Sharma, R. K. (1994) Structural and functional characterization of the rod outer segment membrane guanylate cyclase. Biochem. J. 302, 455–461
20. Brooks, M. J., Rajasimha, H. K., Rogers, J. M., and Swaroop, A. (2011) Next-generation sequencing facilitates quantitative analysis of wild-type and Nrl\(^{-/-}\) retinal transcriptomes. Mol. Vis. 17, 3034–3054
21. Xu, J., Morris, L., Thapa, A., Ma, H., Michalakis, S., Biel, M., Baehr, W., Peshenko, I. V., Dizhoor, A. M., and Ding, X.-Q. (2013) cGMP accumulation causes photoreceptor degeneration in CNG channel deficiency: evidence of cGMP cytotoxicity independently of enhanced CNG channel function. J. Neurosci. 33, 14939–14948
22. Good, N. E., Winget, G. D., Winter, W., Connolly, T. N., Izawa, S., and Singh, R. M. (1966) Hydrogen ion buffers for biological research. Biochemistry 5, 467–477
23. Ferguson, W. J., Braunschweiger, K. I., Braunschweiger, W. R., Smith, J. R., McCormick, J. J., Wasmann, C. C., Jarvis, N. P., Bell, D. H., and Good, N. E. (1980) Hydrogen ion buffers for biological research. Anal. Biochem. 104, 300–310
24. Jin, J., Jones, G. J., and Cornwall, M. C. (1994) Movement of retinal along cone and rod photoreceptors. Vis. Neurosci. 11, 389–399
25. Liebman, P. A., Mueller, P., and Pugh, E. N., Jr. (1984) Protons suppress the dark current of frog retinal rods. J. Physiol. 347, 85–110
26. Koskelainen, A., Donner, K., Kalamkarov, G., and Hemilä, S. (1994) Changes in the light-sensitive current of salamander rods upon manipulation of putative pH-regulating mechanisms in the inner and outer segment. Vision Res. 34, 983–994
27. Kalamkarov, G., Pogozheva, I., Shevchenko, T., Koskelainen, A., Hemila, S., and Donner, K. (1996) pH changes in frog rods upon manipulation of putative pH-regulating transport mechanisms. Vision Res. 36, 3029–3036
28. Sampath, A. P., and Baylor, D. A. (2002) Molecular mechanism of spontaneous pigment activation in retinal cones. Biophys. J. 83, 184–193
29. Duda, T., and Sharma, R. K. (2008) ONE-GC membrane guanylate cyclase, a trimodal odorant signal transducer. Biochem. Biophys. Res. Commun. 367, 440–445
30. Meyertholen, E. P., Wilson, M. J., and Ostroy, S. E. (1980) Removing bicarbonate/CO\(_2\) reduces the cGMP concentration of the vertebrate photoreceptor to the levels normally observed on illumination. Biochem. Bio-

Bicarbonate Modulates Photoreceptor ROS-GC
Bicarbonate Modulates Photoreceptor ROS-GC

phys. Res. Commun. 96, 785–792

31. Meyertholen, E. P., Wilson, M. J., and Ostro, S. E. (1986) The effects of HEPES, bicarbonate and calcium on the cGMP content of vertebrate rod photoreceptors and the isolated electrophysiological effects of cGMP and calcium. Vision Res. 26, 521–533

32. Peshenko, I. V., Olshesvksaya, E. V., Savchenko, A. B., Karan, S., Palczewski, K., Baehr, W., and Dizhooor, A. M. (2011) Enzymatic properties and regulation of the native isoforms of retinal membrane guanylyl cyclase (RetGC) from mouse photoreceptors. Biochemistry 50, 5590–5600

33. Hwang, J.-Y., Lange, C., Helten, A., Höppner-Heitmann, D., Duda, T., Sharma, R. K., and Koch, K.-W. (2003) Regulatory modes of rod outer segment membrane guanylate cyclase differ in catalytic efficiency and Ca2+-sensitivity. Eur. J. Biochem. 270, 3814–3821

34. Musser, G. L., and Rosen, S. (1973) Localization of carbonic anhydrase activity in the vertebrate retina. Exp. Eye Res. 15, 105–119

35. Musser, G. L., and Rosen, S. (1973) Carbonic anhydrase activity in primate photoreceptors. Exp. Eye Res. 15, 467–470

36. Parthe, V. (1981) Histochemical localization of carbonic anhydrase in vertebrate nervous tissue. J. Neurosci. Res. 6, 119–131

37. Wistrand, P. J., Schenholm, M., and Lönnerholm, G. (1986) Carbonic anhydrase isoenzymes CA I and CA II in the human eye. Invest. Ophthalmol. Vis. Sci. 27, 419–428

38. Bok, D., Galbraith, G., Lopez, I., Woodruff, M., Nusinowitz, S., Beltrandel-Rio, H., Huang, W., Zhao, S., Geske, R., Montgomery, C., Van Sligtenhorst, I., Friddle, C., Platt, K., Sparks, M. J., Pushkin, A., Abuladze, N., Ishiyama, A., Dukkipati, R., Liu, W., and Kurtz, I. (2003) Blindness and auditory impairment caused by loss of the sodium bicarbonate cotransporter NBC3. Nat. Genet. 34, 313–319

39. Kao, L., Kurtz, L. M., Shao, X., Papadopoulos, M. C., Liu, L., Bok, D., Nusinowitz, S., Chen, B., Stella, S. L., Andre, M., Weinreb, J., Luong, S. S., Piri, N., Kwong, J. M., Newman, D., and Kurtz, I. (2011) Severe neurologic impairment in mice with targeted disruption of the electrogenic sodium bicarbonate cotransporter NBCe1 (Slc4a5 gene). J. Biol. Chem. 286, 32563–32574

40. Qu, Z., and Hartzell, H. C. (2000) Anion permeation in Ca2+-sensitive Cl- channels. J. Gen. Physiol. 116, 825–844

41. MacLeish, P. R., and Nurse, C. A. (2007) Ion channel compartments in photoreceptors: evidence from salamander rods with intact and ablated terminals. J. Neurophysiol. 98, 86–95

42. Stöhr, H., Heisig, J. B., Benz, P. M., Scholz, M. L., Strauss, O., Aarten, W. M., Wijnholds, J., Weber, B. H., and Schulz, H. L. (2009) TMEM16B, a novel protein with calcium-dependent chloride channel activity, associates with a presynaptic protein complex in photoreceptor terminals. J. Neurosci. 29, 6809–6818

43. Nork, T. M., McCormick, S. A., Chao, G.-M., and Odom, J. V. (1990) Distribution of carbonic anhydrase among human photoreceptors. Invest. Ophthalmol. Vis. Sci. 31, 1451–1458

44. Laser, H. (1937) CCIX. Tissue metabolism under the influence of low oxygen tension. Biochem. J. 31, 1671–1676

45. Craig, F. N., and Beecher, H. K. (1943) The effect of carbon dioxide tension on tissue metabolism (retina). J. Gen. Physiol. 26, 473–478

46. Winkler, B. S., and Riley, M. V. (1977) Na+ - K+ and HCO3- ATPase activity in retina: dependence on calcium and sodium. Invest. Ophthalmol. Vis. Sci. 16, 1151–1154

47. Ogilvie, J. M., Ohlemiller, K. K., Shah, G. N., Ullasow, B., Becker, T. A., Waheed, A., Hennig, A. K., Lukasiewicz, P. D., and Sly, W. S. (2007) Carbonic anhydrase IV deficiency produces a functional defect in the retinal light response. Proc. Natl. Acad. Sci. U.S.A. 104, 8514–8519

48. Broeders, G. C., Parmer, R., and Dawson, W. W. (1988) Electoretinal changes in the presence of a carbonic anhydrase inhibitor. Ophthalmologica 196, 103–110

49. Odom, J. V., Nork, T. M., Schroeder, B. M., Cavender, S. A., van Slycken, S., and Leys, M. (1994) The effects of acetazolamide in albino rabbits, pigmented rabbits, and humans. Vision Res. 34, 829–837

50. Findl, O., Hansen, R. M., and Fulton, A. B. (1995) The effects of acetazolamide on the electoretinographic responses in rats. Invest. Ophthalmol. Vis. Sci. 36, 1019–1026

51. Hetling, J. R., and Pepperberg, D. R. (1999) Sensitivity and kinetics of mouse rod flash responses determined in vivo from paired-flash electroretinograms. J. Physiol. 516, 593–609

52. van Hateren, J. H., and Lamb, T. D. (2006) The photocurrent response of human cones is fast and monophasic. BMC Neurosci. 7, 34

53. Heikkinen, H., Vinberg, F., Pitkänen, M., Kommonen, B., and Koskelainen, A. (2012) Flash responses of mouse rod photoreceptors in the isolated retina and corneal electroretinogram: comparison of gain and kinetics. Invest. Ophthalmol. Vis. Sci. 53, 5653–5664

54. Schwarz, R. K., and Maumenee, I. (2002) Electroretinographic abnormalities in parents of CNGB3 affected individuals. Invest. Ophthalmol. Vis. Sci. 43, 201–210

55. van Hateren, J. H., and Lamb, T. D. (2006) The photocurrent response of the mouse photoreceptor sensory ciliary complex. Mol. Cell. Proteomics 6, 1299–1317

56. Kwok, M. C., Holopainen, J. M., Molday, L. L., Foster, L. J., and Molday, R. S. (2008) Proteomics of photoreceptor outer segments identifies a subset of SNARE and Rab proteins implicated in membrane vesicle trafficking and fusion. Mol. Cell. Proteomics 7, 1053–1066

57. Broeders, G. C., Parmer, R., and Dawson, W. W. (1988) The proteome of the mouse photoreceptor sensory cilium complex. Exp. Eye Res. 48, 211–286

58. Han, J., Dinculescu, A., Dai, X., Du, W., Smith, W. C., and Pang, J. (2013) Review: The history and role of naturally occurring mouse models with PDE6b mutations. Mol. Vis. 19, 2579–2589

59. Kondo, A., and Ramamurthy, V. (2014) AIP1 protein and its indispensable role in cone photoreceptor function and survival. Adv. Exp. Med. Biol. 801, 43–48

60. Bright, S. R., Brown, T. E., and Varnum, M. D. (2005) Disease-associated mutations in CNGB3 produce gain of function alterations in cone cyclic nucleotide-gated channels. Mol. Vis. 11, 1141–1150

61. Liu, C., and Varnum, M. D. (2005) Functional consequences of progressive cone dystrophy-associated mutations in the human cone photoreceptor cyclic nucleotide-gated channel CNGA3 subunit. Am. J. Physiol. Cell Physiol. 289, C187–C198

62. Nork, T. M., Rosen, S. E., and Maumenee, I. (2002) Electroretinographic abnormalities in parents of patients with Leber congenital amaurosis who have heterozygous GUCY2D mutations. Arch. Ophthalmol. 120, 1325–1330

63. Duda, T., Venkataraman, V., Goracznik, R., Lange, K., Koch, K.-W., and Sharma, R. K. (1999) Functional consequences of a rod outer segment membrane guanylate cyclase (ROS-GC1) gene mutation linked with Leber’s congenital amaurosis. Biochemistry 38, 509–515

64. Lange, C., Duda, T., Beyermann, M., Sharma, R. K., and Koch, K.-W. (1999) Regions in vertebrate photoreceptor guanylyl cyclase ROS-GC1 involved in Ca2+-dependent regulation by guanylyl cyclase-activating protein GCAP-1. FEBS Lett. 460, 27–31

65. Han, J., Dinculescu, A., Dai, X., Du, W., Smith, W. C., and Pang, J. (2013) Review: The history and role of naturally occurring mouse models with PDE6b mutations. Mol. Vis. 19, 2579–2589