Bicarbonate Enhances Maximal Response Amplitude and Absolute Sensitivity to Light in Mouse Rod Photoreceptors

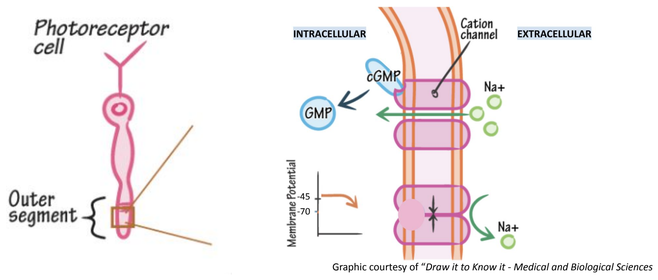
Amanda M Kossoff [1,2], Rajan D Adhikari[2], Clint L Makino[2]

[1]Winston Churchill High School, Potomac, MD

[2]Department of Physiology and Biophysics, Boston University School of Medicine, Boston, MA

1. Introduction

- In the retina, cyclic guanosine monophosphate (**cGMP**) is the second messenger of rod phototransduction.
- Light triggers a photoresponse by causing cGMP levels to decrease, closing cyclic nucleotide-gated channels.
- Closed channels block inward Na^+ flow without inhibiting Na^+/K^+ pumps in the inner segment. This hyperpolarizes the membrane and decreases glutamate release at the synapse.



Graphic courtesy of "*Draw it to Know it - Medical and Biological Sciences*

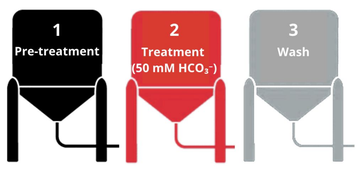
- **Bicarbonate** (HCO_3^-) increases cGMP synthesis by stimulating guanylate cyclase [2], which increases the number of channels open in darkness in amphibian rods [3].
- There has been little research on the effect of bicarbonate on mammalian rods that differ in body temperature, cell size, and other physiological parameters.
- Bicarbonate may play a role in retinitis pigmentosa and other forms of **human blindness** caused by genetic mutations that increase cGMP levels [1].

2. Objectives

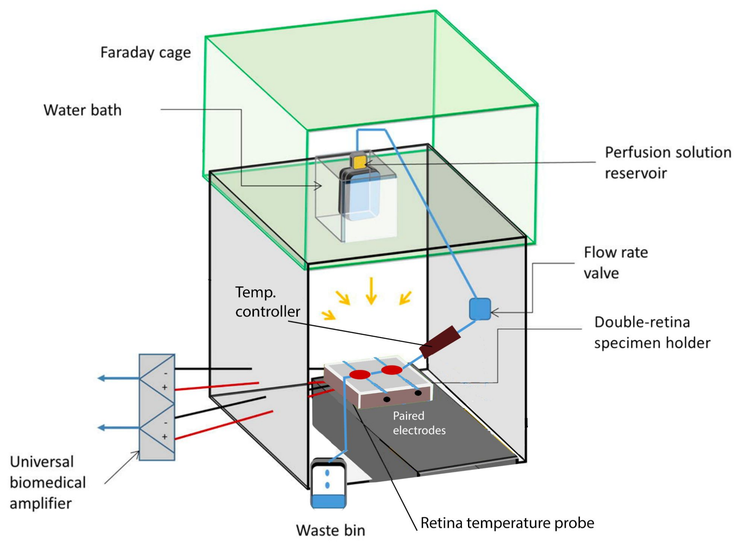
- To determine the **effect of bicarbonate** on the mouse rod photoresponse by quantifying maximal response amplitude, absolute sensitivity, time to recover, time to peak (dim flash kinetics), and to observe changes in light adaptation and dynamic range.
- To gain a greater understanding of differences in rod physiology and function across vertebrates by comparing the relative effect of bicarbonate on mouse rods to that on toad and salamander rods.

3. Methods

- Retinas were isolated from dark adapted mice lacking cone transducin (Gnat2−/−) under dim red light to minimize rod photoexcitation.
- Retinas were perfused continuously with Ames' solution containing $BaCl_2$ (to suppress glial currents) and DL-AP4 (to block synaptic transmission) to isolate rod responses to flashes and steps of light [4].



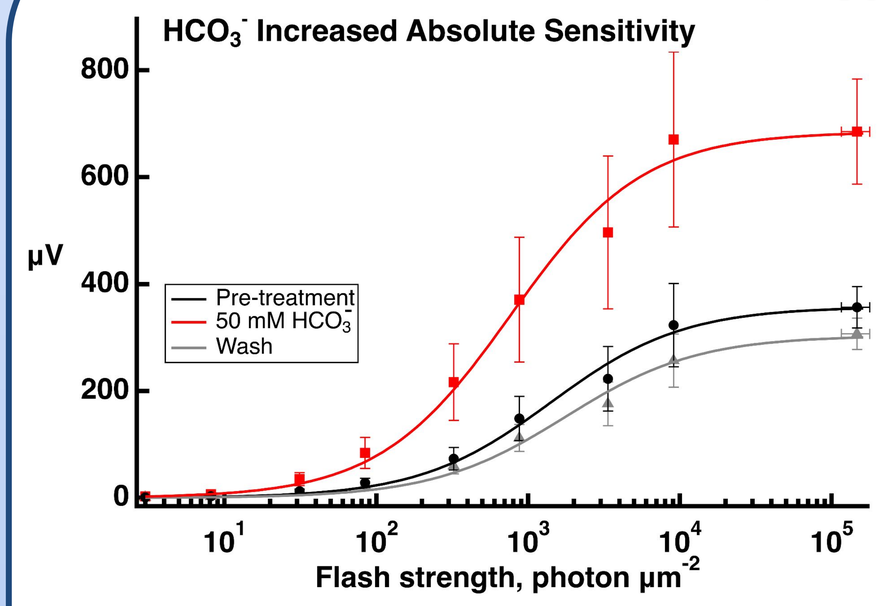
- **Experiment:** Electroretinogram (ERG) recorded transretinal voltage potentials in response to light stimuli to quantify rod response.
- To simulate physiological conditions, perfusate was gassed with 95% O_2 and 5% CO_2, pH 7.45, at 35˚C.
- To minimize electromagnetic noise, the ERG was recorded inside a grounded Faraday cage.



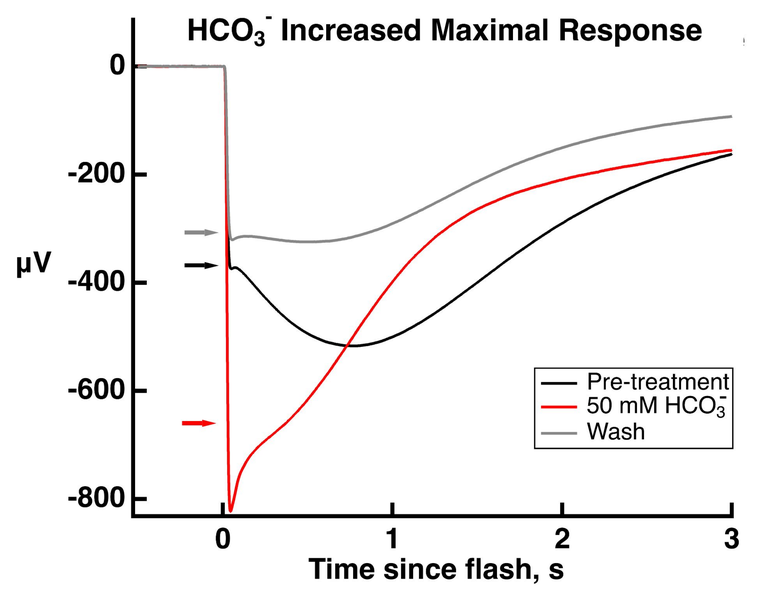
- **Analysis:** Paired t-tests determined statistical significance ($p \leq 0.05$ was considered significant); figures and analysis were conducted on Igor Pro 9.

$$t = \frac{\bar{d}}{S_d / \sqrt{n}}$$

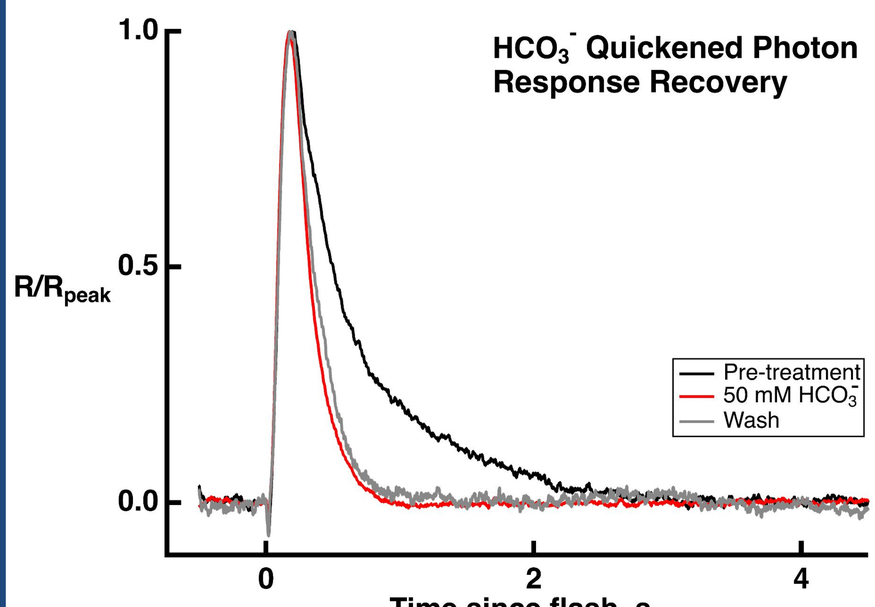
4. Results



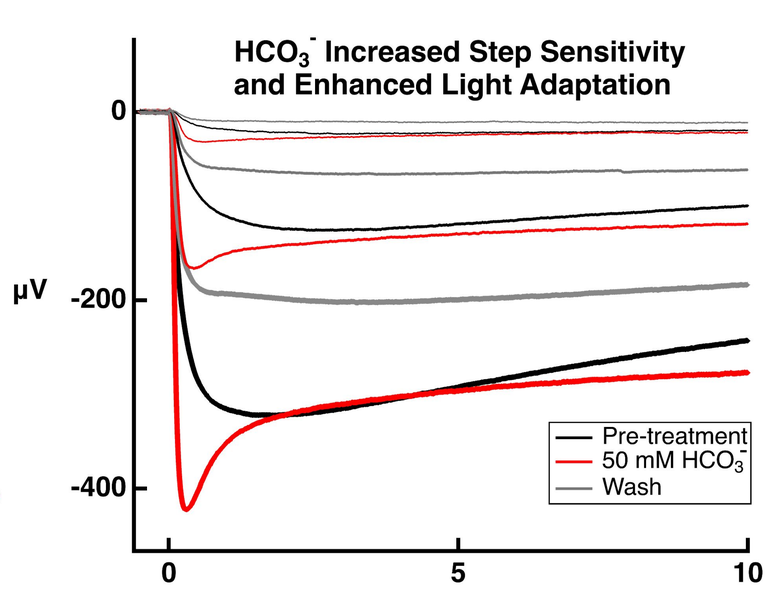
Responses to 20 ms flashes at 500 nm were fitted with a Michaelis function. For dim flashes, response amplitude increased by 153 ± 14% (mean ± SEM, n=5) with bicarbonate.



Saturating flash responses (357,689 photon μm^{-2}) showed a 90% ± 8% increase (n=6) with bicarbonate.



Dim flash responses to 31 and 84 photon μm^{-2} were averaged. The integral of the normalized response was decreased irreversibly by 67 ± 4% (n=6) with bicarbonate. There was no change in time to peak.

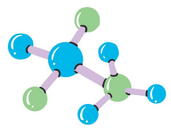


Bicarbonate increased the responses to 10 second steps at three intensities (3, 31, 323 photon μm^{-2}) and resulted in a larger droop to bright steps (n=3).

5. Conclusions

- Bicarbonate more profoundly increased the maximum response of the rod to light (circulating current) in mice compared to that in amphibians, mice having a 90% ± 8% increase and amphibians having a 30 ± 6% increase [5].
- In both mouse and amphibian rods, bicarbonate shortened photon response recovery (by 40 ± 8% in amphibian rods [5] and 67 ± 4% in mouse rods) without affecting time to peak, and enhanced light adaptation.
- Bicarbonate extended the dynamic range to brighter flashes in amphibians and to dimmer flashes in mouse.
- Effects of bicarbonate on absolute sensitivity and maximal response amplitude were reversible, but unexpectedly, the effect on photon response recovery appeared to be irreversible.
- Our study suggests that bicarbonate modulated phototransduction differently in rods of mammals and amphibians.

6. Discussion



1. Physiology: Why does bicarbonate have a differing effect on mammalian and amphibian rods?

- Warm vs cold blooded: with higher body temperature in mice, there are increased rates and changes in biochemical reactions.
- Cell size: toad rod outer segment is 4–5 times larger in diameter than a mouse rod [6].
- Enzymes: the guanylate cyclases expressed in mouse and amphibian rods respond differently to bicarbonate.

2. Therapy: Photoreceptor loss in inherited retinal degeneration-type diseases remains a major unresolved medical problem.

- Dysregulation of cGMP can kill photoreceptors, showing its plausibility as a target for therapeutic interventions [7].
- This study affirmed the significant effect of exogenous bicarbonate, and bicarbonate's short-term irreversible effect on photon response recovery.
- Our study could be grounds for future studies employing bicarbonate regulation in mammals to treat blinding retinal diseases caused by cGMP toxicity.

7. Future Directions

- Carbonic anhydrase catalyzes production of HCO_3^-. Acetazolamide, a carbonic anhydrase inhibitor, could be added to perfusate to quantify the effect of endogenous HCO_3^- present, with comparison to amphibians.
- Studying the effect of HCO_3^- on the photoresponses of mutant mice rods with stimulated guanylate cyclase activity and elevated cGMP levels could better model retinal disease.
- While our study added exogenous HCO_3^-, future research could explore how to promote or repress uptake of the endogenous bicarbonate present in rods.

8. References

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9. Acknowledgments

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