Increased Adaptation, Response Amplitudes, and Dynamic Range in Light Responses of Mouse Rod Photoreceptors with Bicarbonate

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Introduction

- Cyclic guanosine monophosphate (cGMP) plays an important role in opening and closing the cyclic nucleotide-gated (CNG) channel during phototransduction.

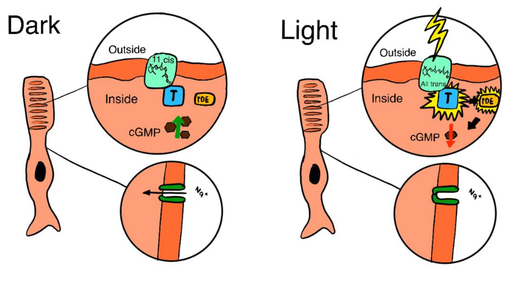


Fig. 1: Visual phototransduction

- CNG channels close when cGMP is hydrolyzed, blocking the entry of Na+. This leads to hyperpolarization, meaning the transmembrane voltage of rods are more negative.
- Dark current is the inward electric current that flows through the CNG channel into the outer segment.
- When dark current is reduced, it hyperpolarizes the rod.
- Bicarbonate stimulates guanylate cyclase, increasing the production of cGMP.
- The imbalance of cGMP can lead to inherited retinal diseases such as Retinitis Pigmentosa and achromatopsia.
- The purpose of this study was to evaluate the effects of bicarbonate on the step responses of murine rod photoreceptors.

Methods

Experiment:

- An ex vivo electroretinogram (ERG) was used to observe the responses of murine rods to steps of various light intensities.
- Each retina was perfused with Locke's solution, containing 20 mM of bicarbonate or an equal amount of Cl- concentration, immediately after its isolation from the eye.
- Perfusates included 50 µM DL-AP4 and 100 µM of $BaCl_2$ to block post-photoreceptoral and glial responses, respectively.
- To mimic physiological conditions, perfusates were heated to 35 ℃ and balanced with 95% O_2 and 5% CO_2.

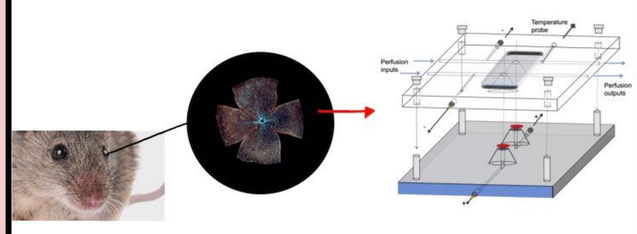


Fig 2: After the dissection of the eye from mice lacking cone transducin, the retina is placed in an ERG perfused continuously with Locke's solution.

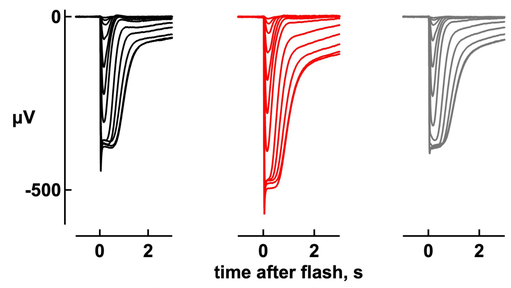
- To stimulate the retina, flashes of light were given with a duration of 20 ms at 500 nm and steps of light were given with 10 s with 540 nm.

Analysis:

- Igor Pro was used to analysis all responses.
- The pre-treatment traces were distinguished with black, bicarbonate with red, and wash with gray.

Results

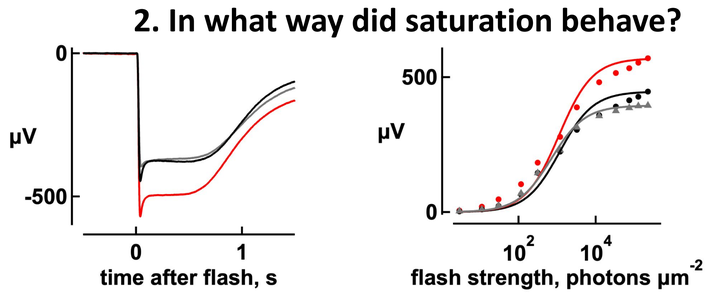
1. What effect did bicarbonate have on rod flash responses?



Bicarbonate had a reversible increase in flash responses (n=6 retinas).

∴ Bicarbonate reversibly amplified the dark current.

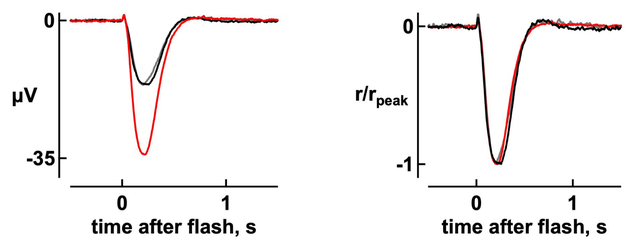
2. In what way did saturation behave?



The saturating flash responses with bicarbonate were 29% larger, but saturation was reached at the same flash strength for both bicarbonate and non-bicarbonate conditions (n=6).

∴ Bicarbonate extended the dynamic range of rods to dimmer flash.

3. How was dim flash response affected by bicarbonate?

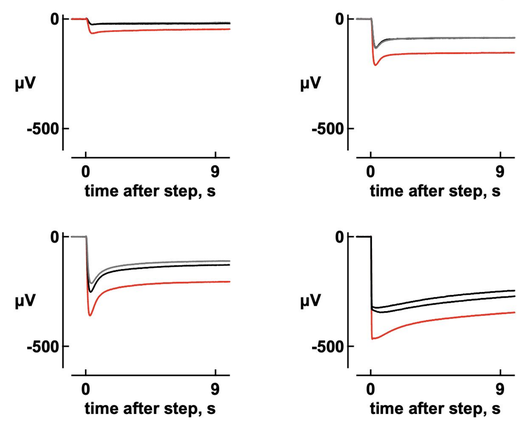


Dim flash responses were 102% larger.

Dim flash responses reached the peak and recovered at the same rate in both conditions.

∴ Absolute sensitivity to flashes improved.
There is no change in dim flash kinetics.

4. What effect did bicarbonate have on the responses to step?

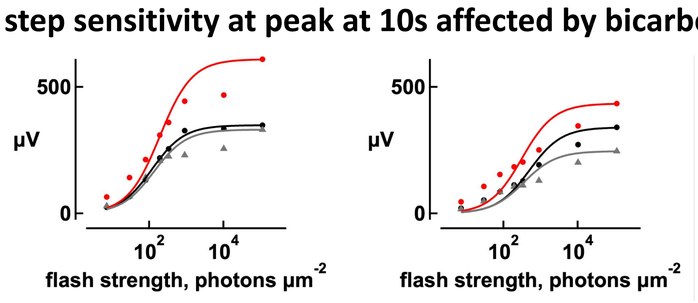


Bicarbonate increased peak responses to all steps.

For brighter and non-saturating intensities, the droop response was more prominent.

∴ Step sensitivity and light adaptation were enhanced by bicarbonate.

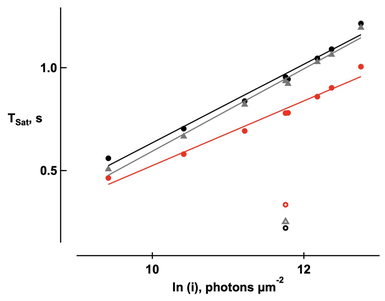
5. How is step sensitivity at peak at 10s affected by bicarbonate?



The responses to dim step were larger, but saturation occurred at same step intensity (n=3).

∴ Bicarbonate expanded the dynamic range of rods to dimmer and brighter steps of light.

6. How was time in saturation affected by bicarbonate?



Saturating time responded fastest with bicarbonate following light adaptation and step of light. Saturation time was shorter in the presence of background light.

∴ Step of light reduced the sensitivity.

Conclusions

- The response amplitudes, which is the measurement of dark current, for both flash and steps of light increased with bicarbonate.
- The absolute sensitivity following both flash and step of light increased, meaning the dynamic range expanded to include dimmer flashes.
- The addition of bicarbonate decreased saturation time but had no significant differences in integration time and time to peak.
- The responses with the presence of bicarbonate had a more prominent and larger droop, signifying an increased adaptation in rods.
- Responses saturated at the same flash intensity, increasing the dynamic range to include brighter flashes as well.
- The presence of bicarbonate was less sensitive to light than without bicarbonate.

Discussion

- The study of adding bicarbonate to rod photoreceptors in mammals has not been researched much.
- There has been previous studies using Ames' solution. The findings have been similar to this study using Locke's solution, however, the integration time was shortened and the response amplitude was higher in the Ame's solution. But, in the Locke's solution, the absolute sensitivity was much higher compared to the Ames' solution.
- Observing that the presence of bicarbonate has increased cGMP and dark current levels, this study can be used as a potential source of developing treatments for retinal diseases related to dysregulation of cGMP.

References

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