

- key eyes after topical anticholinesterase treatment, *Am. J. Ophthalmol.* 82:883, 1976.
5. Kaufman, P.L., and Bárány, E.H.: Cytochalasine B reversibility increases outflow facility in the eye of the cynomolgus monkey, *INVEST. OPHTHALMOL. VISUAL SCI.* 16:47, 1977.
 6. Kaufman, P.L., and Hahnenberger, R.: CI-744 anesthesia for ophthalmological examination and surgery in monkeys, *INVEST. OPHTHALMOL.* 14:788, 1975.
 7. Bárány, E.H.: Simultaneous measurement of changing intraocular pressure and outflow facility in the vervet monkey by constant pressure infusion, *INVEST. OPHTHALMOL.* 3:135, 1964.
 8. Hahnenberger, R.W.: Applanation tonometry in the conscious cynomolgus monkey (*Macaca fascicularis*), *Acta Ophthalmol.* 54:311, 1976.
 9. Cevario, S.J., and Macri, F.J.: The inhibitory effect of pentobarbital Na on aqueous humor formation, *INVEST. OPHTHALMOL.* 13:384, 1974.
 10. Hahnenberger, R.W.: Influence of cataleptoid anaesthetic agents on the intraocular pressure in monkeys (*Macaca fascicularis*), *Acta Ophthalmol.* 54:491, 1976.

Barium delays the onset of rapid dark adaptation in bullfrog cones. A. E. WALTER, D. A. BOLNICK, AND A. J. SILLMAN.

The late receptor potential of the excised, perfused bullfrog retina was isolated with sodium aspartate. By employing a three-flash technique, cone responses were monitored without interference from rods. In cones barium ions were found to delay the onset of rapid dark adaptation, but the rate of recovery, once begun, was unaffected. We propose that barium ions act directly upon the enzyme system postulated to govern the onset of rapid dark adaptation of cones. In addition, barium was found to affect the amplitude of the rod receptor potential differently from that of cones, increasing the former but decreasing the latter. The effect of barium upon photoreceptor potential amplitude is discussed in terms of a reduction in the potassium conductance of the photoreceptors and the mechanisms postulated for photoreceptor excitation and rapid dark adaptation.

Low levels of barium ions (0.1 to 1.2 mM) have been shown to increase the receptor potential amplitude and to mimic dark adaptation in the toad rod.¹ It was suggested that barium ions exert their effect directly upon a nonphotochemical aspect of dark adaptation.¹ In addition, inspection of the data presented by Piccolino and Gerschenfeld² reveals that high levels of barium ions (3 to 6 mM) suppress the receptor potential amplitude of turtle cones. Our own research has indicated that the concentration of another divalent cation, calcium,

plays a role in rapid dark adaptation in bullfrog cones.³ Thus the goals of this study were to explore what effects barium might have on rapid dark adaptation of cones and to further investigate the effect of barium ions upon cone receptor potential amplitude.

Materials and methods. The methods employed here are essentially those described previously.³⁻⁵ In each experiment the late receptor potential of an excised, perfused retina from a bullfrog (*Rana catesbeiana*) was isolated by treatment with 10.0 mM sodium aspartate. In addition, control perfusates contained 100.0 mM NaCl, 2.0 mM KCl, 5.0 mM glucose, 0.4 mM MgCl₂, and 0.4 mM CaCl₂. The test solution differed from the control only in that it contained 0.4 mM BaCl₂. All perfusion media were buffered at pH 7.8 with 20.0 mM Tris-maleate.

Temperature was maintained at 18° ± 0.2° C by a Lauda K-2/R water bath and monitored at the level of the retina with a YSI Model 43 TD electronic thermometer. An IVAC Model 200 gravity flow controller held the perfusion rate at 0.2 ml/min. A Dynatron Model 276 event controller automatically regulated the time interval between and the duration of each stimulus (a flash of white light of 250 msec duration and 47 μW/cm² intensity). Two chlorided silver electrodes, each positioned on opposite sides of the retina, carried responses to a capacitance-coupled amplifier (time constant 0.5 sec). Responses were displayed on a Tektronix Model 5112 oscilloscope, and permanent records were made with a Grass kymograph camera.

Results. A three-flash technique was employed to monitor rapid dark adaptation of cones.³⁻⁵ Thus the response to the first flash contains contributions from both rod and cone photoreceptors. The second stimulus, which follows the first by 10 sec, elicits a pure and maximal cone response. The third flash, given at intervals ranging from 1 to 10 sec following the second flash, evokes a response reflecting the extent of cone recovery. To ensure that the entire system recovers to the fully dark-adapted state, a 2 min interval was employed between each tripartite set of flashes. In each experiment cone rapid dark adaptation was determined first with control Ringer, then 30 min after changing to barium Ringer, and finally 30 min after return to control Ringer.

That the 30 min interval employed between changeover of perfusates was sufficient to allow accurate monitoring of rapid dark adaptation is shown by Fig. 1. This figure depicts the time course of the effect of addition or removal of

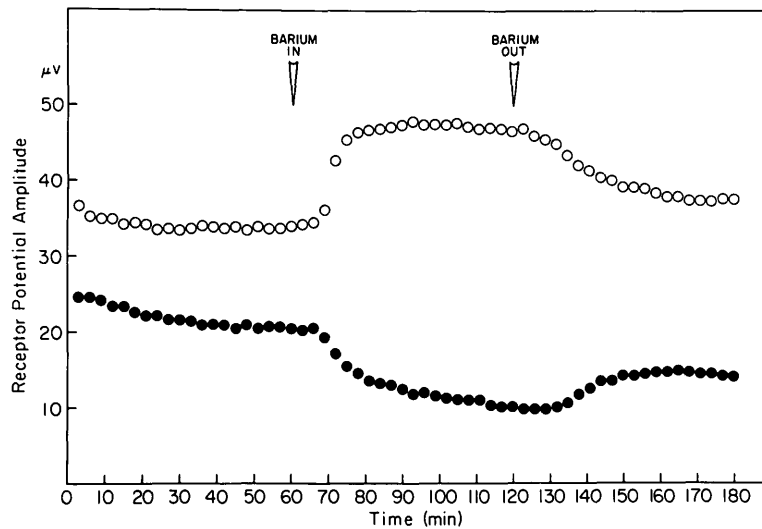


Fig. 1. Effect of barium on receptor potential amplitude. *Open circles*, Responses of the rods; *filled circles*, responses of the cones. Changeover from control to experimental perfusate and back is indicated by the arrows. A 3 min adaptation interval was employed between each pair of stimuli to allow for full recovery. Results are of a typical experiment. Barium ion concentration was 0.4 mM.

barium ions on photoreceptor potential amplitude. The maximum cone response is represented by the filled circles and the amplitude of rod response, computed by subtracting the maximum cone response from the response to the first flash, is illustrated by the open circles. Note that in both cases the effect has plateaued within 30 min. It is very interesting that the effect of barium was not the same in the cones as it was in the rods. In fact, barium decreased the amplitude of the cone receptor potential but increased the amplitude of the rod receptor potential. These findings correlate well with the effect of barium ions upon the turtle cone² and the toad rod.¹

Inspection of Fig. 2 reveals the dramatic effect of barium ions in delaying the onset of rapid dark adaptation of cones. In control perfusate (open circles) the recovery of amplitude was approximately 34% of maximum 1 sec following the second flash. Within 5 sec after the second stimulus, recovery was nearly complete. In contrast, the presence of barium ions (filled circles) shifted the entire recovery curve to the right, and recovery of amplitude did not reach 34% of maximum until 2 sec had elapsed between the second and third stimulus. Thus there was a delay in the onset of recovery, but the rate of recovery, once begun, was unaffected. Since it was consistently observed that the effect of barium ions upon cone rapid dark adaptation was fully reversible, the data obtained

prior to and after monitoring the effect of barium were averaged. The control recovery curve represents this average.

Discussion. The results depicted in Figs. 1 and 2 clearly indicate that barium has profound effects upon the bullfrog retina treated with sodium aspartate. It is doubtful that the effects of barium are mediated through an interaction of the photoreceptors with the more proximal neurons, since the presence of sodium aspartate suppresses the electrical activity of the bipolar and horizontal cells.^{6, 7} Although barium ions are known to suppress the Müller cells,⁸ the effects of the ions observed in the present study are not the result of an action exerted on these glial elements. The reason for this is that slow reversal of such suppression just begins 1 hr after the retina is returned to control Ringer,⁸ whereas in this study, employing the same system, we find that the effects of barium ions on the photoreceptors are fully reversed 30 min after the retina is returned to control Ringer. We conclude therefore that barium ions act directly upon the photoreceptors to produce the delay in the onset of rapid dark adaptation of cones and the changes in rod and cone receptor potential amplitude.

Certainly it is provocative that in the presence of barium ions the receptor potential amplitude of the rods increases but that of the cones decreases. Since barium ions are known to decrease the po-

tassium conductance of other excitable cells,^{9, 10} it is possible that the potassium conductance of the photoreceptors is also reduced. Although the potassium conductance of the cones has not been measured, it is assumed that there is a continuous outward potassium current which leads to hyperpolarization when light excites the cone and inactivates the inward sodium current.¹¹ Therefore it is conceivable that barium ions, through a blockage of potassium conductance, produce a decrease in cone hyperpolarization and so reduce the receptor potential amplitude of cones.

Interestingly, it has been proposed that barium ions cause a reduction in the potassium conductance of rods.¹ However, despite the fact that a diminished potassium conductance accounts for the observed depolarization in the resting membrane potential of rods, the change in membrane potential cannot fully explain the increase in rod receptor potential amplitude.¹ Thus the action of barium ions upon the rods must involve more than a reduction in potassium conductance.¹

Perhaps the ability of barium ions to affect the receptor potential amplitude of rods differently from that of cones is best interpreted in terms of the mechanisms proposed for photoreceptor excitation and rapid dark adaptation. For the rods it is postulated that a stimulus of light allows the saccules to release a blocking substance which then diffuses through the cytoplasm to the sodium channels.¹² To maintain both a constant store of blocking agent within the saccules and a reduced level of this agent in the cytoplasm, it is suggested that some form of retrieval mechanism exists in the saccule.¹² Brown and Flaming¹ postulate that barium impedes the reaccumulation of the blocking substance, thereby increasing the amount delivered to the sodium channels following a light stimulus. This hypothesis predicts that a larger hyperpolarization should result and certainly is in accord with the increase in rod receptor potential amplitude observed in both their study and ours.

Unlike the rods, the saccules of the cones are exposed to the extracellular fluid¹³ and it is advocated that the source of the blocking agent is the extracellular fluid.¹² In addition, it is proposed that following excitation a conformational change occurs in the membrane of the outer segment, so that the blocker gains access to the sodium channels.¹² Since a temperature study showed that the kinetics of cone rapid dark adaptation, following onset of recovery, are independent of temperature, Sillman et al.⁵ suggested that a sodium channel inactivator exerts its effect not by entering the cone cytoplasm but rather by binding to the ex-

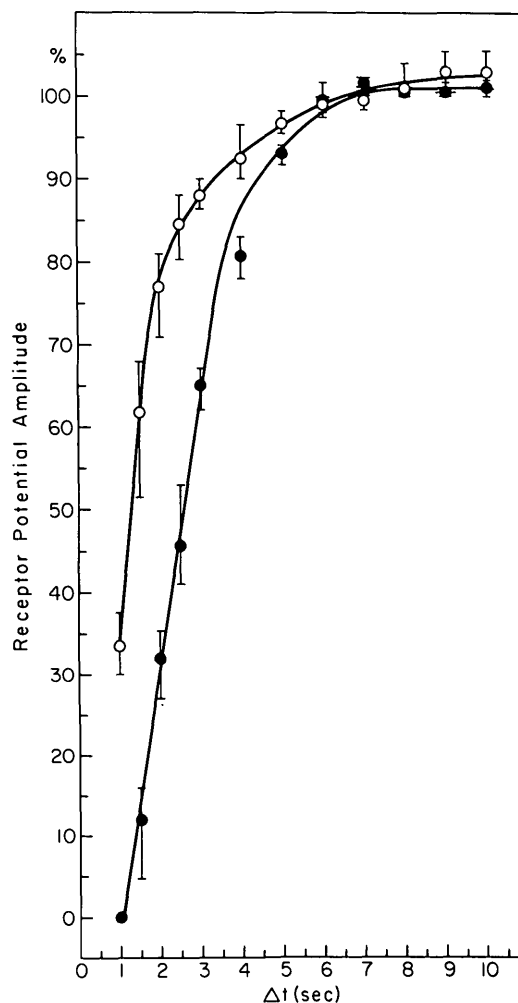


Fig. 2. Time course of adaptation of the cone response as affected by 0.4 mM barium ion treatment. The amplitude of the second cone response as a percentage of the first (maximum) cone response is shown. *Open circles*, Mean values obtained with control Ringer; *filled circles*, mean values obtained with 0.4 mM barium Ringer. Error bars indicate the range. Data are from three experiments conducted on retinas from three frogs.

terior surface of the cone saccules. Rapid dark adaptation, then, entails the enzymatic removal of the inactivator followed by passive redistribution of the substance into the extracellular fluid. In terms of the mechanism envisioned for rapid dark adaptation of cones there is no means for barium ions to induce the accumulation of the sodium channel inactivator once it has left the membrane. Thus barium would not be expected to increase

the hyperpolarization of cones. This proposal together with the suggestion that barium ions reduce the potassium efflux in cones predicts that the receptor potential amplitude of cones should decline in the presence of barium. Such a decrease is noted in this study and elsewhere.²

Also consistent with this interpretation is our present finding that barium ions delay the onset of rapid dark adaptation but that the rate of recovery, once begun, is unaffected. The onset of this adaptation process is highly temperature-sensitive and therefore may represent an enzymatic process which removes the blocking agent or the inhibitory substance from the sodium channels.⁵ It is not unreasonable that barium may act directly on this proposed enzymatic process so as to impede the removal of the sodium channel inactivator. Such a possibility is not remote since barium is known to affect enzyme systems.^{14, 15} Because the onset of the adaptation process in rods is also postulated to be governed by the enzymatic removal of an inactivator from the sodium channels,⁵ we anticipate that barium ions will increase the latency of the recovery process in rods as well. Research in our laboratory is currently under way to test this hypothesis.

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Key words: barium, rapid dark adaptation, cones, bullfrog, retina, photoreceptor

REFERENCES

1. Brown, K.T., and Flaming, D.G.: Opposing effects of calcium and barium in vertebrate rod photoreceptors, *Proc. Natl. Acad. Sci. U.S.A.* 75:1587, 1978.
2. Piccolino, M., and Gershenfeld, H.M.: Activation of a regenerative calcium conductance in turtle cones by peripheral stimulation, *Proc. R. Soc. Lond. [Biol.]* 201:309, 1978.
3. Bolnick, D.A., Clinite, E.W., Walter, A.E., and Sillman, A.J.: The influence of calcium and magnesium on rapid dark adaptation in bullfrog retinal cones, *Fed. Proc.* 37:528, 1978 (abst.).
4. Sillman, A.J.: Rapid dark-adaptation in the frog cone, *Vision Res.* 14:1021, 1974.
5. Sillman, A.J., Bolnick, D.A., Clinite, E.W., and Rudert, K.S.: The effect of temperature on rapid dark adaptation in bullfrog photoreceptors—a difference between rods and cones, *Vision Res.* 18:1375, 1978.
6. Murakami, M., Ohtsuka, T., and Shimazaki, H.: Effects of aspartate and glutamate on the bipolar cells in the carp retina, *Vision Res.* 15:456, 1975.
7. Murakami, M., Ohtsu, K., and Ohtsuka, T.: Effects of chemicals on receptors and horizontal cells in the retina, *J. Physiol. (Lond.)* 227:899, 1972.
8. Bolnick, D.A., Walter, A.E., and Sillman, A.J.: Barium suppresses slow PIII in perfused bullfrog retina, *Vision Res.* (in press).
9. Magura, I.S.: Long-lasting inward current in snail neurons in barium solutions in voltage-clamp conditions, *J. Membr. Biol.* 35:239, 1977.
10. Henderson, E.G., and Volle, R.L.: Ion exchange in frog sartorius muscle treated with 9-aminoacridine or barium, *J. Pharmacol. Exp. Ther.* 183:356, 1972.
11. Cervetto, L.: Influence of sodium, potassium and chloride ions on the intracellular responses of turtle photoreceptors, *Nature* 241:401, 1973.
12. Yoshikami, S., and Hagins, W.A.: Light, calcium, & the photocurrent of rods & cones. *Biophys. Soc.* 15:47a, 1971 (abst.).
13. Cohen, A.I.: New evidence supporting the linkage to extracellular space of outer segment saccules of frog cones but not rods. *J. Cell. Biol.* 37:424, 1968.
14. Sperelakis, N., and Lee, E.C.: Characterization of (Na⁺, K⁺)-ATPase isolated from embryonic chick hearts and cultured chick heart cells. *Biochim. Biophys. Acta* 233:562, 1971.
15. Yazawa, M., Morita, F., and Yagi, K.: Role of divalent metal cations in heavy meromyosin-adenosine triphosphate complex. *J. Biochem.* 71:301, 1972.

An estimate of image quality in the rat eye.

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The point spread function on the optic axis of the rat eye is indirectly estimated to increase from a half-height width no greater than 12.5' to not less than 36' after dilation of a 0.33 mm pupil diameter to 3.0 mm.

A modulation transfer function (MTF) curve is not available for the rat. The following measurements provide an estimate of the *minimum* spatial frequency at which image contrast disappears close to the optic axis of the rat eye.

Methods. Observations were made on the eyes of five D.A. rats of between 115 and 130 days of age (215 to 230 gm weight). The animals were anesthetized by the intraperitoneal injection of 1 cc of 25% urethane solution and mounted in a head holder. Saline irrigation was employed instead of a contact lens. At first, the natural pupil was used, but later it was dilated by atropine. The apparatus of Fig. 1 is essentially that of Wässle.¹ A grating of transparent and opaque bands was