Rapid dark adaptation of bullfrog rods is delayed by barium

A. E. Walter, D. A. Bolnick, L. W. Haynes, and A. J. Sillman

With use of sodium aspartate, the late receptor potential of the excised, perfused bullfrog retina was isolated. By means of a two-flash technique, rapid dark adaptation of rods was monitored. As in cones, barium ions were found to delay the onset of rapid dark adaptation of rods, but the rate of recovery, once begun, was virtually unaffected. The effect of barium on the amplitude-intensity relationship of rods was also determined. Unlike its ability to dramatically increase the amplitude of the receptor potential of rods, barium had no effect on the absolute threshold of rods. We propose that barium ions act on the enzyme system postulated to govern the onset of rapid dark adaptation of rods and suggest that a reduction in the activity of an ATP protein kinase might be the basis for this effect. (INVEST OPHTHALMOL VIS SCI 23:351-356, 1982.)

Key words: barium, rapid dark adaptation, rods, bullfrog, ATP protein kinase

When a dark-adapted retina is exposed to a light stimulus that bleaches only a negligible amount of visual pigment, the sensitivity of the retina is immediately reduced and then returns to its original level within seconds.1, 2 One important site of the phenomenon is the photoreceptor,3-6 with both rods3, 4 and cones5 exhibiting rapid recovery of their ability to generate a receptor potential. Just as the time course of the cone receptor potential is faster than that of rods,6 the rate of rapid dark adaptation is faster in cones than in rods.5 It has been shown that rapid dark adaptation consists of a latency phase and a recovery phase in both rods and cones.7 Although the recovery phase is highly temperature sensitive only in the rods, the latency phase is temperature dependent in both rods and cones.7 Thus the major similarity between rod and cone rapid dark adaptation may lie in the latency phase. Recent work concerned with the ionic basis of rapid dark adaptation of cones has shown that the addition of barium ions to the perfusate lengthens the latency of recovery.8 If the latency phases of rod and cone rapid dark adaptation involve similar mechanisms, one would expect that barium ions should lengthen the latency phase of the rods. The present study will show that barium ions do indeed lengthen the latency phase of rapid dark adaptation in rods but leave the recovery phase virtually unaffected.

Methods
The methods employed in this study were essentially the same as those described previously.4, 7 Prior to each experiment, a bullfrog (Rana catesbeiana) was dark adapted overnight. Under dim red illumination the animal was decapitated, after which an eye was enucleated and hemisected. The eyecup was then immersed in Ringer’s solution and the retina and pigment epithelium were dis-
sected away. The retina, minus the pigment epithelium, was then mounted in the perfusion chamber, which in turn was placed in a metal block. Control and experimental solutions were held in reservoirs above the perfusion chamber and were passed through separate water-jacketed condensers before entering the chamber. The temperature of water circulating through the jacketed condensers and the metal block was controlled by a Lauda K-2/R water bath. A YSI Model 43TD electronic thermometer monitored the temperature at the level of the retina and the Lauda was set so that the temperature of the preparation was maintained at 18°C ± 0.2°C. An IVAC Model 200 gravity flow controller held the perfusion rate at 0.2 ml/min.

To isolate the late receptor potential of the excised, perfused retina, both control and experimental solutions contained 10.0 mM sodium aspartate. These solutions also contained 100.0 mM NaCl, 2.0 mM KCl, 5.0 mM glucose, 0.4 mM MgCl₂, and 0.4 mM CaCl₂ and were buffered at pH 7.8 with 20.0 mM Tris-maleate. The test solution differed from the control only in that it contained 0.4 mM BaCl₂.

A Dynatron Model 276 event controller automatically regulated both the time interval between the stimuli and the duration of each stimulus, a 250 msec flash of white light. The light source was a quartz-iodine lamp that delivered an unattenuated flash with an intensity of 4700 μW/cm² to the retina. Appropriate neutral density filters were placed in the light path to produce the desired degree of attenuation. Responses were carried to a capacitance-coupled amplifier (time constant 0.5 sec) by two chlorided silver electrodes positioned on opposite sides of the retina. A Grass kymograph camera was used to make permanent records of the responses displayed on a Tektronix Model 5112 oscilloscope.

Results

Effect of barium ions on rapid dark adaptation of rods. To monitor rapid dark adaptation of rod photoreceptors, a two-flash method described by Sillman et al. was employed. The first stimulus, which elicits a combined response from the rods and cones, served as the conditioning stimulus by depressing the activity of the photoreceptors. A second stimulus, identical to the first, presented to the preparation at various time intervals, acted as the test stimulus. Between each pair of flashes, a 3 min interval was employed to ensure that the photoreceptors recovered fully. By comparison of the response to the conditioning stimulus with the response to the test stimulus for each respective time interval, it was possible to assess the extent of recovery of the photoreceptors. In each experiment rapid dark adaptation of the rods was determined first with control Ringer's, then 30 min after changing to barium Ringer, and finally 30 min after returning to control Ringer. That the 30 min interval employed between changeover of perfusate was sufficient to allow accurate monitoring of rod receptor potential amplitude was shown in a previous report, in which the time course of the effect of barium ions on the amplitude response of rod photoreceptors was determined with the same system. Since the effect of barium ions on rapid dark adaptation of rods was fully reversible, data obtained prior to and after the exposure of the retina to barium were averaged and the control recovery curves represent this average.

At a stimulus intensity of 0.47 μW/cm², recovery in control Ringer followed the time course depicted by the open circles in Fig. 1. When the interval (Δt) between the two flashes of light was 5 or 10 sec, the amplitude of the response to the test stimulus was approximately 17% of that to the conditioning stimulus. This initial plateau has been shown to represent a period during which the cones have recovered fully and respond maximally but the activity of the rods remains suppressed. The termination of this initial plateau period or latency phase marks the onset of the recovery phase of the rods. Thus, in the present study, the latency phase lasted 10 sec under control conditions. Once initiated, recovery of the rods proceeded linearly at 2.4%/sec but began to plateau when Δt was 40 sec and eventually reached completion when Δt was 80 sec. The filled circles in Fig. 1 illustrate the effect of barium perfusate on the time course of recovery of the rods at a stimulus intensity of 0.47 μW/cm². In the presence of barium ions the latency phase was about 20 sec; therefore, the delay in the onset of rapid dark adaptation of the rods was about twice that in control Ringer. Once be-
Barium delays rod adaptation

Fig. 1. Time course of rod recovery at a stimulus intensity 0.47 μW/cm² as affected by 0.4 mM barium ions. The amplitude of the second response as a percentage of the standard response that preceded it is displayed. Open circles, Mean values obtained with control Ringer's solution; filled circles, mean values obtained with 0.4 mM barium Ringer. Lines are eye fit. Error bars represent standard deviation. Data are from seven experiments conducted on retinas from seven frogs.

gun, however, recovery in the presence of barium proceeded at 2.0%/sec, a rate 17% lower than that in control Ringer.

To determine whether barium delays the onset of recovery of sensitivity as well as that of amplitude, we conducted an experiment similar to that outlined above but designed to determine the stimulus intensities necessary to elicit a criterion response equal to 12.5% of the response to the conditioning stimulus. The time course of recovery of criterion sensitivity in control and barium Ringer is depicted in Fig. 2 by the open and filled circles, respectively. In control Ringer, recovery began when Δt was about 10 sec, while in barium perfusate the onset of recovery began after Δt was greater than 10 sec. These data show that barium delays the onset of recovery of rod activity when measured in terms of criterion sensitivity.

To determine whether the effects produced by barium ions were dependent on the stimulus intensity, we repeated the above experiment with a lower light intensity of 0.047 μW/cm². The time course of recovery in control and barium Ringer is shown in Fig. 3 by the open and filled circles, respectively. In control solution, recovery of the rods had already begun when Δt was 5 sec, while in barium perfusate the onset of recovery began after Δt was greater than 10 sec. In other words, barium ions more than doubled the time of the delay in the onset of rapid dark adaptation of rods. The respective slopes of recovery in the control and barium solutions were 4.5%/sec and 3.9%/sec, a difference of 13%.

The effect of barium on the onset of recovery was further explored in two experiments conducted at stimulus intensities of 4.7 and 47.0 μW/cm². At both light intensities it was found that barium delayed the onset of recovery by about 100%. Therefore, irrespective of the light intensity, barium increases the time to the onset of recovery by about 100%.

Effect of barium ions on the absolute sensitivity of rods. If barium ions were to alter the absolute threshold of the rods, the time course of rapid dark adaptation of these photoreceptors could be obscured. For example, when the absolute threshold of the photoreceptors is elevated, the rate of rapid dark
adaptation is slowed considerably. Therefore the effect of barium ions on the absolute sensitivity of dark-adapted rods was examined by determining the relationship between the receptor potential amplitude and light intensity. Responses to single stimuli were recorded as the flash intensity was increased in increments of 0.4 log units from —log 10.0 to —log 6.0 and then decreased in increments of 0.4 log units from —log 6.2 to —log 9.8. The circles in Fig. 4 represent the average of the data obtained prior to and after exposure of the retina to barium ions, while the triangles illustrate the data in the presence of barium ions. It is clear from Fig. 4 that the enhancing effect of barium on rod response amplitude lessens at lower light intensities. This is in agreement with the observations of Brown and Flaming on the toad Bufo marinus. In both control and experimental solutions the rods first responded when the level of attenuation of the light intensity approached —log 8.0. Therefore the absolute threshold of the rods was not altered by 0.4 mM barium chloride.

Discussion

Clearly, barium ions lengthen the latency phase of rapid dark adaptation of rods and, in so doing, delay the onset of recovery. Although the rate of recovery is also somewhat reduced, the effect is subtle by comparison and may not be significant. It is improbable that these effects of barium are mediated through an interaction of the rods with more proximal neurons, since the perfusate contained sodium aspartate, a chemical that suppresses the electrical activity of the bipolar and horizontal cells. However, in the aspartate-treated retina of the bullfrog, the slow PIII wave persists and barium ions suppress this wave. Even so, the changes in the recovery process of the rods are not the result of barium acting on the mechanism generating the slow PIII. The reason for this is that 1 hr after returning to control Ringer there is just a hint of recovery of the slow PIII, while in this study utilizing the same system, the effects of barium on rapid dark adaptation of rods are reversed within 30 min after returning to control Ringer. There is the remote possibility that rod recovery is linked...
to cone recovery and that barium delays rod recovery by acting on the cones. However, the fact that the cones are fully recovered long before the onset of rod recovery, and the fact that barium delays cone recovery only about 1 sec but delays rod recovery by at least 5 sec, argue against this possibility. It is likely, therefore, that barium ions affect rods directly.

The mechanism by which barium ions act to delay the onset of rapid dark adaptation of rods is not known. However, we can say that barium does not act by affecting the mechanism responsible for absolute sensitivity, since barium ions do not shift the absolute threshold of rods. Since the recovery process is probably dependent on an enzymatic event, it is possible that barium depresses the activity of the enzyme system responsible for regulating the onset of the adaptive process. The question, then, arises as to what enzyme(s) might be acted on by barium so as to produce a delay in the onset of rapid dark adaptation of rods. Inasmuch as barium inhibits the activity of an ATP protein kinase isolated from the outer segments of rods, it seems appropriate to examine a phosphorylation reaction that has been proposed to occur in rods and that may involve an ATP protein kinase. In the scheme set forth by Leibman and Pugh, a trigger molecule formed from the photoreception of rhodopsin is inactivated by a phosphorylation reaction involving an ATP protein kinase. Until the trigger molecule is inactivated, it catalyzes the initial reaction in a cascade of reactions that lead to the closure of the sodium channels. With a reduction in the activity of ATP protein kinase the sodium channels would be expected to remain closed for a longer period of time. If the onset of rapid dark adaptation involves the reopening of the sodium channels, then prolonging the duration of closure of the sodium channels would postpone the onset of the adaptive process. Therefore it is possible that, by depressing the activity of ATP protein kinase, barium prolongs the period of closure of the sodium channels and thereby delays the onset of rapid dark adaptation.

This mechanism proposed to explain the action of barium on the onset of recovery is speculative but it is consistent with other observations made regarding the effects of barium on the receptor potential of the rods. For example, slowing the inactivation of the trigger molecules would lengthen the duration of closure of the sodium channels and, as a consequence, prolong the period of hyperpolarization of the receptor potential of rods. If so, this may account for the fact that barium ions slow the decay of the rod receptor potential. It seems reasonable that slowing the inactivation of the trigger molecules would have the same effect as increasing their formation. As a result, a greater number of sodium channels would be closed. It follows, then, that should barium impede the inactivation of the trigger molecules, the amplitude of the receptor potential of the rods would increase. The amplitude of the rod receptor potential does increase in the presence of barium. Moreover, the effect appears to be saturable, suggesting the involvement of an enzyme system. On the other hand, increasing barium beyond 2.0 mM results in a decrease in the amplitude of the receptor potential of rods. Thus, although it appears that some of the effects of barium on the rods are explicable in terms of a reduction in the activity of the ATP protein kinase proposed to catalyze the inactivation of the trigger molecule, this ATP protein kinase cannot be the sole locus at which barium acts.

Barium may, in fact, decrease the potassium conductance of rods. Certainly a reduction in the outward movement of potassium is consistent with the fact that the resting membrane potential of dark-adapted rods depolarizes in barium Ringer. In view of the fact that efflux of potassium appears to be necessary for the generation of the rod receptor potential, it is conceivable that a reduction in the potassium conductance would reduce the amplitude of the receptor potential of rods. Of course, this would oppose the increase in the receptor potential that may result from the proposed ability of barium to reduce the activity of the ATP protein kinase that participates in the inactivation of the trigger molecules. Perhaps, then, the ef-
fect of barium on the amplitude-generating mechanism of rods represents the net effect of barium on ATP protein kinase and on potassium conductance.

We thank Steven Grupenhagen and Margaret Park for their excellent technical assistance and the IVAC Corporation of San Diego for their gift of the Model 200 gravity flow controller used in this study.

REFERENCES