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Barium Removes the Ouabain-Induced Increase in the Rod Response to Light

A. E. Walter* and A. J. Sillman

The mass receptor potential of the excised, superfused retina of the bullfrog was isolated with aspartate. Rods were selectively stimulated by using very dim flashes of light. In the presence of 0.1 mM ouabain, the amplitude of the receptor response was found first to increase transiently and, subsequently, to decrease progressively. The ouabain-induced transient increase in receptor response was completely eliminated by 0.4 mM barium chloride. However, barium did not affect the rate at which the response decayed in the presence of ouabain. The ability of barium to remove the ouabain-induced transient increase in the amplitude of the receptor response is discussed in terms of reducing the coupling ratio of the postulated electrogenic sodium–potassium pump of rods. Invest Ophthalmol Vis Sci 26:782–785, 1985

It is commonly known that cardiac glycosides such as strophanthidin or strophanthin (ouabain) poison sodium–potassium pumps. It is not surprising, therefore, that such substances cause a decrease in the amplitude of the photoreceptor potential, since suppression of the pump causes the collapse of the sodium and potassium gradients that are necessary to generate the response.[1,2] What is surprising, however, is that when rod photoreceptors are exposed to strophanthidin and stimulated with dim flashes of light, the amplitude of the receptor potential first increases and, then, decreases.[2] This transient increase in the receptor potential amplitude may be explicable in terms of the postulated electrogenicity of the sodium–potassium pump of rods.[2,3] If so, then altering the electrogenicity should also alter the transient. Interestingly, barium ions have been shown to make the sodium–potassium pump in muscle less electrogenic.[4] Thus, the present study was undertaken to determine whether barium removes the transient increase in rod receptor response amplitude that is induced by cardiac glycosides.

Materials and Methods. The methods employed in this study have been described before[5,6] and will not be repeated in detail here. Experimental animals were treated in conformity with the ARVO Resolution on the Use of Animals in Research. Prior to each experiment a bullfrog, *Rana catesbeiana,* was dark-adapted overnight. Under dim red illumination the animal was decapitated and double-pithed, after which an eye was enucleated and hemisected. The eyecup was then immersed in control Ringer solution where the retina and pigment epithelium were dissected out. The retina, minus the pigment epithelium, was then mounted in the superfusion chamber which, in turn, was placed in a metal block. Control and experimental solutions were held in reservoirs above the perfusion chamber and before entering the chamber passed through separate water-jacketed condensers. The temperature of water circulating through the jacketed condensers and the metal block was controlled such that the temperature of the preparation was maintained at 18 ± 0.2°C. The base of each condenser fit into a manifold that conducted the solutions to a common outlet that was connected, in turn, to the inlet of the chamber. To decrease turnover time, the valves that controlled the flow from each of the condensers were positioned as close as possible to the common outlet. An IVAC Model 230 gravity flow controller (IVAC Corp; San Diego, CA) held the perfusion rate constant at 0.4 ml/min.

Double-distilled water was used to make up all solutions. The solutions contained 100.0 mM NaCl, 2.0 mM KCl, 5.0 mM glucose, 0.4 mM $MgCl_2$, and 0.4 mM $CaCl_2$ and were buffered at pH 7.8 with 20.0 mM Tris-Maleate. Tris-Maleate was selected as a buffer since it does not precipitate with $BaCl_2$ at the concentrations employed in this study. To isolate the late receptor potential of the excised, perfused retina, both control and experimental solutions contained

10.0 mM sodium aspartate. Although the slow PIII is not suppressed by sodium aspartate, this potential is undoubtedly much too slow to have any significant effect on the initial phase of the photoresponse.[7]

The stimulus was a 250-msec flash of white light. The light source, a quartz-iodine lamp, was calibrated to deliver an unattenuated flash with an intensity of 4700 $\mu W/cm^2$ to the retina. Appropriate neutral density filters were placed in the light path to attenuate the light by six log units. In this system a −log 6.0 stimulus is below the absolute threshold of bullfrog cones but elicits a good response from the rods. In addition, a dim stimulus intensity was chosen because Torre[2] showed that cardiac glycoside produces the transient increase in the rod receptor response only at dim stimulus intensities. Responses were carried to a Dynatron Model 977 instrumentation amplifier (Dynatron; Los Angeles, CA) by two chlorided silver electrodes positioned on opposite sides of the retina. Amplification was always capacitance-coupled with a time constant of 0.8 sec. A Grass kymograph camera (Grass Corp; Quincy, MA) was used to make permanent film records of the responses displayed on a Tektronix Model 5112 oscilloscope (Tektronix Corp; Beaver, OR).

Prior to actually beginning an experiment, a stabilization period of 1 hr was allowed, during which the superfused retina remained in the dark.

Results. Displayed in Figure 1 are the results of an experiment conducted to determine the effects of ouabain on the amplitude of the mass rod response. With the onset of light stimulation, the amplitude of the response increased with each successive flash from an initial value of 13.5 μV and eventually reached a stable plateau of 21.0 μV. This enhancement of the rod response is identical to that shown in previous work from this laboratory.[8] Shortly after addition of 0.1 mM ouabain (arrow), the receptor response first increased in amplitude from 21.0 μV to 28.5 μV and, then, progressively decayed in an exponential manner to almost zero. This finding qualitatively agrees with the intracellular work of Torre[2] on the toad, *Bufo marinus*.

Figure 2 shows the influence of barium on ouabain's effects. For each experiment, all values were normalized to the mean value of the twenty responses prior to changeover to ouabain. Each of the data points represents the average of three experiments. Following the standard 1-hr stabilization period in control Ringer, the retinas were superfused with solution from a second water jacketed condenser and light stimulation was begun. In the case of the retinas of the control group (filled circles), the second condenser contained the same control Ringer. In the case of the retinas of the experimental group (filled triangles),

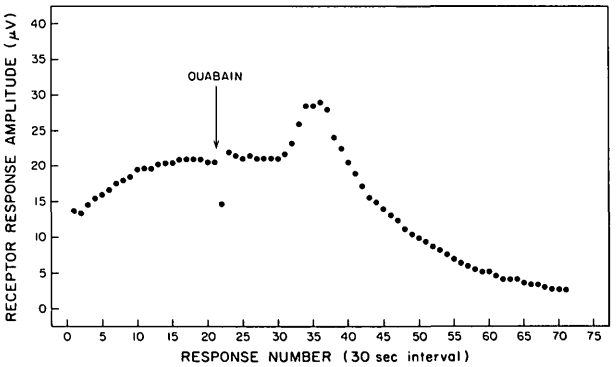

Fig. 1. Effect of ouabain on rod response amplitude. Changeover from control to experimental superfusate is indicated by the arrow. The severely depressed response recorded near the time of changeover is artifactual and due to manipulation of the valves. Ouabain concentration was 0.1 mM in the experimental superfusate. A 30-sec adaptation interval was employed between each stimulus.

the second condenser contained 0.4 mM barium Ringer. (To economize on space within the figure only the last 20 of the 60 responses prior to changeover to ouabain are shown.) In the absence of barium (filled circles in Fig. 2), changing over to a superfusate containing ouabain (arrow) resulted, as usual, in an increase in receptor response amplitude followed by a progressive decline. In sharp contrast, in the presence of 0.4 mM barium (filled triangles in Fig. 2), changing over to a superfusate containing ouabain (arrow) did not result in an increase in receptor response amplitude but did result in the progressive decline. Clearly, then, barium removed the ouabain-induced transient increase in rod receptor response amplitude.

The inset of Figure 2 shows the same data but shifted along the X-axis so that the decay portions of the two sets of points superimpose. This shows that while barium completely removed the ouabain-induced transient increase in the amplitude of the rod response, it had little, if any, effect on the rate of decay of the receptor response amplitude that is caused by ouabain.

Discussion. This study shows that barium ions remove the ouabain-induced increase in the amplitude of the rod receptor response. Barium does not prevent ouabain from ultimately eliminating the rod response and, in fact, does not even alter the rate of decay of the response due to ouabain. Thus, barium is not interfering with the ability of ouabain to block the sodium–potassium pump and to, thereby, eventually collapse the sodium and potassium gradients. Barium's effect on the ouabain-induced transient is consistent with an ability to reduce the electrogenicity of sodium–potassium pumps[4] and we find this possibility intriguing. Electrogenic sodium–potassium pumps have a coupling ratio greater than 1:1, ie, the net number of sodium ions transported out of the cell is greater than

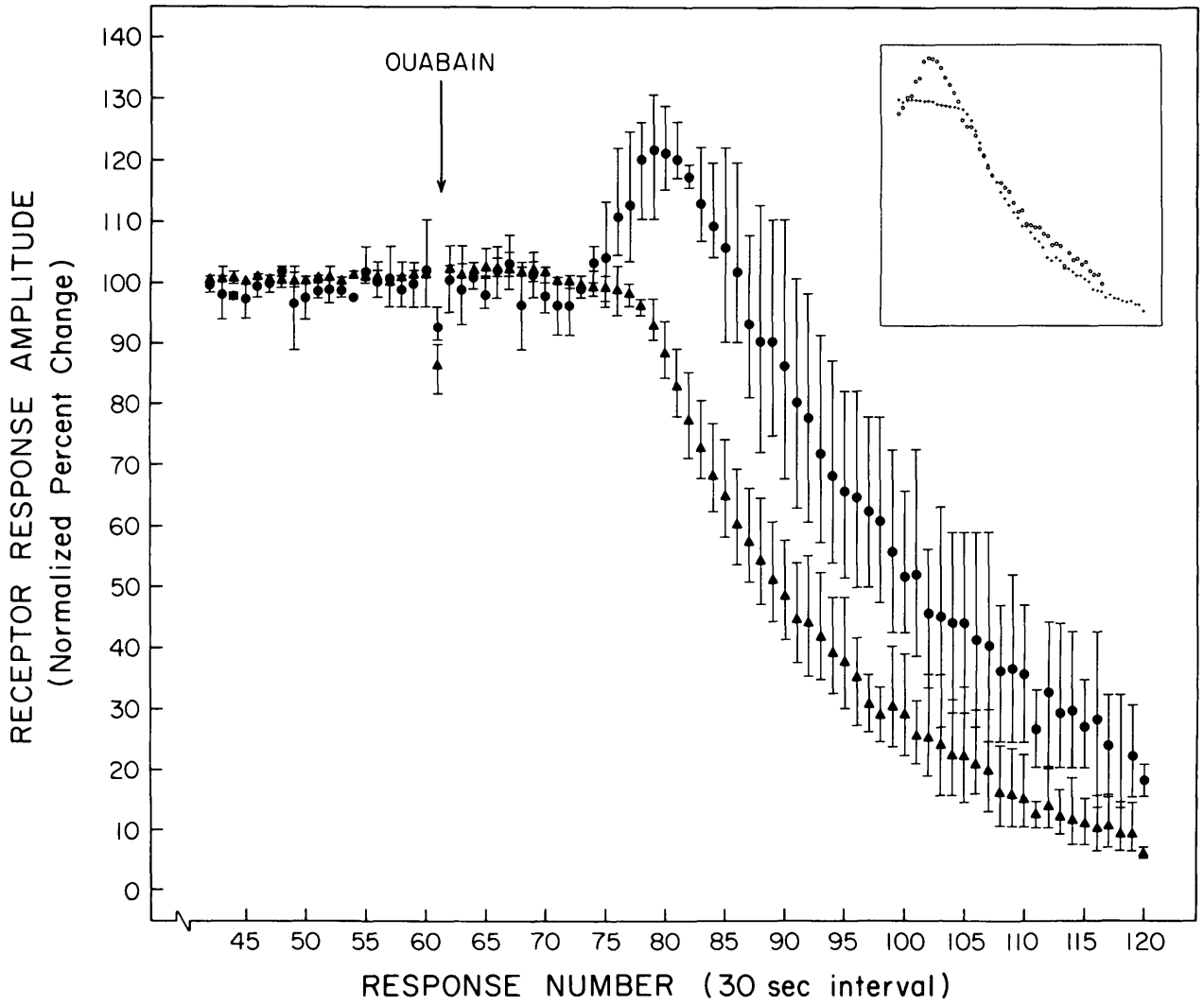

Fig. 2. Influence of 0.4 mM barium on ouabain's effect on the receptor response amplitude. For each experiment all values were normalized to the average of the 20 responses prior to changeover to ouabain. Each data point represents the average of three experiments. The filled circles represent mean values obtained with control Ringer and the filled triangles represent mean values obtained with barium Ringer. Error bars indicate ranges. Changeover to ouabain is indicated by the arrow. The severely depressed responses recorded near the time of the changeover are artifactual and due to manipulation of the valves. Ouabain and barium concentrations were 0.1 mM and 0.4 mM, respectively. A 30-sec adaptation interval was employed between each stimulus. The inset of the figure shows the data shifted along the X-axis so as to superimpose. Data obtained with control Ringer are represented by the open circles; data with barium Ringer, by the crosses.

the net number of potassium ions transported into the cell.[9] As a result of the difference in the exchange of ions, this type of pump acts directly to polarize the cell membrane and, therefore, has an effect on the potential difference beyond that which is due to the establishment of diffusion gradients.[9] Ouabain's earliest effect in blocking an electrogenic pump would be to reduce the membrane potential to the extent that it is due to uneven ion exchange. Such a depolarization, with the gradients still intact, would result in an increase in rod response amplitude. Only later, when the gradients begin to collapse, would the rod response decrease. This pattern of events is exactly what is seen upon treatment of the retina with ouabain or strophanthidin. However, if barium were to decrease the coupling ratio of the pump so that it approached 1:1, then ouabain's only effect would be to collapse the ionic gradients and no transient increase in rod response amplitude would be seen. The results of the present study, then, may indicate that barium acts by modifying the coupling ratio of an electrogenic sodium–potassium pump. However, other possible actions of barium that affect voltage-sensitive channels of the rod membrane and that, therefore, may have altered the rod receptor potential cannot be excluded at this time.

Key words: barium, ouabain, rods, electrogenic sodium–potassium pump

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From the Department of Animal Physiology, University of California, Davis, California. Supported in part by Grant No. ES-02444 from the National Institute of Environmental Health Sciences.* Present address: Dr. A. E. Walter, Jules Stein Eye Institute, School of Medicine, University of California, Los Angeles, CA 90024. Submitted for publication: May 1, 1984. Reprint requests: Dr. Arnold J. Sillman, Department of Animal Physiology, University of California, Davis, CA 95616.

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Auditory Testing of Dogs with Inherited Retinal Degeneration

Gregory M. Acland,*† Roger R. Marsh,‡ and Jerry W. Northington†

Auditory function was tested by brainstem-evoked response (BSR) audiometry in dogs affected by hereditary retinal degeneration (HRD). Comparison of BSR thresholds and latency–intensity functions revealed no significant difference between progressive rod–cone degeneration (PRCD) affected and unaffected miniature poodles, and no evidence of sensorineural hearing loss in HRD-affected English cocker spaniels and miniature schnauzers. The authors conclude that hearing loss is not a feature of the retinal degenerations in these dogs. Invest Ophthalmol Vis Sci 26:785–788, 1985

Hereditary retinal degenerations in the dog are known collectively as progressive retinal atrophy (PRA).[1] Specific forms of PRA have been well characterized in some breeds (eg, progressive rod cone degeneration (PRCD) in the miniature poodle[2] and are recognized but not fully characterized in other breeds.[1] The inference that PRA in different breeds represents different diseases has been proven in some cases[1] and is supported by the different ages of onset and rates of progression of the syndrome among the different breeds. PRA in the miniature poodle, the English cocker spaniel, and the miniature schnauzer is recessively inherited. In poodles and spaniels, PRA is late in onset and slowly progressive, but in schnauzers the onset is earlier and the progression more rapid. Affected dogs exhibit night blindness progressing to total blindness, abnormal electroretinograms, morphologic abnormalities of the photoreceptors and, ophthalmoscopically, progressive retinal thinning, attenuation of the retinal vasculature and pallor of the optic disc.[1] In all these respects PRA mimics retinitis pigmentosa (RP), a syndrome of hereditary retinal degeneration of man.[3]

Hearing loss is the most frequently noted extraocular symptom of persons affected with RP.[3] The association of sensorineural hearing impairment and RP defines Usher syndrome,[4] although this diagnosis is often reserved for cases where the hearing loss is both congenital and profound.[4] Nonetheless, regardless of the degree of hearing loss and the probability that some cases represent chance association, segregation analysis indicates that hearing impairment is significantly associated with RP is recessively inherited.[5] In differing clinical populations, the incidence of hearing impairment among RP patients ranges from 20 to 30%.[3–6]

Since PRA in the dog models RP in humans and because of the association between RP and hearing loss, we evaluated auditory function, by brainstem-evoked response audiometry (BSR), in dogs affected with PRA.

Materials and Methods. *Animals:* Seventeen miniature poodles, seven English cocker spaniels, and two miniature schnauzers were tested (Table 1). All were cared for, in a research colony for the study of