Photoreceptor-Bipolar Cell Transmission in the Perfused Retina Eyecup of the Mudpuppy

Abstract. *The hypothesis that a synaptic transmitter is released by photoreceptors in the dark is supported by experiments in which cobalt was used as a synaptic blocking agent, while intracellular recordings of receptors and neurons that are directly postsynaptic to receptors were maintained. In the dark the depolarizing bipolars are hyperpolarized, whereas the hyperpolarizing bipolars are depolarized.*

Experimental studies of the vertebrate retina suggest that photoreceptors release a transmitter in the dark and that light stimulation, which hyperpolarizes the receptors, leads to a reduction in the rate of transmitter release (*1*). At present, this evidence is primarily based on studies of horizontal cells which, because of their large size, provide a favorable target for intracellular electrophysiological studies. The light-evoked hyperpolarization of horizontal cells is associated with an increase in input resistance (*2*). In the skate retina, an increase in external Mg^{2+} abolishes the light response and hyperpolarizes horizontal cells (*3*). Presumably the effects of Mg^{2+} are due to a block of calcium-dependent transmitter release (*4*) from receptors to horizontal cells. Thus, conductance measurements and Mg^{2+} experiments suggest that receptors release a transmitter in the dark which depolarizes horizontal cells by a conductance increase mechanism.

The other cell receiving input from the receptors is the bipolar cell (BC), of which two types have been revealed by intracellular recording experiments (*5, 6*): one type (the HPBC) hyperpolarizes, and the second type (the DPBC) depolarizes in response to light stimulation. Conductance measurements show that the hyperpolarizing response of the HPBC is associated with an increase in input resistance (*7*), in agreement with the concept of a dark-released depolarizing transmitter. On the other hand, the light-evoked depolarization of the DPBC is associated with a resistance decrease (*7*), raising the possibility that this cell type is activated by a depolarizing transmitter released during light stimulation. An alternative suggestion that could account for this observation has also been advanced (*8*). A transmitter released in the dark could hyperpolarize the DPBC by a conductance decrease mechanism (that is, by closing ionic channels). Other possibilities should also be considered: the DPBC could be influenced by more than one synaptic mechanism, with the net result of these interactions producing a light-evoked conductance increase despite the fact that the receptor bipolar cell interaction results in a decreased conductance during light.

We have investigated some properties of synaptic transmission in the perfused mudpuppy (*Necturus maculosus*) eyecup, using Co^{2+} as a synaptic blocking agent while intracellularly recording from receptors and neurons that receive direct synaptic input from receptors (horizontal cells, DPBC's, and HPBC's). Details of the methods have been described elsewhere (*9*). A freshly excised mudpuppy eyecup was mounted in a chamber and continuously perfused with a Ringer solution (*10*), using HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer to maintain a *p*H of 7.8. The test solution contained 2 m*M* $CoCl_2$ in a Ringer solution that was otherwise identical to the control medium. Intracellular recordings were obtained with high-resistance, beveled micropipettes filled with 2*M* KCl or 2*M* potassium acetate. The use of small spot, diffuse, and annular light stimulation made it possible to identify the cell type according to the criteria of Werblin and Dowling (*5*). However, for the purpose of clarity, the responses illustrated in Fig. 1 were recorded during a period of intermittent diffuse light stimulation.

Figure 1a shows an example of a receptor recording during a 3-minute exposure to 2m*M* Co^{2+}. During the period of Co^{2+} perfusion the receptor response decreased in amplitude and was slightly hyperpolarized: a more prolonged Co^{2+} exposure (not shown) eventually abolished activity of some receptors (probably cones), which recovered slowly after a normal perfusate was introduced (*11*). This is an intriguing effect when one considers that calcium has been postulated as an intermediate ion in the mechanism of phototransduction (*12*) and that Co^{2+} blocks Ca^{2+} channels (*13*). The postreceptor neurons, however, were affected much more rapidly by Co^{2+}. Cobalt exposures of less than 1 minute abolished light-evoked activity of postreceptor neurons but had minor effects on receptors. Figure 1b illustrates the action of Co^{2+} on a horizontal cell. The loss of light responsiveness is associated with a large hyperpolarization of the cell, as previously reported in skate and turtle (*3, 14*). These effects are reversible upon introduction of a normal perfusate. Figure 1, c and d, were recorded from an HPBC and a DPBC, respectively. In these cells a diffuse light stimulus produced an initial transient response, followed by a sustained component due to interaction of

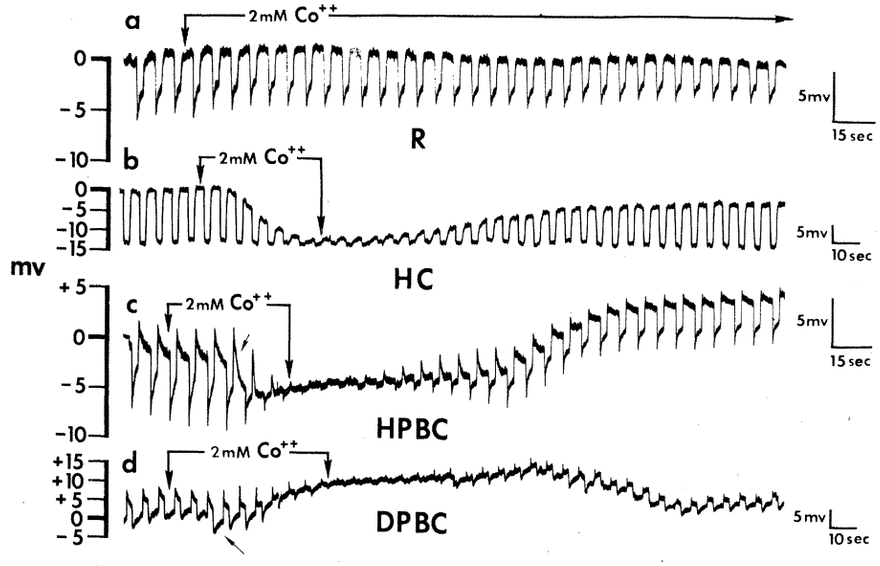

Fig. 1. Effects of 2 m*M* cobalt on intracellularly recorded responses obtained from a receptor (*R*), horizontal cell (*HC*), hyperpolarizing bipolar cell (*HPBC*), and depolarizing bipolar cell (*DPBC*). A diffuse intermittent light stimulus (irradiance 8.25×10^{-6} watt/cm^2) was used in all studies. Each trace is an uninterrupted display of the effects on the light response and membrane potential of changing the perfusate from a normal Ringer solution to a Ringer solution with 2 m*M* Co^{2+} added. The duration of Co^{2+} exposure is indicated. A 3-minute exposure had relatively little effect on receptors (some reduction in amplitude is observed). However, very brief (1 minute or less) exposures abolished the light-evoked activity of the postreceptor neurons. In the horizontal cell (b) and the HPBC (c) Co^{2+} application is associated with hyperpolarization. However, Co^{2+} perfusion results in a depolarization of the DPBC (d). In both bipolar cells the initial effect of Co^{2+} is an enhancement of transient on and off responses. Recovery of response amplitude in bipolar cells was incomplete after return to the initial perfusate, probably because of some deterioration of the cell. Positivity is indicated by an upward deflection.

center surround antagonism (*5, 6*). Application of Co^{2+} resulted in a hyperpolarization of the HPBC and a depolarization of the DPBC, associated with the loss of light response in both cells. During the early Co^{2+} exposure the transient "on" and "off" polarizations of these cells (arrows) were briefly exaggerated. With return to the normal perfusate, the light-evoked activity of both cell types did not recover to control response amplitudes and the membrane potentials were somewhat more depolarized than those observed before cobalt exposure. These effects were probably the result of cell deterioration, a common problem encountered with bipolar cell recordings.

To the extent that the principal effect of a brief exposure to Co^{2+} is to block synaptic transmission, the findings reported here are consistent with the view that a dark-released receptor transmitter depolarizes both horizontal cells and HPBC's but that it has a hyperpolarizing action on DPBC's. These findings do not support the idea that the DPBC is activated by a light-evoked transmitter release mechanism. If this were the case, Co^{2+} should abolish the light response but have a minimal effect on the membrane potential.

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11. The loss of light-evoked receptor activity has thus far been observed in recordings from receptors that are probably cones. The response of these cells is abolished within 6 to 10 minutes. Probable rod recordings have been maintained in Co^{2+} for periods up to 25 minutes with no detectable decrease in light-evoked response amplitude. Also, we have continuously perfused the isolated retina eyecup of the rabbit with 2 m*M* Co^{2+} and have noted the persistence of the rod-dominated PIII component for 45 minutes. Thus if rods are affected by Co^{2+}, an exposure of more than 25 minutes (mudpuppy) to 45 minutes (rabbit) is required.
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Horizontal Cell Potentials: Dependence on External Sodium Ion Concentration

Abstract. *The membrane potential of the horizontal cell of the axolotl is highly dependent on the extracellular concentration of sodium. Experimental results reported here are consistent with the suggestion that in the dark the receptors release a synaptic transmitter which increases primarily the sodium conductance of the postsynaptic membrane. Externally applied aspartate or glutamate depolarizes the horizontal cell membrane and eliminates the light response of the horizontal cell. However, it appears to increase the conductances of the postsynaptic membrane to several ions rather than just to sodium ions.*

One of the most useful techniques for investigating the ionic mechanism of neural potentials is to manipulate the ionic environment of a nerve cell while recording its membrane potential intracellularly. Recently this technique has been used to study the neurons in the vertebrate retina (*1–4*). We describe here our use of this technique to study the ionic mechanism of horizontal cell potentials in the isolated retina of the axolotl (*Ambystoma mexicanum*). The fact that the horizontal cell is a second-order neuron complicates the interpretation of results obtained by altering the ionic environment. Manipulations of ionic environment may influence receptor potentials and the synaptic interactions between receptors and horizontal cells, as well as the ionic mechanism of horizontal cells themselves. Therefore, we recorded intracellularly from both receptors and horizontal cells while we changed the ionic composition of the medium flowing over the receptor side of the retina.

In both rods and cones, illumination elicits a membrane hyperpolarization termed the receptor potential. Several studies have indicated that the receptor potential is caused by a decrease in the sodium conductance of the receptor membrane (*4, 5*). In horizontal cells, light elicits a hyperpolarization of the membrane (which we will call the horizontal cell potential), usually accompanied by a decrease in membrane conductance (*6*). It has been proposed (i) that in the dark, receptors release a transmitter which depolarizes the horizontal cells, and (ii) that the light-elicited hyperpolarization of the receptor terminals decreases transmitter release and thereby hyperpolarizes the horizontal cells (*7*). In support of this proposal, an increase in the external concentration of magnesium ions has been shown to hyperpolarize the horizontal cell membranes (*3, 8, 9*). This is the expected result if an increase in external magnesium ion concentration interferes with the release of transmitter in the photoreceptor synapse, as in the case of other chemical synapses (*10*).

For this study, young adult axolotls were dark-adapted for at least 8 hours and partially anesthetized by placing them on ice for an hour. The eye was removed and the eyeball hemisected under dim, red light. The eyecup was submerged in a pool of fresh, oxygenated solution in a bathing chamber. The retina was gently pulled away from the pigment epithelium and positioned receptor side up. The control bathing solution was composed of 109 m*M* NaCl, 2.4 m*M* KCl, 5 m*M* dextrose, 0.5 m*M* $MgCl_2$, 0.85 m*M* $CaCl_2$, 0.6 m*M* Na_2SO_4, 0.32 m*M* $NaHCO_3$, and 2.8 m*M*

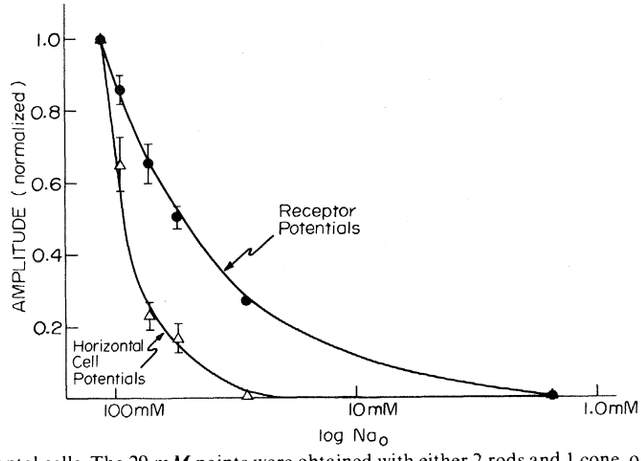

Fig. 1. Effect of decreasing Na_o on receptor and horizontal cell potentials. The horizontal cell potentials displayed much stronger dependence on Na_o than the receptor potentials. Data are from cells that recovered completely upon return to the control solution after being bathed in several different Na_o solutions. All points, except those for 29 m*M* Na_o (26 percent normal), were based on data obtained from either 14 rods and 3 cones, or 13 horizontal cells. The 29 m*M* points were obtained with either 2 rods and 1 cone, or 2 horizontal cells. For each cell, the amplitudes of the light responses were normalized with respect to the response amplitude for the same cell in the control bathing solution.