Ionic Mechanisms Underlying the Responses of Off-Center Bipolar Cells in the Carp Retina

II. Studies on Responses Evoked by Transretinal Current Stimulation

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ABSTRACT Transretinal current pulses flowing from the receptor side to the vitreous side of the retina cause transient release of transmitter from the photoreceptor terminals, and in off-center bipolar cells they evoke transient depolarizations with a brief (<1 ms) synaptic delay. Since it is known that the presence of Na⁺ in the external medium is not essential for this type of transmitter release, we used this procedure to examine the role of \([\text{Na}^+]_o\) in the generation of light-evoked responses (hyperpolarizing to spot illumination in the receptive field center and depolarizing to an annulus in the surround) of this type of bipolar cell. When the cell membrane was steadily depolarized by current injection through the recording microelectrode, the depolarizing response evoked by the transretinal current pulses decreased in amplitude and reversed its polarity at above +45 mV. Conversely, the response amplitude increased when the cell was steadily hyperpolarized. The reversal potential seems to be lowered in low \([\text{Na}^+]_o\) (28 mM). Removal of Na⁺ from the superfusate hyperpolarized the cell and both the light-evoked and current-evoked responses disappeared. From these observations, it is hypothesized that the hyperpolarizing center response of the off-center bipolar cells is a result of removal of sustained depolarization produced by sodium permeability increase.

INTRODUCTION

It is widely believed that the neurotransmitter of photoreceptors in vertebrate retinas is tonically released during darkness, and that when the retina is illuminated, photoreceptors hyperpolarize and the amount of transmitter release reduces (Trifonov, 1968; Dowling and Ripps, 1973; Cervetto and...
Piccolino, 1974; Kaneko and Shimazaki, 1975). In the receptive field center of carp off-center bipolar cells, a spot of white illumination evokes a hyperpolarizing response. In the preceding paper (Saito and Kaneko, 1983), we postulated that the transmitter of the photoreceptor depolarizes off-center bipolar cells in the dark by increasing membrane permeability to Na⁺, and that the light-evoked hyperpolarization is generated by a decrease of Na⁺ permeability as a result of removal of endogenous transmitter.

The evidence that supports the above hypothesis is as follows: first, the resistance of off-center bipolar cells mainly increases during spot illumination; second, the reversal potential of the light-evoked response is above zero membrane potential and close to the presumable equilibrium potential of Na⁺.

We hoped to demonstrate that substitution of external Na⁺ with impermeable cations would eliminate the light response of off-center bipolar cells if the response is Na⁺ dependent. However, if the external Na⁺ concentration is lowered, photoreceptors hyperpolarize and their light responses disappear (Cervetto 1973; Brown and Pinto, 1974; Kaneko and Shimazaki, 1975). Therefore, even if membrane hyperpolarization is seen in Na⁺-free medium, it could be an indirect effect secondary to changes occurring in photoreceptors. It is the aim of the present study to examine the direct role of [Na⁺]₀ in off-center bipolar cells.

Studies of vertebrate neuromuscular junctions have shown that release of the endogenous transmitter can be induced by placing the presynaptic terminal in an electric field in such a way that it is depolarized (Katz and Miledi, 1967a, b). For this type of release, Ca²⁺ must be present in the external medium, whereas the presence of Na⁺ is not essential (Katz and Miledi, 1967b). Since photoreceptors are elongated cells lying perpendicular to the retinal layers, it is possible that receptor terminals could be depolarized when a current was passed across the retina from the receptor side to the vitreous side. It has been shown that transretinal current pulses in this direction can evoke depolarizing responses in horizontal cells (Trifonov, 1968; Byzov and Trifonov, 1968). Similar transient depolarizations have also been observed in off-center bipolar cells (Kaneko and Shimazaki, 1976; Toyoda et al., 1977). These transient depolarizations have been abolished by the application of synaptic blocking agents, such as Co²⁺ ions (Toyoda et al., 1977). It is therefore probable that the transient depolarizations are induced by the endogenous transmitter released from receptor terminals.

To examine the role of [Na⁺]₀ in the generation of the light-evoked responses of off-center bipolar cells, we studied the properties of these current-evoked transient responses.

A preliminary report has been presented at the Japanese Neuroscience Society Meeting (Kaneko and Saito, 1980).

METHODS

Materials and methods were similar to those in the preceding paper (Saito and Kaneko, 1983), adapted as follows for transretinal current stimulation (Kaneko and
Shimazaki, 1975, 1976). Fig. 1 illustrates the recording setup. The bottom of the recording chamber was inlaid with a circular silver plate (5 mm diam). The surface of the plate was chlorided and used as one of a pair of current electrodes. A chlorided silver wire, immersed in 3 M KCl solution and connected to the recording chamber with an agar bridge, served as the second current-passing electrode. The amount of current was monitored from the voltage drop across a 100-Ω resistor inserted in the current circuit. In this setup, it seems likely that the current passed through the retina almost radially and with uniform density at least in the central portion of the preparation, since the retina (~10 mm diam, ~250 μm thick) was mounted flat, so as to cover the entire surface of the circular silver plate. Recordings were made in the central part of the retina, i.e., above the center of the silver plate. To minimize the stimulus artifacts, two microelectrodes were connected differentially to the preamplifier: one, the active electrode, penetrated a bipolar cell; the other, the reference electrode, was placed in the medium near the surface of the preparation. The minimal current to evoke responses in bipolar cells was on the order of \(10^{-4} \text{ A/cm}^2\) (~5 ms duration), close to the value necessary for evoking transient depolarizing responses in horizontal cells (Kaneko and Shimazaki, 1976).

The isolated retina was superfused with artificial salines of various ionic compositions. The volume of the chamber was ~0.2 ml and the medium (20 ± 2°C) was continuously replaced at a flow rate of 0.5–1.0 ml/min. Only the receptor side of the

![Diagram showing the setup. The isolated retina was mounted, receptor side up, in a chamber where oxygenated artificial saline was continuously supplied. Intracellular responses were recorded differentially between the two microelectrodes, one penetrating the cell and the other placed in the bath near the penetrated cell. Current pulses were applied between the saline and the inlaid Ag-AgCl plate below the retina. Saline in the recording chamber is connected to the current source through an agar bridge, a KCl chamber, and an Ag-AgCl wire dipped in the KCl. A voltage drop across the resistor (100 Ω) in series with the current source is used to monitor the amount of current.](image-url)
RESULTS

Response of Off-Center Bipolar Cells to Transretinal Current Pulses

In response to a brief current pulse (~1 ms duration) flowing from the receptor side to the vitreous side, i.e., in the direction to depolarize receptor terminals, the off-center bipolar cell showed a transient biphasic response (Fig. 2A). The threshold in the current intensity was ~2.5 mA/cm² (0.5 ms duration), and the response amplitude increased with the current strength, within a certain range. The threshold and the effective range of stimulus intensities were similar to those required to elicit similar responses in horizontal cells (Kaneko and Shimazaki, 1976).

Illumination affected the waveform of the current-evoked responses. In the dark, the electrically evoked response consisted of an initial depolarization...
followed by a hyperpolarization. During illumination covering the receptive field center, the amplitude of the initial depolarizing component of the current-evoked response was notably enlarged, particularly in comparison with the subsequent hyperpolarizing component.

Brief current pulses flowing from the vitreous side to the receptor side were ineffective (Fig. 2B), as they were ineffective in horizontal cells (Kaneko and Shimazaki, 1976). They could not evoke any detectable responses even at an intensity 100 times higher than the threshold intensity delivered in the effective direction.

![Graph showing membrane polarization effects on the light-evoked and current-evoked responses of an off-center bipolar cell. Control membrane potential in the dark was -21 mV. The cell was hyperpolarized by injecting -3.5 nA negative current through the recording microelectrode. Note that both the light-evoked and current-evoked responses were enhanced. When the membrane was depolarized by injecting +6.0 nA current, both the light-evoked and current-evoked responses reversed their polarities. Other conditions are identical to those of Fig. 2A.]

**Figure 3.** Effects of membrane polarization on the light-evoked and current-evoked responses of an off-center bipolar cell. Control membrane potential in the dark was -21 mV. The cell was hyperpolarized by injecting -3.5 nA negative current through the recording microelectrode. Note that both the light-evoked and current-evoked responses were enhanced. When the membrane was depolarized by injecting +6.0 nA current, both the light-evoked and current-evoked responses reversed their polarities. Other conditions are identical to those of Fig. 2A.

**Reversal Potential of the Current-evoked Responses**

To determine the ionic species that is responsible for the current-evoked responses of off-center bipolar cells, the reversal potential was determined by shifting the membrane potential. Fig. 3 shows the current-evoked responses of
an off-center bipolar cell both in the dark and during illumination, and the
effect of membrane polarization on these responses, as an example of a similar
experiment on seven cells. When the membrane was hyperpolarized, both the
light-evoked hyperpolarization and the current-evoked response were en-
hanced. Of the two components of the current-evoked responses, the initial
depolarization was markedly enhanced, whereas the amplitude of the after-
hyperpolarization was not as strongly enhanced. Both the light-evoked and
the current-evoked responses were suppressed by membrane depolarization.
When the cell was strongly depolarized, their response polarities could be
reversed, as seen in Fig. 3. The reversal potential was almost equal for both
the light-evoked and current-evoked responses.

To increase the chance of penetration of off-center bipolar cells, which are
generally small, we used single pipettes with fine tips. These pipettes, however,
often showed rectification by themselves, or became noisy when a large
amount of current was passed, as seen in the example of Fig. 3. It is nevertheless
apparent that both the photoresponse and the current-evoked response re-
versed their polarities. In these experiments, the reversal potential was esti-
lated as follows:

\[ E_R = E_D + I \cdot R, \]

where \( E_R \) is the reversal potential, \( E_D \) is the dark membrane potential, \( I \) is the
amount of injected current with which potential reversal was observed, and \( R \)
is the input resistance of the cell. For \( R \), we used a value of 15 M\( \Omega \), the mean
slope resistance obtained in the preceding paper (Saito and Kaneko, 1983).
For the recording illustrated in Fig. 3, \( E_D \) was -30 mV and \( I \) was +5 nA;
thus, \( E_R \) was estimated to be +45 mV. Reversal potential in another cell was
about +20 mV. We were unable to depolarize the remaining five cells enough
to invert the responses.

Effect of Na\(^+\) Removal on the Current-evoked Responses

Fig. 4 shows one example of four similar experiments in which the effects of
Na\(^+\)-free solution were examined on the current-evoked responses in off-center
bipolar cells. Within 150 s after the medium was switched from normal saline
to low-[Na\(^+\)]\(_o\) medium, the dark membrane potential became slightly hyper-
polarized and the amplitudes of the light-evoked responses decreased. How-
ever, the amplitude of the current-evoked responses was enhanced. This
contradictory phenomenon is understandable if we assume that the [Na\(^+\)]\(_o\) of
the medium around the photoreceptors decreased more rapidly than that
around the bipolar cell dendrites. At \( \sim 200 \) s after the solution change, the
current-evoked response also decreased in amplitude and eventually became
undetectable. When the superfusing medium was returned to the normal
saline, the dark membrane potential, the light-evoked responses, and the
current-evoked responses all reappeared in a sequence opposite to the order in
which they were altered after Na\(^+\) removal.

Next, we examined the reversal potential of current-evoked responses in
low-[Na\(^+\)]\(_o\) media. Fig. 5 illustrates one of the two cells tested in low [Na\(^+\)]\(_o\).
After exposure of the retina for 10 min to superfusate whose \([\text{Na}^+]_0\) medium was 28 mM, the light-evoked responses of this cell became undetectable. At the same time, current-evoked responses could still be observed. Furthermore, as in Fig. 3, hyperpolarization of this off-center bipolar cell by steady extrinsic current increased the amplitude of the current-evoked responses, and a steady depolarization completely reversed the response polarity. The reversal potential was approximately +5 mV. The reversal potential estimated in this experiment may include some errors, but it was less positive than the reversal potential obtained in normal Ringer. It was also noted that the response time course became slower when the membrane was polarized in either direction. The change in time course is particularly obvious in the reversed responses.

**DISCUSSION**

*The Nature of the Responses*

The response evoked in off-center bipolar cells by a brief transretinal current stimulation consisted of a transient depolarization followed by a hyperpolarizing transient. It has been also reported (Byzov and Trifonov, 1968; Kaneko and Shimazaki, 1976) that horizontal cells respond to the same kind of electric stimulation with similar transient depolarizations. It has been suggested (Byzov and Trifonov, 1968) that the stimulating current pulse depolarized the photoreceptor terminals, letting them release the endogenous transmitter. Although no direct demonstration is available for polarization of photoreceptor terminals by the transretinal current, this idea has widely been accepted because the photoreceptors have a morphology and location most suitable to be affected by the transretinal current stimulation.

It is also believed that photoreceptors release their endogenous transmitter substance tonically during darkness and that the release decreases in amount when they are hyperpolarized by illumination. By applying current pulses of relatively long duration, it might be possible to control the amount of
transmitter release. In fact, in horizontal cells, it has been shown that application of transretinal current pulses of relatively long duration (>100 ms) can evoke more or less sustained potential changes in the postsynaptic cells: the current flowing from the receptor side to the vitreous side evokes a steady depolarization and that flowing from the vitreous side to the receptor side evokes a steady hyperpolarization (Kaneko and Shimazaki, 1976). However, if the current duration is limited within a few milliseconds, only the current flowing from the receptor side to the vitreous side is effective, and the current flowing in the opposite direction (vitreous to receptor side) becomes ineffective. The nature of this difference in effectiveness of currents of the opposite direction is not well understood, but it might be related to the current-voltage relationship of photoreceptor terminal membrane, which is more readily depolarized than hyperpolarized by the same amount of current.

The notion that the current-evoked brief depolarization of off-center bipolar cells is the postsynaptic response to depolarization of photoreceptor terminals is supported by the present findings. First, the current-evoked response has a

![Figure 5](image-url)
latency of \( \sim 0.5 \) ms, comparable to that needed to cross a single synapse. This value is close to the latency found in horizontal cells (Kaneko and Shimazaki, 1976). Second, the initial depolarization of the current-evoked response of off-center bipolar cells had a reversal potential close to that of the light-evoked responses. Furthermore, the current-evoked responses of both off-center bipolar cells and horizontal cells disappear after the application of \( \text{Co}^{2+} \) into the superfusate (Kaneko and Shimazaki, 1976; Toyoda et al., 1977). \( \text{Co}^{2+} \) ions are known to block the transmitter release from the presynaptic terminals.

The origin of the late component seems more complicated. Because its polarity is opposite to the initial component, it is natural to correlate it with the light-evoked response in the receptive field surround. It is supposed that for responses in the receptive field surround, bipolar cells are thought to be driven by horizontal cells (Toyoda and Tonosaki, 1978). In fact, the time course of the late component is similar to that of the current-evoked transient depolarization of horizontal cells. The pathway from horizontal cells to bipolar cells may involve the feedback route from horizontal cells to photoreceptors, but the presence of a direct pathway from horizontal to bipolar cells cannot be ruled out. Furthermore, it has been shown morphologically (Witkovsky and Dowling, 1969; Kaneko et al., 1980) that amacrine cells make numerous feedback contacts on bipolar cells axon endings. The physiology of these synapses is not yet understood. From the response polarity and longer latency of the late component, we have speculated that the late component might be produced by the activation of some (or all) of these lateral pathways.

**Ionic Mechanisms of Current-evoked Responses**

The initial depolarizing component of the current-evoked responses reversed their polarities at \( \sim 50 \) mV above zero potential level. For technical reasons accompanying single-electrode experiments, the reversal potential was estimated from the amount of passed current on the assumptions that the cell's input resistance was 15 M\( \Omega \), that the cell's \textit{I-V} relationship was linear within the range we tested, and that the electrode was not rectifying. We anticipated that these conditions might not be fully satisfied, but the reversal potentials estimated in the present experiments were close to those of the light-evoked response obtained in the preceding work (Saito and Kaneko, 1983) using double-barreled electrodes.

The fact that the reversal potential of current-evoked responses of off-center bipolar cells is above zero membrane potential and that it decreased in low \([\text{Na}^+]_o\), is a good indication that the initial depolarizing component is produced by a transient increase in the conductance of the off-center bipolar cell to \( \text{Na}^+ \). The present observations are consistent with the hypothesis that the transmitter released from photoreceptor terminals depolarizes off-center bipolar cells by increasing membrane permeability mainly to \( \text{Na}^+ \), and that hyperpolarizing light-evoked responses are generated by lowering membrane permeability to \( \text{Na}^+ \).

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