Ionic Mechanisms of Two Types of On-Center Bipolar Cells in the Carp Retina

I. The Responses to Central Illumination

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ABSTRACT   Properties of the depolarizing response of on-center bipolar cells to a light spot stimulus were studied in the carp retina. On-center bipolar cells were classified into two types, cone-dominant and rod-dominant, according to their major input from cones and rods. Cone-dominant bipolar cells responded to spectral light with the maximum amplitude near 625 nm, suggesting major input from red cones. The response was accompanied by a resistance increase and showed a reversal potential at -63 ± 21 mV when the membrane was hyperpolarized by current. The results suggest that the photoresponse of cone-dominant cells is due to a decrease of g_K and/or g_{Cl}, membrane conductances to potassium and chloride, respectively. Rod-dominant bipolar cells responded to spectral light with the maximum amplitude near 525 nm under scotopic conditions and near 625 nm under photopic conditions, providing evidence that they receive input from rods and red cones. In the scoptopic condition their response was accompanied by a resistance decrease and showed a reversal potential at 29 ± 13 mV, whereas in the photopic condition the response in most of them was accompanied by a resistance increase, at least in their initial part and showed a reversal at -53 ± 11 mV. The results suggest that the photoresponse activated by rod input is due to an increase in g_{Na}. In the mesopic condition rod-dominant cells showed complex electrical membrane properties as the result of electric interaction between the above two different ionic mechanisms activated by rod and cone inputs.

INTRODUCTION

Bipolar cells respond to light with graded potentials. They have a receptive field organization consisting of concentric and antagonistic center and surround zones. It is generally accepted that their response to central illumination is the result of a direct synaptic input from photoreceptors, whereas the response to surround illumination is mediated by the activity of horizontal cells. (Werblin and Dowling, 1969; Kaneko, 1970; Matsumoto and Naka, 1972; Toyoda, 1973; Schwartz, 1974; Fain, 1975; Yazulla, 1976).

In the fish retina, bipolar cells that respond to a center spot of light with depolarization and to annulus with hyperpolarization have been termed the

"on-center bipolar cells" (Kaneko, 1973). On-center bipolar cells identified by procion yellow staining (Kaneko, 1970; Famiglietti et al., 1977) include large and small bipolars of Cajal (1892). Cajal considered that the small bipolar cells have connection exclusively with cones, whereas the large bipolar cells have connection exclusively with rods. The former are often referred to as "cone bipolars" and the latter as "rod bipolars" (cf. Parthe, 1972). Recent studies with Golgi-electron microscope (EM) technique, however, have demonstrated that large bipolar cells make synaptic contacts with both rods and cones (Stell, 1967; Stell et al., 1977; Scholes and Morris, 1973; Scholes, 1975). Physiological studies also suggest a convergence of rod and cone signals onto some of the large bipolar cells in the fish retina (Saito et al., 1978; Kaneko and Tachibana, 1978).

The present experiments are concerned with the ionic mechanisms of depolarizing center response of on-center bipolar cells in the carp. Previous studies on the electrical properties of on-center bipolar membrane (Toyoda, 1973; Nelson, 1973; Toyoda et al., 1977) have shown that the depolarizing response is accompanied by a resistance decrease, is augmented by hyperpolarization of the membrane by current, but is suppressed or inverted by depolarization of the membrane. These results suggest that the depolarizing response is due to an increase in the membrane permeability to ions having an equilibrium potential toward or beyond the zero potential level. Kaneko and Shimazaki (1975) suggested from perfusion experiments of the carp retina that sodium ions are important in the generation of the depolarizing response, whereas Miller and Dacheux (1976) suggested from similar experiments on the mud-puppy retina that the response is sensitive to chloride.

Recently (Toyoda et al., 1977), it has been reported that on the carp retina more than one ionic mechanism is involved in the depolarizing response of on-center bipolar cells. Further experiments (Saito et al., 1978), though preliminary, suggested two ionic mechanisms, one related to rod inputs and the other to cone inputs. The present experiments are an extension of our preliminary analyses concerning the ionic mechanisms specific to rod and cone inputs.

METHODS

Preparation

Carps (*Cyprinus carpio*) of 25–30 cm in length were used in the present experiments. They were kept in cold (10°C) aerated tap water and dark-adapted for at least 30 min before experiments. Under dim red light, the eye was excised and the retina was isolated from the pigment epithelium. The isolated retina was set receptor-side up in a moist chamber provided with a Ag-AgCl wire serving as the indifferent electrode. The chamber was placed on an X-Y micrometer-driven stage.

Light Stimulus

Fig. 1 shows a schematic diagram of an experimental setup. The test light beam from a quartz-iodine lamp was guided to the retina through a lens and mirror system. The intensity of light was attenuated by inserting neutral density filters, covering a range of 6.0 log units, in the light path. An intensity of light of −4.0 log units, which roughly corresponds to 3 lm/m², was commonly used during penetration because it activated both rod and cone systems without changing much the state of dark adaptation at stimulus

intervals of 2.5 s generally used in these experiments. The size of the light spot on the retinal surface was controlled by inserting diaphragm into the light path and was variable from 350 μm to 2.5 mm in diameter. The smallest spot was usually used for central illumination. The annular illumination occasionally used to test the center-surround organization of bipolar receptive fields was 600 μm in inner and 2.5 mm in outer diameter. Monochromatic lights (475, 525, 575, 625, and 675 nm) were obtained by narrow-band interference filters in the light path. Their quantal flux has been adjusted equal by neutral density filters. Some experiments were performed under diffuse background light of 500 nm from a small tungsten lamp with an interference filter.
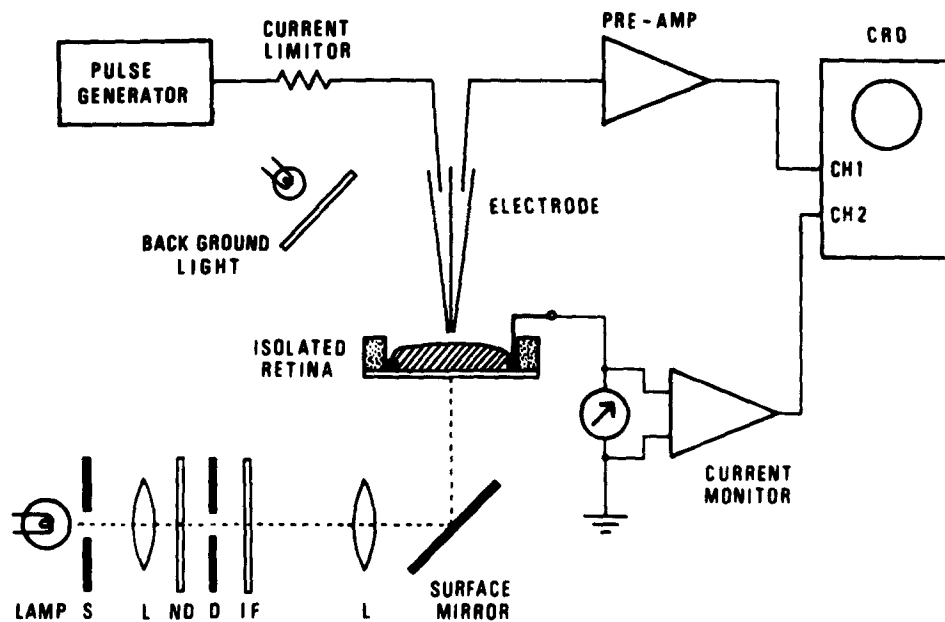
FIGURE 1.   Schematic diagram of experimental arrangement. S, shutter; L, lens; ND, neutral density filter; D, diaphragm; IF, interference filter; CRO, dual-beam cathode-ray oscilloscope.

Electrode

Micropipettes were made from a glass capillary tubing with a thin fiber fused to the inside wall. Single- or double-barreled microelectrodes, both filled with 2.5 M KCl solution and of 60–150 MΩ resistance, were used for intracellular recording and for current injection. The coupling resistance of the double-barreled electrodes measured in the vitreous was usually 0.5–2 MΩ. One barrel of double-barreled electrode was connected to a high impedance negative-capacitance preamplifier (M701, W-P Instruments, Inc., New Haven, Conn.) and the other to a current generator through a 100-MΩ resistor. The current was measured by an electronic galvonometer with an input impedance of 1 KΩ (Yokogawa 2709, Yokogawa Corp. of America, Elmsford, N.Y.) placed between preparation and ground. For intracellular marking experiments, electrodes were filled with 6% procion yellow. The resistance of these electrodes was 200–500 MΩ.

The retina was penetrated from the receptor side. An electrode placed at the center of

the light spot was advanced vertically. Penetration of the electrode into a neuron was facilitated by bringing the electrode tip into oscillation by increasing the capacity-compensating feedback (Baylor et al., 1971). Experiments were performed in a room temperature of about 21°C.

RESULTS

Classification of On-Center Bipolar Cells According to Their Response Properties

Responses of on-center bipolar cells were usually recorded at a depth of 70–100 μm from the receptor surface, just distal to the level of horizontal cells. They were easily identified from other responses by their polarity and wave form, and also by their receptive field organization.

The on-center bipolar cells could be grossly classified into two types, cone-dominant and rod-dominant according to the wave form of response, sensitivity to light, and spectral response characteristics. The following are some details of the response properties of these two types of on-center bipolar cells.

INTENSITY-RESPONSE AMPLITUDE CURVE Fig. 2 shows the relation between the peak amplitude of the response and the stimulus intensity for the two types of
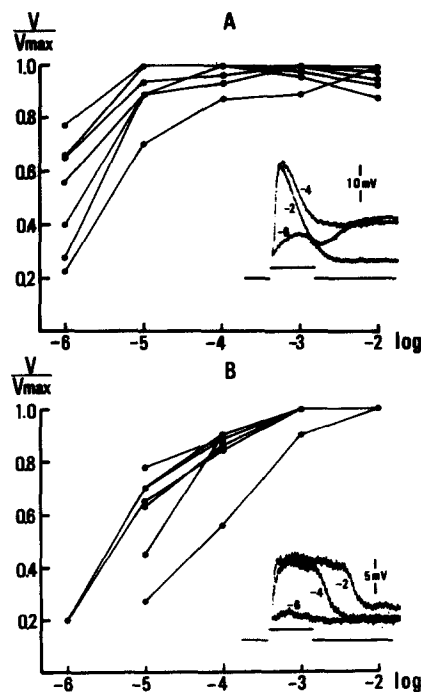

FIGURE 2. Relation between the response amplitude and stimulus intensity for two types of on-center bipolar cells. (A) Records from seven rod-dominant bipolar cells. (B) Records from seven cone-dominant bipolar cells. Sample records of responses from two types of bipolar cells are shown in the superimposed tracings of the inset in A and B. Each light stimulus of 350 μm diameter was applied after the response returned to the base line.

bipolar cells recorded in the dark-adapted retina. Each light stimulus was applied after the preceding response returned to the base-line. The amplitudes plotted were normalized with respect to the maximum response amplitude of the unit. The responses increased in amplitude with increasing intensity of light until they were saturated at certain intensities. Fig. 2 A shows the intensity-amplitude curves of rod-dominant cells. They reach a saturation at about −4.0 log units, although there is a slight variation among units. At higher intensities, some units showed a slight decrease in amplitude rather than an increase. This is probably due to scattering light which tends to activate the antagonistic surround field. Fig. 2 B shows the intensity-amplitude curves of cone-dominant cells. Their responses reach the maximum amplitude at a light intensity about 1.0 log unit higher than for the former type.

The two types of bipolar cells were also different in the wave form of response as illustrated by superimposed tracings in the *insets* of Fig. 2. Rod-dominant cells responded to light, except at low light intensities, with a transient depolarization followed by a plateau. Although the transient became more prominent with increasing light intensities, its amplitude and duration slightly decreased by further increase in the light intensity, probably reflecting the effect of scattering light. The level of the plateau was also decreased slightly, but there was a prominent prolongation of the plateau at high light intensities. The higher the light intensity, the more prolonged the plateau. At a light intensity of −2.0 log units, the response returned to the base line with a time-course of over 15 s. Cone-dominant cells responded to light with more or less rectangular wave forms. At an intermediate light intensity, the duration of response was about the same as that of illumination. The response of cone-dominant cells was also prolonged at very high intensities, outlasting the period of illumination, but it returned to the base line much faster than that of rod-dominant cells.

Except for the experiments described above, light stimulation was usually repeated at a constant interval of about 2.5 s. The two types of bipolar cells under this stimulus condition were distinguished when the light intensity was increased in steps of 1.0 log units. Examples are shown in Fig. 3. The response of rod-dominant bipolar cells (Fig. 3 A) begins to be saturated at an intensity of −4.0 log units as in the previous experiments. Further increase in the intensity results in a marked decrease in the response amplitude partly because the response to each stimulus begins to be superimposed on the prolonged plateau depolarization of the preceding response and partly because of the effect of light adaptation. The response to each stimulus becomes barely discernible except for the early transient at the light intensity of −2.0 log units. The response of cone-dominant bipolar cells (Fig. 3 B) saturated at the light intensity of −3.0 log units. Although there is a slight decrease in amplitude and a slight prolongation of the response at higher intensities, their response to each stimulus is clearly seen even at the light intensity of −2.0 log units.

In 64 rod-dominant bipolar cells which gave stable intracellular recordings, the membrane potential in the dark was −37 ± 8 mV (mean ± SD). The membrane potential of 13 cone-dominant bipolar cells was −28 ± 7 mV.

SPECTRAL RESPONSE CURVE Response patterns of the two types of bipolar cells to a series of monochromatic lights are shown in Figs. 4 and 5. Spectral
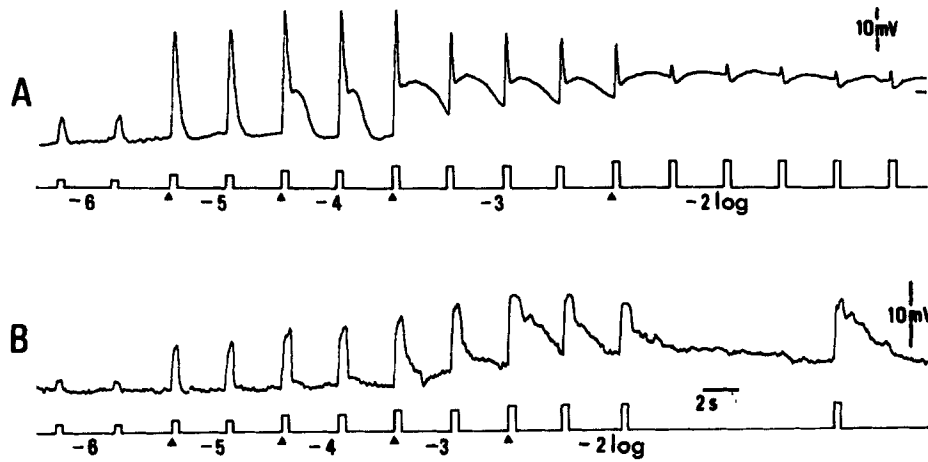
FIGURE 3. Responses of two types of bipolar cells to repetitive light stimuli increasing in intensity in steps of 1.0 log units. (A) Responses of rod-dominant bipolar cell. (B) Responses of cone-dominant bipolar cell. The retina was illuminated every 2.5 s by a white light spot of 350 μm diameter. The relative intensity is indicated in log units below each stimulus trace and is also indicated by the height of stimulus marks. The light intensity was increased at a point indicated by a triangle.
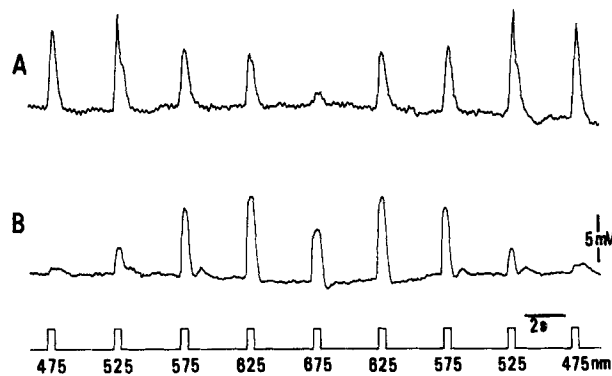

FIGURE 4. Spectral-response curves of two types of on-center bipolar cells recorded under mesopic conditions. (A) The response of a rod-dominant bipolar cell. (B) The response of a cone-dominant bipolar cell. A series of monochromatic lights of equal quantal flux were presented to the retina starting at 475 nm, increasing in 50-nm steps to 675 nm and then decreasing back to 475 nm.

responses were obtained under mesopic conditions in 16 rod-dominant bipolar cells and 5 cone-dominant bipolar cells. In Fig. 4, monochromatic lights were successively applied from 475 to 675 nm and back in 50-nm steps. In 4 of 16 rod-dominant cells the response amplitude peaked at 525 nm (Fig. 4 A), corresponding approximately to the rod pigment of the goldfish retina measured by microspectrophotometry (cf. Liebman, 1972). In 12 other cells, however, the

response to 525 nm light was almost the same amplitude as that to 575 nm (Fig. 5 A). Under diffuse chromatic adaptation with 500 nm, the maximum response amplitude of these cells shifted to either 575 or 625 nm (Fig. 5 B). This shift suggests that they receive inputs from both rods and red cones. Additional evidence in support of this will be given later when the electrical properties of these cells are discussed. In three of five cone-dominant cells, the response amplitude peaked at 625 nm (Fig. 4 B) which fits the data of the spectral absorbance and the spectral response of red cones (Marks, 1965; Tomita et al., 1967). The other two cells showed a response maximum at 575 nm, indicating that they receive inputs at least from two kinds of receptors, such as red cones, and rods or green cones.

The procion yellow dye was injected iontophoretically into rod- and cone-dominant bipolar cells after studying their response properties. All of six rod-dominant cells and two cone-dominant cells identified by the dye injection corresponded to Cajal's large and small bipolar cells, respectively. There is anatomical evidence that Cajal's large bipolars receive inputs from rods and red cones (Scholes, 1975; Scholes and Morris, 1973; Stell, 1967). Anatomical (Lasansky, 1973) and physiological (Fain, 1975) evidence that rod and cone signals converge onto single bipolars has also been shown in the amphibian retina.

FIGURE 5. Spectral-response curves of a rod-dominant bipolar cell recorded under the two different states of adaptation. Upper trace shows the spectral-response curve obtained under low mesopic condition. Lower trace shows the spectral-response curve of the same unit recorded in the presence of a diffuse background light of 500 nm.

Electrical Membrane Properties of On-Center Bipolar Cells

To determine ionic mechanisms underlying the bipolar cell response, it is useful to study the membrane resistance changes associated with the photoresponse and to determine the membrane potential level at which the response reverses its polarity.

MEMBRANE RESISTANCE CHANGE Fig. 6 shows membrane resistance changes accompanying responses of rod- and cone-dominant bipolar cells. Resistance changes were measured using a bridge circuit built in the preamplifier (M701, W-P Instruments) designed to apply negative current pulses through the intracellular electrode. A control and bridge record of one of the rod-dominant bipolar responses to −4.0 log units light stimulus is shown in Fig. 6 A. Rod-dominant bipolar response is accompanied by a resistance decrease as judged by
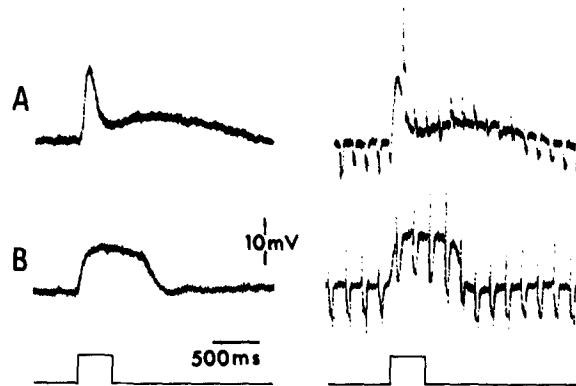
FIGURE 6. Comparison of membrane resistance changes accompanying responses recorded in two types of bipolar cells. (A) The control response (left) of a rod-dominant cell to a light intensity of −4.0 log units and its bridge record (right). A train of negative current pulses of about 1.2 nA and of 35 ms duration was applied at 7 Hz. (B) The control response (left) of a cone-dominant cell to light of −3.0 log units and its bridge record (right). A train of negative current pulses of about 1.0 nA and of 45 ms duration was applied at 6 Hz. An increase of the negative pulse height in the bridge record indicates an increase in the membrane resistance. The 10 mV change in the pulse height corresponds to the change in the resistance of 10 MΩ.
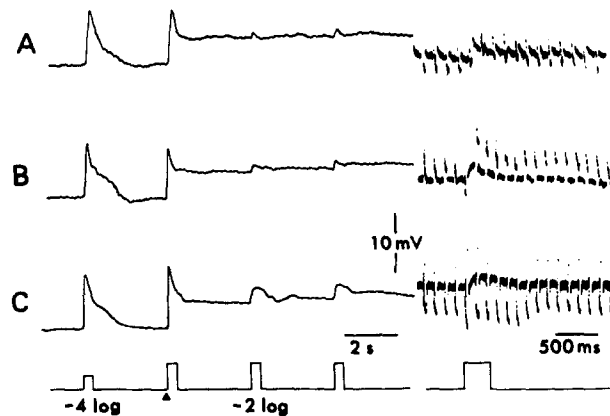

FIGURE 7. Variety of membrane resistance changes of rod-dominant bipolar cells recorded at a high intensity of light. The control responses first at a light intensity of −4.0 log units and then three successive responses at −2.0 log units are shown on the left side in records A, B, and C. Resistance changes accompanying the response to −2.0 log units are shown at a faster sweep speed on the right side.

a decrease in the negative pulses of the bridge record. In contrast, cone-dominant bipolar response shown in Fig. 6 B is accompanied by a resistance increase. The result suggests that the ionic mechanism underlying the response of cone-dominant bipolar cells is quite different from that of rod-dominant bipolar cells.

Test of membrane resistance changes at a higher light intensity revealed three subtypes of rod-dominant bipolar cells as shown by sample records in Fig. 7.

Control responses to light stimulus of −4.0 log units followed by responses to test light of −2.0 log units are shown on the left side of the figure. Bridge records of resistance changes accompanying responses to −2.0 log units are shown on the right at a faster sweep speed. In record A, the response is accompanied by a decrease in the membrane resistance. Of 20 cells studied, 3 were of this type. In record B, the resistance increases at an initial part of the response and then decreases during the rest of the response. 13 cells were of this type. In record C, the resistance increases during the whole response. Four cells were of this type.

Inasmuch as the response of cone-dominant cells was accompanied by a resistance increase instead of decrease (Fig. 6 B), it seems reasonable to consider that the variety of resistance changes in rod-dominant bipolar cells reflects the different ratio of contribution from rods and red cones onto individual cells. Further evidence in favor of this will be given in the next section.
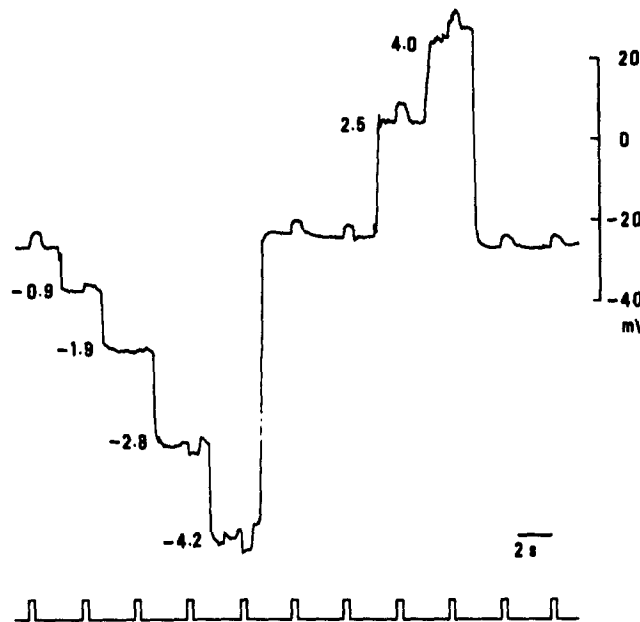

FIGURE 8. Effect of polarizing current on a cone-dominant bipolar cell response. A light spot of about 500 μm in diameter and 350 ms in duration was presented every 2.5 s at an intensity of −3.0 log units. Numbers at the beginning of each polarization indicate the strength of current in nanoamperes. The response reversed its polarity at about −54 mV.

REVERSAL POTENTIAL Fig. 8 shows effects of membrane polarization on the response of a cone-dominant bipolar cell. When the membrane is hyperpolarized by steps, the response decreases in amplitude and eventually reverses its polarity. The estimated value of the reversal potential, after substraction of a voltage drop across the coupling resistance of the electrode was −54 mV in this case. The reversal potential measured for seven cells ranged from −49 to −80 mV and was −63 ± 21 mV (mean ± SD).

Three of seven cone-dominant cells responded to hyperpolarization of the membrane with a complex wave form. An example is shown in Fig. 9, in which the effect of hyperpolarizing current was studied after testing the type of the cell by increasing the stimulus light intensity in steps of 1.0 log units from −5.0 to −3.0 log units. At a certain hyperpolarization of the membrane two voltage components, depolarizing and hyperpolarizing, are discerned. The depolarizing component became less prominent by a strong hyperpolarization of the membrane, because the hyperpolarizing component was augmented more than the depolarizing one. One possible explanation for the two components is that each component is generated by different kinds of photoreceptors. It is difficult, at present, to prove this because of infrequent penetration of this type of cell.
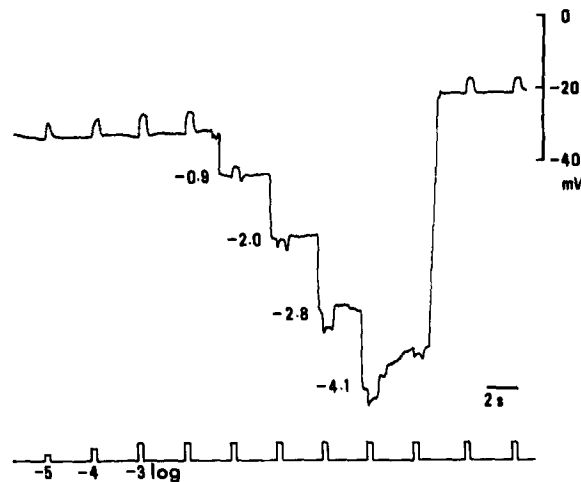

FIGURE 9. Another example of the effect of hyperpolarizing current on a cone-dominant bipolar cell response. The light intensity was first increased in steps of 1.0 log units from −5.0 to −3.0 log units as indicated under the stimulus marks. The two voltage components, hyperpolarizing and depolarizing, were revealed in this unit at certain levels of the membrane hyperpolarization.

Fig. 10 shows the effects of membrane polarization on the response of rod-dominant bipolar cells. Studies on 49 rod-dominant cells in the mesopic condition revealed two types from the response patterns to membrane polarization. The response shown in Fig. 10 A simply increases in amplitude by hyperpolarization of the membrane from −27 (resting potential in the dark) to −92 mV, but decreases in amplitude and eventually reverses its polarity by membrane depolarization to +40 mV. Of 49 cells, 7 were of this type. The unit in Fig. 10 B shows, in response to increasing hyperpolarization from −40 (resting potential in the dark) to −95 mV, an initial transient hyperpolarization followed by an enhanced depolarization but a transient depolarization followed by hyperpolarization when the membrane is depolarized to +48 mV. The result suggests that the response consists of two voltage components; one showing reversal at a positive and the other at a negative potential. 42 cells were of this type.

It is possible that the two voltage components of these bipolar cell responses reflect the synaptic inputs from rods and cones, and if so it should be possible to isolate the two components by changing stimulus conditions such as the state of adaptation of the retina. Fig. 11 shows a separation of the two components at different light intensities. The responses were obtained first to a light intensity of −4.0 log units. When the membrane is hyperpolarized, the response to −4.0 log units shows the initial transient hyperpolarization followed by depolarization. The stronger the membrane hyperpolarization, the more enhanced the hyperpolarizing component (record A). When the light intensity was increased to −2.0 units, there was a marked change in the ratio of the depolarizing and
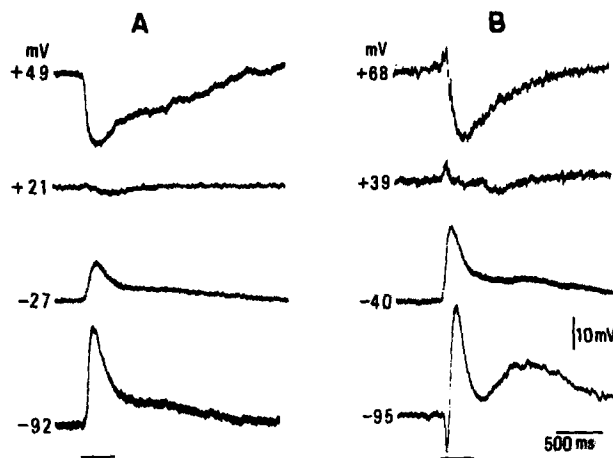

Figure 10. Two types of rod-dominant bipolar cells which respond to membrane polarization differently. The responses were recorded at a light intensity of −4.0 log units. (A) Membrane potential in the dark was −27 mV. The response simply increased in amplitude by membrane hyperpolarization and decreased by membrane depolarization. The response reversed its polarity near +20 mV. (B) Membrane potential in the dark was −40 mV. The response showed complex wave forms during membrane polarization suggesting that it was composed of two voltage components different in their time-course and in their reversal potential.

the hyperpolarizing components. The sample response (record B), which was recorded at almost the same level of the membrane hyperpolarization as in record A, is composed of a prominent hyperpolarizing component followed by a small depolarizing component. Most likely, this depolarizing component is rod-related because background illumination of 500 nm completely suppresses it (record C).

The results of separation of the two components showed that the reversal potential in the scotopic condition was +29 ± 13 mV (mean ± SD, value from 24 cells) and that in the photopic condition was −53 ± 11 mV (value from 21 cells). In our previous paper (Saito et al., 1978), the negative reversal potential was reported to be about −70 mV. The difference occurred probably as a result of insufficient isolation of the hyperpolarizing component in the previous experiments.

Figs. 12 and 13 show the result of separation of the two components using light of different wavelengths. The upper trace in Fig. 12 A shows a spectral response pattern in the absence of polarizing current. Each spectral response is affected differently by membrane hyperpolarization (Fig. 12 B). The response to 475 nm increases in its amplitude, whereas the response to 675 nm reverses its polarity under membrane hyperpolarization. The responses to other wave-
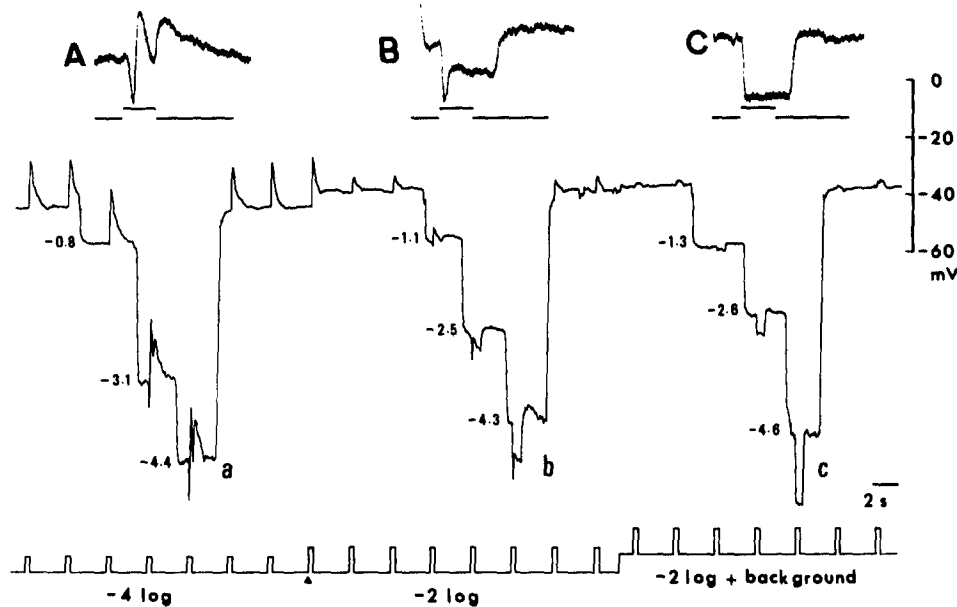
FIGURE 11.   Effect of hyperpolarizing current on the responses of a rod-dominant bipolar cell under different light intensities. The response to a light intensity of −4.0 log units showed the initial transient hyperpolarization followed by depolarization as the membrane was hyperpolarized. The depolarizing component under hyperpolarization of the membrane became less prominent on the response to −2.0 log units. Under a diffused background illumination of 500 nm, the depolarizing component was almost undetectable during membrane hyperpolarization. Three responses (A, B, and C) sampled at different light conditions are shown above with an expanded time scale. Numbers at the beginning of each polarization indicate the strength of current in nano amperes. Conditions of light stimulus are indicated under the stimulus trace.

lengths are composed of the two components of which the hyperpolarizing component is prominent at longer wavelengths. Another example of the same experiments is shown in Fig. 13. In this example, the amplitude of the control responses to 525 and 675 nm was equalized by adjusting the light intensities. When the membrane is hyperpolarized, the response to 525 nm increases in amplitude, whereas the response to 675 nm reverses its polarity.

DISCUSSION

Two types of on-center bipolar cells were distinguished in the carp retina on the basis of their electrophysiological properties.
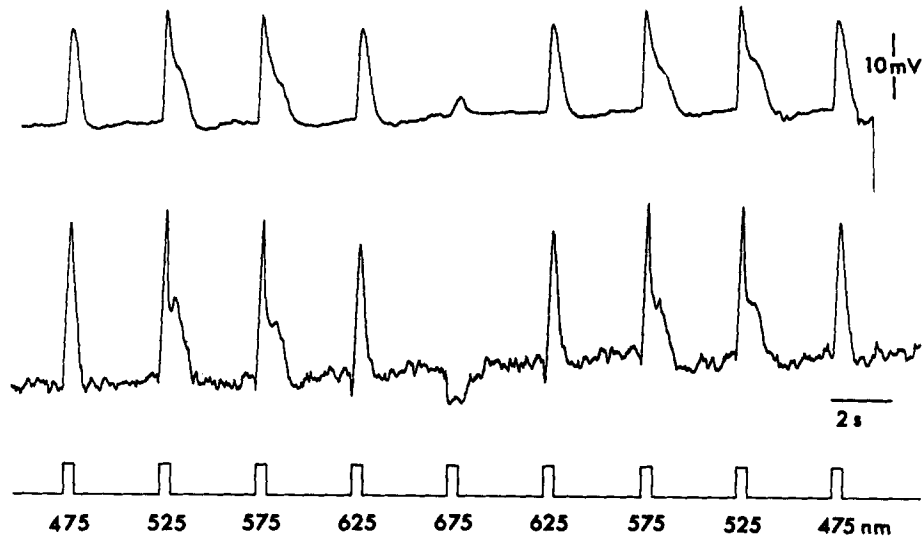
FIGURE 12.   Effect of hyperpolarizing current on the spectral responses of a rod-dominant bipolar cell. Upper trace shows a spectral response pattern without hyperpolarizing current. Lower trace shows a spectral response pattern during hyperpolarization of the membrane by current. Note that each spectral response was affected differently by membrane polarization.

FIGURE 13.   Comparison of rod-dominant bipolar cell responses to 525 (left) and 625 nm (right) with and without hyperpolarizing current. The amplitude of the control responses to both wavelengths was equalized by adjusting the light intensities. Numbers at the beginning of each polarization indicate the strength of current in nano amperes.

Cone-dominant bipolar cells showed a high sensitivity to red region of spectrum, suggesting that they receive major input from red cones. Their depolarizing response to a light spot was accompanied by a resistance increase, and was suppressed or inverted in polarity by hyperpolarization of the membrane. The reversal potential was −63 ± 21 mV. Such electrical membrane properties of these cells are quite in contrast to those of on-center bipolar cells

so far reported (Nelson, 1973; Toyoda, 1973; Werblin, 1977). At the present experiment, bipolar cells with the main input from green or blue cones were not recorded, although such types of bipolar cells have been described in the rudd retina by morphological studies (Scholes, 1975). Their synaptic mechanisms remain to be determined in the future.

Rod-dominant bipolar cells showed high sensitivities to green region of spectrum in the scotopic condition and to red region of spectrum in the photopic condition. This Purkinje shift is consistent with the anatomical connections demonstrated for large bipolar cells in the cyprinid fish retina (Stell, 1967; Scholes and Morris, 1973).

Further evidence suggesting the convergence of rod and red cone signals onto rod-dominant cells came from the results of their electrical properties in the present study. A large number of rod-dominant cell responses was composed of the two-voltage components; one shows a reversal potential at +29 ± 13 mV and the other at −53 ± 11 mV. The component with a positive reversal was dominant in the scotopic condition and was sensitive to green light, whereas the component with a negative reversal was dominant in the photopic condition and was sensitive to red light. If the latter component is generated by red cone inputs, the reversal potential value of this component should be the same as that of the cone-dominant bipolar cells. However, there was a slight difference between them. The difference is probably not essential but apparent, coming from various technical problems in the measurement of reversal potential, such as a complex geometry of the bipolar cells or a coupling between barrels of the double-barreled electrode. A complex dendritic ramification of the bipolar cells makes difficult an adequate and uniform current clamp of these cells because of electronic decrement between electrodes and synaptic sites. Accordingly, the reversal potential values are often overestimated. This overestimate must be more prominent for cone-dominant bipolar cells which have delicate dendritic processes. The coupling resistance of the electrode measured in the vitreous may not be the same as that in the cytoplasm. This kind of error would often be larger for cone-dominant bipolar cells, because finer electrodes are needed for the recording.

There were a few rod-dominant cells showing only the component with a positive reversal. This finding may suggest the existence of on-center bipolar cells receiving inputs only from rods. However, it is also possible that minor cone inputs were deteriorated in these cells during the experiment.

Ionic mechanisms responsible for generating on-center bipolar cell responses are schematically shown in Fig. 14 by an electrical circuit model. The batteries E_K, E_{Cl}, and E_{Na} represent the potassium, chloride, and sodium concentration cells, and g_K, g_{Cl}, and g_{Na} represent the potassium, chloride, and sodium conductances. Ionic channels in the subsynaptic membrane of bipolar cells activated by red cones are shown on the left half of the model. Assuming that the K^+ concentration is much higher inside the cell than outside and that the Na^+ and Cl^- concentrations are much higher outside, ions that could have a negative reversal potential are K^+ and Cl^-. It is suggested, therefore, that the photoresponse of red cone-dominant bipolar cells is generated by a decrease in g_K and/or g_{Cl}. Recently, Miller and Dacheux (1976) reported that the response of

depolarizing bipolar cells in the mudpuppy retina is sensitive to Cl^-. However, their bipolar cells must be different from our cone-dominant bipolars because their response was accompanied by a resistance decrease instead of an increase. Ionic channels activated by rods are shown on the right half of the model. The response of rod-dominant bipolar cells in the scotopic condition was accompanied by the resistance decrease with a positive reversal potential. Kaneko and Shimazaki (1975) reported that removal of Na^+ from the external media hyperpolarizes the membrane and abolishes the photoresponse of on-center bipolar cells. These results suggest that the photoresponse of the bipolar cell activated by rod inputs is generated by an increase in g_{Na}.
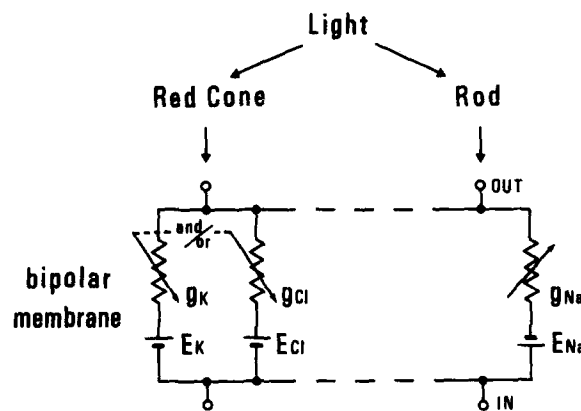

FIGURE 14. Electrical circuit diagram of ionic mechanisms for generation of on-center bipolar cell responses. E_{Na}, E_K, and E_{Cl} are sodium, potassium, and chloride equilibrium potentials. g_{Na}, g_K, and g_{Cl} are membrane conductances to sodium, potassium, and chloride ions, respectively. The left half of the diagram shows the synaptic input from red cones and the right the input from rods. In the light, g_{Na} is increased, whereas g_K and/or g_{Cl} are decreased.

In most rod-dominant bipolar cells, rod and cone signals converge on the same unit. In the mesopic condition, therefore, ionic mechanisms underlying their responses must be expressed by an appropriate combination of the two ionic channels shown in the model. The complex membrane properties of rod-dominant bipolar cells, as revealed by hyperpolarization of the membrane by current, can be explained by an algebraic sum of these two subsynaptic events which respond to light with different latencies and durations. The difference in latency and wave form between rod and cone potentials in a certain range of intensities has been described in the turtle (Baylor and Hodgkin, 1973) and in the mudpuppy (Normann and Werblin, 1974; Fain, 1975).

The permeability of Na^+ channels and that of K^+ and/or Cl^- channels undergoes changes in opposite directions, namely, g_{Na} increases but $g_{K\ and/or}\ g_{Cl}$ decrease in response to light. The change in g_{Na} seems to play a dominant role in the mesopic condition, judging from our result that the depolarizing response to light is accompanied by a prominent decrease in the input resistance. When the retina is adequately light-adapted by background light so as to saturate the

rod response but not the cone response, the g_{Na} remains high and the g_K and/or g_{Cl} becomes low. An increase in the light intensity under this condition brings about a further decrease in g_K and/or g_{Cl}. The resulting depolarization is relatively small because of the low input resistance of the cell due to high g_{Na}. However, the present system of two ionic channels working in opposite directions has a certain merit to maintain the sensitivity to cone signals beyond the level of the rod saturation. Because a relatively large steady sodium current is flowing in the light adaptation, a further change in the membrane conductances is accompanied by a change in the voltage drop across the membrane. Thus, a decrease in g_K and/or g_{Cl} is more effective in depolarizing the membrane than would be the case if the cone signals, as well as the rod signals, were mediated by an increase in g_{Na}.

Trifonov (1968) first proposed a hypothesis from his experiments on horizontal cells that the release of the transmitter substance from photoreceptors continues in the dark and is inhibited by light. Based on this hypothesis, Kaneko and Shimazaki (1976) and Toyoda et al. (1977) tried to explain the depolarizing response of on-center bipolar cells by further assuming that the transmitter released in the dark acts to decrease the permeability of Na^+ channels and that inhibition of the transmitter release in the light results in a depolarizing response by increase in Na^+ permeability of the subsynaptic membrane. This assumption is applicable to the rod signals of rod-dominant bipolar cells described in the present experiments. The hypothesis proposed by Trifonov is also applicable to the cone signals if it is further assumed that the transmitter released in the dark acts to increase the permeability of K^+ and/or Cl^- channels of the subsynaptic membrane. This type of synaptic activity is identical to the conventional inhibitory postsynaptic potential.

This study raises an important question as to whether rod and red cone terminals possess different transmitter substances or the subsynaptic elements of the bipolar cells respond differently to a single transmitter substance. The answer to this question awaits identification of transmitter(s) in the photoreceptor terminals.

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