Application of Transretinal Current Stimulation for the Study of Bipolar-Amacrine Transmission

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ABSTRACT Transretinal current flowing from the receptor side to the vitreous side depolarizes the axon terminals of retinal cells and facilitates the release of transmitter. Such current elicited a depolarizing response in off-center bipolar cells and a hyperpolarizing response in on-center bipolar cells. It also elicited a response of relatively complex waveform in amacrine cells. The responses elicited in bipolar cells were suppressed in the presence of 5–10 mM glutamate in the perfusing Ringer solution, while the responses of amacrine cells persisted, although their waveform changed to a simple one that showed monotonic depolarization irrespective of the type of amacrine cell and were accompanied by a decrease in the membrane resistance. The results indicate excitatory synaptic transmission from bipolar cells to amacrine cells. Since the response elicited by current in ON-OFF cells was almost identical to those elicited in ON or OFF amacrine cells, the transient nature of their light response cannot be due to their membrane properties. ON-OFF cells responded to transretinal current flowing in the opposite direction with a small hyperpolarization accompanied by a resistance increase. The hyperpolarizing response was suppressed by the addition of GABA in glutamate Ringer solution. The results suggest an activation by the current of GABA-ergic feedback pathways from amacrine cells to bipolar cells.

INTRODUCTION

The amacrine cells in the retina are the third-order neurons since they receive inputs from bipolar cells, but they also serve as higher-order neurons since they receive inputs from other amacrine cells. They send their information to ganglion cells and neighboring amacrine cells and partly back to bipolar cells (Witkovsky and Dowling, 1969). From such connections they are considered to modify bipolar-ganglion transmission. Amacrine cells of the teleost retina can be classified into sustained and transient types according to their response to light (Kaneko, 1973; Chan and Naka, 1976). The sustained type responds to light either with sustained depolarization (ON type) or with sustained hyperpolarization

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(OFF type), while the transient type responds to light with transient depolarization at both on and off of light (ON-OFF type).

From the analysis of membrane conductance changes accompanying the response, Toyoda et al. (1973) postulated that the bipolar-amacrine transmission is excitatory. Morphological studies on the stratification of amacrine cell dendrites supported this conclusion (Famiglietti et al., 1977). Namely, the synaptic contacts between on-center bipolar cells and ON amacrine cells were located at sublamina b of the inner plexiform layer, while those between off-center bipolar cells and OFF amacrine cells were confined to sublamina a. ON-OFF amacrine cells extended their dendrites in both sublaminae a and b. Slaughter and Miller (1981) found that in the mudpuppy retina, 2-amino-4-phosphonobutyric acid selectively blocked the ON channel without affecting the OFF channel. It also suppressed the ON response of ON-OFF amacrine cells without affecting the OFF response.

These results are consistent with the hypothesis that the ON-OFF amacrine cells receive inputs from both on-center and off-center bipolar cells, and that the depolarization of on-center bipolar cells is responsible for the ON response and the depolarization of off-center bipolar cells for the OFF response of these units (Toyoda et al., 1973; Marchiafava and Torre, 1978). However, the characteristics of the synaptic transmission, especially to ON-OFF amacrine cells, are not well understood. It is still puzzling why the response is so transient. The present experiments attempted to solve whether the transient nature of ON-OFF cells is due to their membrane properties or to their neuronal circuitry.

Trifonov (1968) first applied transretinal current to study the synaptic transmission. Brief transretinal current from the receptor side to the vitreous side elicited a depolarizing response of horizontal cells. It was postulated that the current depolarized photoreceptor terminals and facilitated the release of the transmitter, which acted to depolarize the horizontal cell. Transretinal current was also effective in eliciting a response in bipolar cells (Kaneko and Shimazaki, 1976; Toyoda et al., 1978) and in amacrine cells. In this paper, the bipolar-amacrine transmission elicited by such current stimulation was analyzed while the receptor-bipolar transmission was blocked by addition of glutamate.

METHODS

The experimental setup is schematically illustrated in Fig. 1. The retina was isolated from the carp (*Cyprinus carpio*) and was put on a piece of filter paper with its receptor side up. It was then placed on a chlorided silver plate in a perfusion chamber. The plate served as an indifferent electrode. The oxygenated Ringer solution was introduced to the chamber through a peristaltic pump and superfused the retina at a flow rate of ~1 ml/min. The composition of normal Ringer solution used was (mM): 110 NaCl, 2.5 KCl, 2.2 $CaCl_2$, 5 $NaHCO_3$, 20 glucose, 5 HEPES, adjusted to pH 7.7 by NaOH. To abolish bipolar cell responses, glutamate Ringer solution containing 5–10 mM glutamate was used. The effect of several other drugs was tested by adding these in the normal Ringer or in the glutamate Ringer solution. Each solution was kept in a separate reservoir and the switching of the perfusing solution was aided by an electromagnetic valve. Another chlorided silver ring, serving as a current electrode, was placed in the solution on the receptor side of the retina for transretinal stimulation.

Two glass microelectrodes were used for recording. They were set vertically on a manipulator and lowered through the center of the ring of current electrode. One of

them was positioned on the surface of the retina, and the other was inserted into the retina for intracellular recording. The electrodes were filled with 4 M potassium acetate and had a resistance measuring 40–80 MΩ in Ringer solution. The potential recorded by these two microelectrodes was differentially recorded in order to reduce the stimulus artifact. The transretinal current was a train of rectangular pulses of 2.5 mA and of 0.2–0.5 ms duration. It was applied for 1 s at a rate of 100 pulses/s. Under this condition, it was sufficient to evoke an almost maximal response in horizontal cells. In some experiments, positive current pulses of 1 nA and 1 ms duration were applied through the recording electrode and the bridge circuit of the preamplifier to test the membrane resistance changes of the cell recorded.

For photic stimulation, diffuse white light of 1 s duration and 0.4 lm/m^2 was used. Responses of amacrine cells were identified by the depth of recording and the response

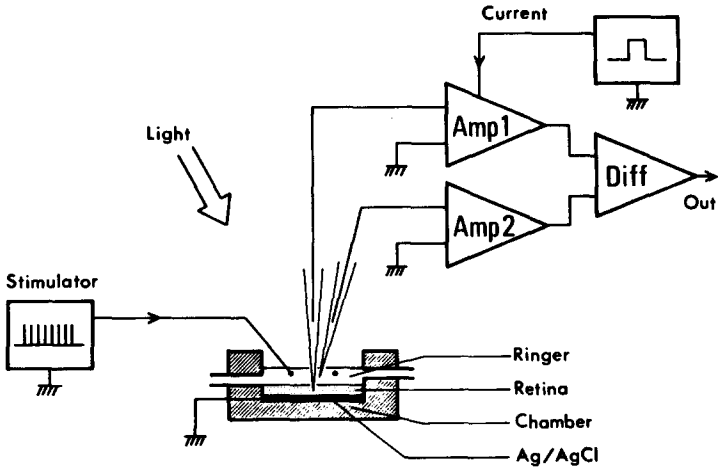

FIGURE 1. Schematic illustration of experimental setup. Transretinal repetitive current supplied from a stimulator was passed between an Ag/AgCl wire immersed in the perfusing solution and an Ag/AgCl indifferent electrode.

shape. These criteria were adopted on the basis of staining experiments with Procion Yellow (T. Saito, unpublished observation). Although it was difficult to distinguish responses of displaced ganglion cells from those of amacrine cells, the recording from the former was infrequent (<10%). Responses were amplified and monitored on an oscilloscope and at the same time recorded on tape for further processing of the data. Experiments were performed at room temperature, 20–24°C.

RESULTS

Effect of Repetitive Current Stimulation on Bipolar Cells

The effect of transretinal current stimulation on two types of bipolar cells was studied first. The current pulse flowing in the direction to depolarize photoreceptor terminals elicited a transient hyperpolarizing response in on-center bipolar cells and a depolarizing response in off-center cells. Repetitive application of current pulses resulted in sustained potential changes due to the fusion of each transient response as shown in Fig. 2. Thus, the transmitter released from

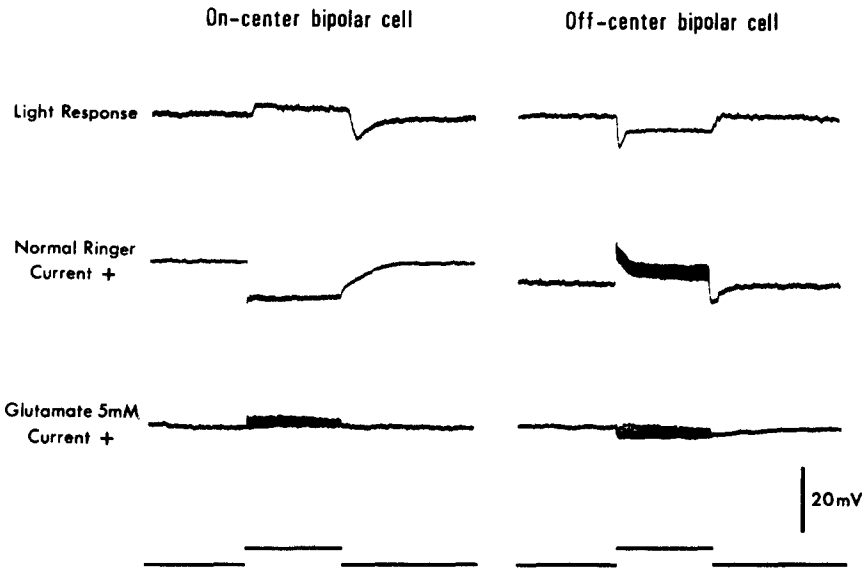

FIGURE 2. Responses of an on-center (left) and off-center (right) bipolar cells to diffuse white light and to repetitive transretinal current stimulation. The third trace indicates that the responses elicited by terminal depolarizing current are abolished by the addition of 5 mM glutamate in the perfusing Ringer solution. The duration of light and repetitive current stimulation is indicated by upward displacement of the bottom trace.

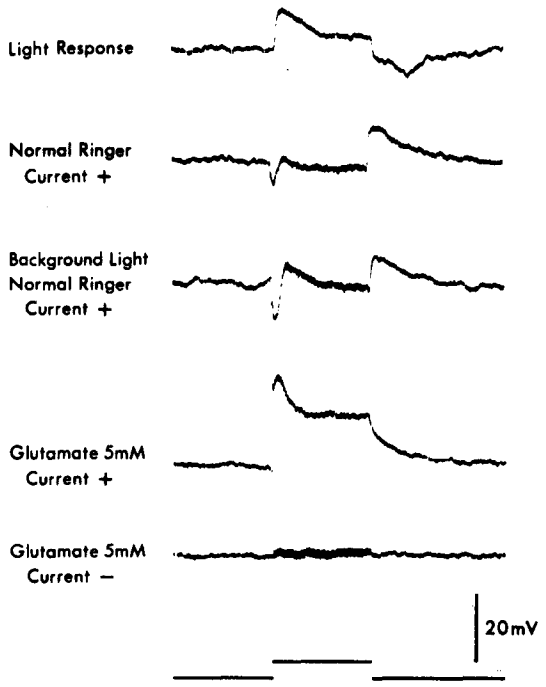

FIGURE 3. Responses of ON amacrine cells to light and to current stimulation. "Current +" indicates the terminal depolarizing current, and "current −" is the terminal hyperpolarizing current. The response shown in the third trace was recorded in the presence of diffuse white background light.

photoreceptor terminals acts to hyperpolarize on-center cells and to depolarize off-center cells, as has been suggested in previous studies. The current flowing in the opposite direction did not elicit a detectable response in the present experiments.

It is known that glutamate mimics the action of the photoreceptor transmitter. It depolarizes horizontal cells (Cervetto and MacNichol, 1972) and off-center bipolar cells and hyperpolarizes on-center bipolar cells (Murakami et al., 1975; Kaneko and Shimazaki, 1976; Toyoda et al., 1978). At a relatively high concentration, glutamate also blocks the response evoked by light as well as by transretinal current (Toyoda et al., 1978). Fig. 2 (bottom) shows the effect of terminal

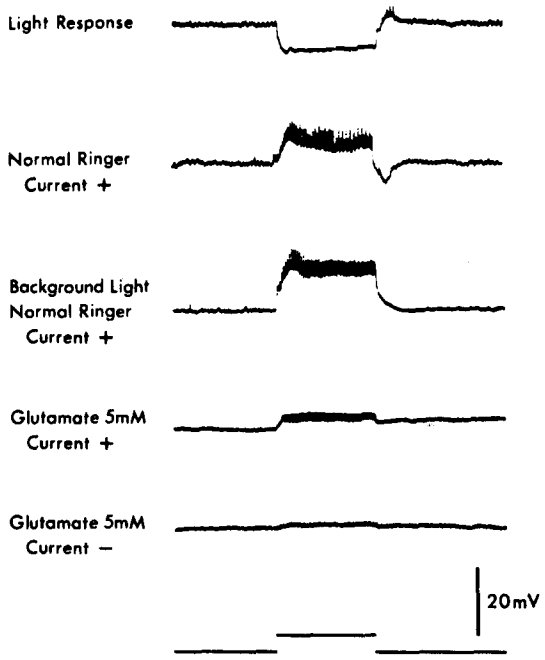

FIGURE 4. Responses of OFF amacrine cells. Recording conditions are the same as in Fig. 3.

depolarizing current after the addition of 5 mM glutamate to the perfusing solution. The current was no longer effective. The result indicated that glutamate can be used to inactivate receptor-bipolar transmission. The membrane potential of amacrine cells, on the other hand, was not markedly affected by glutamate. Thus, it is reasonable to assume that bipolar-amacrine transmission can be studied without disturbance of bipolar cell activities when transretinal current is applied after blocking bipolar cell responses by addition of glutamate.

Effect of Repetitive Transretinal Current on Amacrine Cells

ON AMACRINE CELLS The effect of repetitive transretinal current was studied in 27 ON amacrine cells. The terminal depolarizing current elicited in ON amacrine cells a response of complex waveform. A sample record is shown in

Fig. 3. Although on-center bipolar cells are hyperpolarized by the current, as shown in the previous figure, the current will also directly depolarize the axon terminals of bipolar cells. Thus, the response of amacrine cells receiving inputs from on-center bipolar cells is expected to be complex since they receive two opposing effects from the latter. When a steady background light was applied, the resting potential of the ON amacrine cells was slightly depolarized, but the complexity of the response elicited by current was not affected.

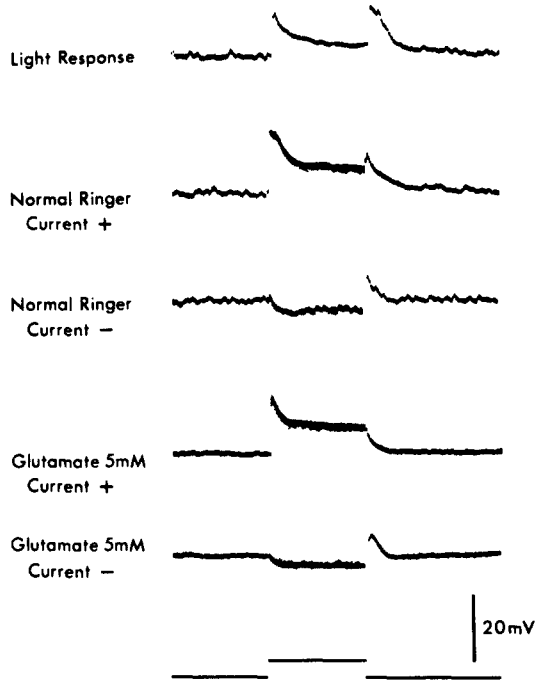

FIGURE 5. Responses of an ON-OFF amacrine cell. The effect of background light was not tested. The terminal hyperpolarizing current elicited a hyperpolarizing response with a rebound at cessation of the stimulus in both normal and glutamate Ringer.

When glutamate was added to the perfusing solution to block receptor-bipolar transmission, the complex response elicited by current changed to a simple one—a sustained depolarization with an initial transient, as shown in Fig. 3. The results suggest that the transmitter from bipolar cells to ON amacrine cells is excitatory; in other words, the synapse is of the sign-conserving type. The current of opposite direction did not elicit a detectable response.

OFF AMACRINE CELLS A total of nine OFF amacrine cells was studied in the present experiments. In normal Ringer, terminal depolarizing current elicited in OFF amacrine cells a sustained depolarization with an initial transient, which is almost a mirror image of the light response. An example is shown in Fig. 4. When glutamate was added to the solution, the response became small, without prominent changes in the waveform. These results are easily accounted

for because the current not only depolarizes off-center bipolar cells through the depolarizing action of photoreceptor transmitter, but also depolarizes their terminals directly. The sum of these two effects will give a larger response than the effect of current on the bipolar cell terminals alone, which remains after the

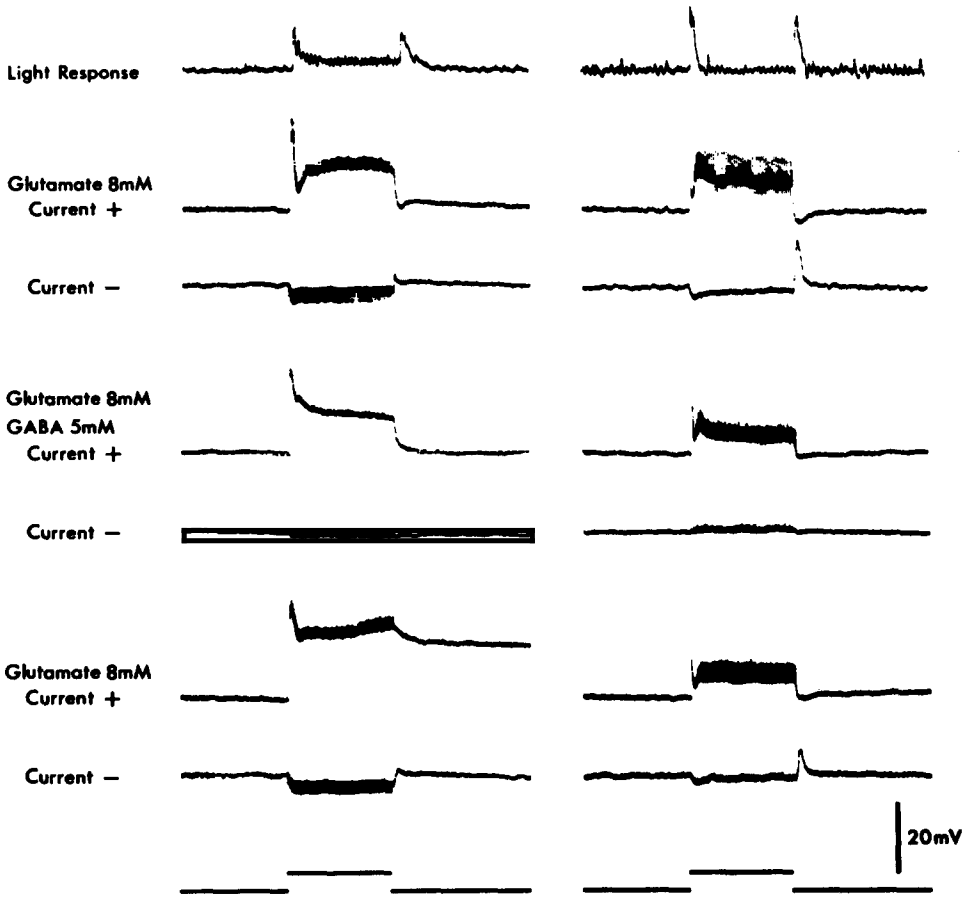

FIGURE 6. Effect of GABA on the current evoked responses of ON-OFF amacrine cells. Two examples of ON-OFF amacrine cells are shown. The uppermost trace is their light response. The upper pair of traces shows the control responses to + and − currents in the glutamate Ringer. The next pair shows the responses after the addition of 2 mM GABA in the glutamate Ringer. The hyperpolarizing response to − current was abolished and the depolarizing response to + current often became less steep as in the left-hand record. The lowest pair is the control after removing GABA from the perfusing medium.

suppression of receptor-bipolar transmission by glutamate. The results suggest that the synaptic transmission from bipolar cells to OFF amacrine cells is also excitatory. Transretinal current of opposite polarity was not effective in evoking a detectable response.

ON-OFF AMACRINE CELLS The effect of current stimulation was tested on 39 ON-OFF amacrine cells. In normal Ringer solution, the terminal depolarizing current elicited in these cells a transient depolarization followed by a sustained phase but with another depolarizing transient at the current cessation. An example is shown in Fig. 5. The response resembled the light response, although the OFF transient was relatively small. This OFF transient was selectively sup-

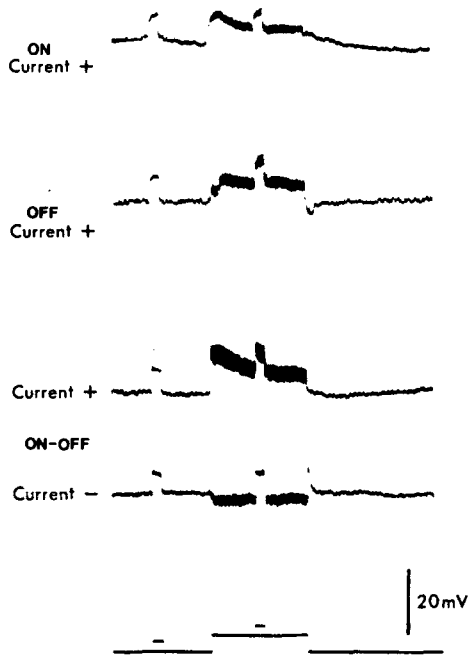

FIGURE 7. Input resistance changes during amacrine cell responses elicited by repetitive current stimulation. The type of amacrine cell is indicated at the left. A positive pulse of 1 nA and 100 ms duration was injected to the cell through the recording electrode before and during the transretinal current stimulation. The depolarizing responses were accompanied by a decrease in membrane resistance as indicated by the decrease in pulse height during the response. The hyperpolarizing response of ON-OFF cells was accompanied by a resistance increase. A resistance change of >1 MΩ was detectable in all of the responses shown in this figure.

pressed when glutamate was introduced to the medium. The waveform of the response elicited in glutamate Ringer was almost identical to that observed in ON and OFF amacrine cells. The results indicate an excitatory transmission from bipolar to ON-OFF amacrine cells and suggest that the properties of the subsynaptic membrane of ON-OFF amacrine cells are not different from other amacrine cells.

Transretinal current of opposite direction, however, elicited a small sustained hyperpolarization in ON-OFF amacrine cells even in the presence of glutamate (Fig. 5). This hyperpolarizing response was suppressed when GABA was added to the medium at a concentration of >2 mM, as shown in the two sample records of Fig. 6. In both examples, the hyperpolarizing response to terminal hyperpo-

larizing current recorded in glutamate Ringer was reversibly blocked by the addition of GABA. Often the time course of the initial transient part of the response became slower and less oscillatory.

Membrane Resistance Changes of the Response

Membrane resistance changes accompanying the response elicited by transretinal current were studied by passing a positive current pulse to the amacrine cell through the recording electrode and the bridge circuit of the preamplifier. An increase in the positive pulse height indicated an increase in the membrane resistance. Sample records are shown in Fig. 7. The depolarizing response recorded in the presence of glutamate was accompanied by a decrease in the membrane resistance, irrespective of the type of amacrine cell. On the other hand, the hyperpolarizing response of ON-OFF amacrine cells was accompanied by an increase in the membrane resistance.

DISCUSSION

It is generally accepted that transmitter substances in the nervous systems are liberated by depolarization of presynaptic terminals. It is expected that transretinal current flowing from the receptor side to the vitreous side of the retina elicits depolarization of axon terminals of bipolar cells as well as photoreceptors and consequently enhances the liberation of transmitter substances. By terminal depolarizing transretinal current, on-center bipolar cells were hyperpolarized and off-center cells were depolarized. This may add a piece of direct evidence that the transmission from receptors to on-center bipolar cells is sign-inverting and that to off-center cells is sign-conserving.

The amacrine cells receive direct input from these bipolar cells. To examine the effect of the transmitter liberated only from the bipolar cell terminals onto amacrine cells, the synaptic transmission from photoreceptors to bipolar cells should be blocked to avoid confusion. For this purpose, glutamate was added in the perfusing solution. The results of these experiments showed that the transmitters from bipolar cells were depolarizing to any type of amacrine cell.

It is possible that amacrine-amacrine synapses are also activated by transretinal current, provided that the synaptic complex is vertically arranged. However, since the processes of amacrine cells are more or less horizontally oriented, it is difficult to assess the effect of current.

Glutamate affects the membrane potential of bipolar cells. On-center cells are hyperpolarized and off-center cells are depolarized. The release of transmitter from bipolar cell terminals must be influenced by this shift in the membrane potential. It is expected that the transmitter release from off-center cells is enhanced in the presence of glutamate because of its depolarizing effect. Therefore, the terminal depolarizing current would be less effective in increasing the release. On the other hand, the current would be more effective in releasing the transmitter from on-center bipolar cells, which are hyperpolarized in the presence of glutamate. This explains the relatively large current-evoked response of ON amacrine cells, in contrast to the relatively small response of OFF amacrine cells in glutamate Ringer. The same explanation would apply for the effect of background light on the current-evoked response. Namely, the transmitter

release will be enhanced from the neuron hyperpolarized by background light as in the OFF amacrine cell shown in Fig. 4.

The excitatory transmission from bipolar cells to amacrine cells gives further support for the hypothesis that ON amacrine cells receive inputs from on-center bipolar cells, OFF amacrine cells from off-center bipolar cells, and ON-OFF amacrine cells from both types of bipolar cells. Since the response waveform elicited in ON-OFF cells is not markedly different from that elicited in other types of amacrine cells, the transient response to light of the former cannot be due to their specific membrane property. The characteristics of the response must be formed in the neuronal circuitry.

The transretinal current flowing from the vitreous side to the receptor side evoked a hyperpolarizing response in ON-OFF amacrine cells, although it was not effective in eliciting a detectable response in other types. This hyperpolarizing response was abolished when 2 mM GABA was added to the glutamate Ringer. GABA was preferably used because its effect was more rapidly reversible than its antagonists'. Since the GABA-ergic pathways were desensitized in the presence of excess of GABA (Murakami et al., 1982), it is probable that the GABA-ergic pathway is responsible for the generation of this response. It is not likely, however, that the response is due to the direct GABA-ergic input to the ON-OFF cells, since this hyperpolarizing response is accompanied by an increase in the membrane resistance. We suppose that GABA-ergic feedback synapses onto bipolar cells are activated by this current and hyperpolarize bipolar cells, resulting in a decreased release of the excitatory transmitter.

In the teleost retina, GABA applied near the bipolar cell terminals causes hyperpolarization of on-center bipolar cells but does not affect off-center bipolar cells (Kondo and Toyoda, 1983). Morphological studies also show that GABA-ergic amacrine cells make contact with on-center bipolar cells but not with off-center bipolar cells (Marc, 1982). The hyperpolarizing response of ON-OFF amacrine cells could be mediated by such on-center bipolar cells. However, what puzzles us is the fact that this current did not elicit a detectable response in both on-center bipolar cells and ON amacrine cells. It is possible that the membrane potential of on-center cells in glutamate Ringer is kept hyperpolarized close to the reversal potential of GABA-mediated response so that the response is rather small. Another possibility is that the current-evoked response in the GABA-ergic pathway is confined in the local synaptic area within the dendrites of ON-OFF amacrine cells and does not spread into other terminal branches or to bipolar cell soma.

For further analysis of bipolar-amacrine transmission, it is necessary to record simultaneously from both neurons and study the interaction between them. However, this type of experiment has not been successful so far.

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