Reciprocal Synaptic Interactions Between Rod Bipolar Cells and Amacrine Cells in the Rat Retina

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Hartveit, Espen. Reciprocal synaptic interactions between rod bipolar cells and amacrine cells in the rat retina. J. Neurophysiol. 81: 2923–2936, 1999. Reciprocal synaptic transmission between rod bipolar cells and presumed A17 amacrine cells was studied by whole cell voltage-clamp recording of rod bipolar cells in a rat retinal slice preparation. Depolarization of a rod bipolar cell evoked two identifiable types of Ca\(^{2+}\) current, a T-type current that activated at about –70 mV and a current with L-type pharmacology that activated at about –50 mV. Depolarization to greater than or equal to –50 mV also evoked an increase in the frequency of postsynaptic currents (PSCs). The PSCs reversed at \(E_{\text{Cl}}\) (the chloride equilibrium potential), followed changes in \(E_{\text{Na}}\) and were blocked by \(\gamma\)-aminobutyric acid \(_A\) (GABA\(_A\)) and GABA\(_C\) receptor antagonists and thus were identified as GABAergic inhibitory PSCs (IPSCs). Bipolar cells with cut axons displayed the T-type current but lacked an L-type current and depolarization-evoked IPSCs. Thus L-type Ca\(^{2+}\) channels are placed strategically at the axon terminals to mediate transmitter release from rod bipolar cells. The IPSCs were blocked by the non-N-methyl-D-aspartate (non-NMDA) receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione, indicating that non-NMDA receptors mediate the feed-forward bipolar-to-amacrine excitation. The NMDA receptor antagonist 3-((RS)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid had no consistent effect on the depolarization-evoked IPSCs, indicating that activation of NMDA receptors is not essential for the feedforward excitation. Tetrodotoxin (a blocker of voltage-gated Na\(^+\) channels) reversibly suppressed the reciprocal response in some cells but not in others, indicating that graded potentials are sufficient for transmitter release from A17 amacrine cells, but suggesting that voltage-gated Na\(^+\) channels, under some conditions, can contribute to transmitter release.

INTRODUCTION

Despite detailed morphological information available about the individual components, it has been difficult to obtain clear evidence regarding the function of specific reciprocal synapses in the mammalian retina. Bipolar cell synapses in the inner plexiform layer occur as dyads where a synaptic ribbon in the bipolar cell axon terminal is apposed simultaneously to two postsynaptic processes. A reciprocal synapse occurs when an amacrine cell process provides a synapse back onto the bipolar cell axon terminal (Dowling and Boycott 1966; Raviola and Raviola 1967). For rod bipolar cells, both postsynaptic processes (AI and AII) are those of amacrine cells, but only one of the postsynaptic processes (AI) provides a reciprocal synapse onto the rod bipolar terminal (Kolb and Famiglietti 1974). In the cat retina, the AI process has been identified as generally belonging to the type A17 wide-field amacrine cell, which lacks an axon but has a large number of thin, radially oriented dendrites of considerable lateral extension (Nelson and Kolb 1985). In the rat retina, A17-like amacrine cells have been described in Golgi-material (Perry and Walker 1980), but their involvement in rod bipolar cell dyads has not been demonstrated directly. However, the circuitry of the rod pathway in the rat retina has been shown to be similar to that of other mammals (Chun et al. 1993; Kim et al. 1998); thus in this study, the amacrine cell providing the reciprocal process in rod bipolar dyads in the rat retina for simplicity will be called the A17 cell.

It generally is thought that the bipolar cell-to-amacrine cell connection is glutamatergic (Dong and Werblin 1998; Ehinger et al. 1988; Tachibana and Okada 1991) and that the reciprocal amacrine cell-to-bipolar cell connection is GABAergic (Chun and Wässle 1989; Dong and Werblin 1998; Freed et al. 1987). In terms of function, one idea is that feedback from amacrine cells to bipolar cells may expand the operating range of the bipolar cells (e.g., Tachibana and Kaneko 1988). Little is known, however, about the functional characteristics of this reciprocal synaptic interaction in the mammalian retina. It is not known, for example, whether transmitter release from a single rod bipolar axon terminal is sufficient to trigger a feedback response onto the same bipolar terminal from which transmitter was released, whether synaptic release of GABA from A17 amacrine cells activates both GABA\(_A\) and GABA\(_C\) receptors on rod bipolar terminals, or whether GABA is released from A17 amacrine cells by a conventional vesicular mechanism or a nonvesicular, transporter-dependent mechanism as has been suggested for some amacrine cells (O’Malley et al. 1992; Vardi and Auerbach 1995).

In the present study, the functional properties of the synaptic transmission between rod bipolar cells and A17 amacrine cells were examined directly by whole cell voltage-clamp recordings from rod bipolar cells in the rat retinal slice preparation. The main findings were that rod bipolar cells display both T- and L-type voltage-gated Ca\(^{2+}\) currents, transmitter release is elicited from the axon terminals by depolarization greater than or equal to –50 mV, and transmitter release is mediated by the L-type Ca\(^{2+}\) current. Furthermore synaptic input from rod bipolar cells to A17 amacrine cells is mediated by ionotropic glutamate receptors and the reciprocal inhibitory feedback is most likely mediated by vesicular release of GABA, acting on both GABA\(_A\) and GABA\(_C\) receptors on the rod bipolar axon terminals. A brief account of some of these findings has been published in abstract form (Hartveit 1997b).
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M E T H O D S

The methods have been described previously in detail (Hartveit 1996, 1997a). Albino rats (4–8 wk postnatal) were anesthetized deeply with halothane in oxygen and killed by cervical dislocation. After the retina was dissected free, vertical slices were cut by hand.

Electrophysiology and infrared video microscopy

During experiments, slices were viewed with infrared differential interference contrast (Nomarski) video microscopy and epifluorescence optics. The extracellular perfusing solution was bubbled continuously with 95% O2-5% CO2 and had the following composition (in mM): 125 NaCl, 25 NaHCO3, 2.5 KCl, 1 MgCl2, 1.25 Na2HPO4, and 10 glucose, pH 7.4 (18–22°C). In all but a few experiments, 0.5 mM strychnine was added to the extracellular solution. In experiments where Ca2+ was added to the extracellular solution (replacing Ca2+), NaH2PO4 was omitted.

All recordings were made in the whole cell configuration of the patch-clamp technique. The electrode resistance was 5–6 MΩ except for a few experiments where the resistance was increased to 7–9 MΩ. For standard experiments with high intracellular chloride concentration, the recording electrodes were filled with a solution of the following composition (in mM): 125 CsCl, 1 MgCl2, 15 tetraethylammonium (TEA) chloride, 10 N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES), 0.1 ethylene glycol-O,O,N,N'-tetraacetic acid (EGTA), 12 phosphocreatine, and 4 ATP, pH 7.3 with CsOH. EGTA was added at a low concentration to reduce the contamination of Ca2+ in the pipette solution, while at the same time minimally disturb the endogenous Ca2+ buffering systems. In some experiments, the pipette solution also contained 10 mM CsOH and 10 mM glutamic acid, replacing an equimolar amount of CsCl. A high internal chloride concentration was used to enhance GABA-gated chloride currents at a holding potential (Vh) of −70 mV (chloride equilibrium potential (ECl) = +2.1 mV; +0.3 mV when 10 mM Cs-glutamate replaced an equivalent amount of CsCl intracellularly). Because the recordings depended on a high internal chloride concentration, it was not feasible to use the perforated-patch technique. In some recordings, the extracellular chloride concentration was reduced from 134.5 to 20.5 mM by substituting 114.5 mM NaCl with an equimolar amount of sodium isethionate. This recording condition was used to enhance GABA-gated chloride currents at a Vh of −20 or −30 mV (ECl = +49.6 mV). For experiments with low intracellular chloride concentration, the solution had the following composition (in mM): 125 CsOH, 15 Cs gluconic acid, 1 MgCl2, 15 TEA, 10 HEPES, 0.1 EGTA, and 4 ATP, pH 7.3 with CsOH. Lucifer yellow was added at a concentration of 1 mg/ml to all intracellular solutions. Theoretical liquid junction potentials of extracellular solutions against the internal solutions were calculated with the computer program JPCalcW (Axon Instruments, Foster City, CA) according to the generalized Henderson equation (Barry and Lynch 1991). The software (Pulse; HEKA elektronik, Lambrecht/Pfalz, Germany) controlling the amplifier (EPC-9; HEKA Elektronik) automatically corrected the holding potentials for the liquid junction potentials on-line.

To reduce electrode capacitance, some electrodes were coated with dental wax (Kerr’s sticky wax) and gently fire polished immediately before usage. Seal resistances were in the gigaohm range (≥10 GΩ for electrodes with heat-polishing, 2–6 GΩ for electrodes without heat-polishing). Series resistances were usually between 10 and 30 MΩ and were in about one-third of the recordings compensated by 50–90%, with a lag of 2 or 10 μs. Because of the small magnitude of the evoked currents, the steady-state voltage error at the soma caused by the series resistance is likely to have been small. Nevertheless, I sometimes observed sustained tail currents after depolarization-evoked Ca2+ currents, indicating considerable escape from dynamic voltage control (cf., Matsui et al. 1998). These cells are excluded from the material reported here. Depending on the experimental protocol, the digital sampling interval was varied between 10 μs and 1.1 ms. Before sampling, the signal was low-pass filtered (analog 3- and 4-pole Bessel filters in series) with a corner frequency (~3 dB) automatically adjusted to 1/3 of the sampling frequency. Capacitative currents caused by the recording pipette and the cell membrane were measured with the automatic capacitance neutralization network feature of the EPC-9 amplifier (Sigworth et al. 1995). With this method, the average cell capacitance was 3.23 ± 0.07 (SE) pF (n = 152). This is likely to be an underestimate of the true capacitance, as values obtained from charging transients (Mennnerick et al. 1997), after disabling the Cslow capacitance neutralization circuitry of the EPC-9, result in higher capacitance values (M. Veruki and E. Hartveit, unpublished results).

In experiments where Ca2+ currents were isolated pharmacologically, potassium currents were blocked by recording with the intracellular solutions above and by replacing 20 mM NaCl by 20 mM TEA in the extracellular solution. The extracellular solution also contained picrotoxin (1 mM). In all but a few recordings of voltage-gated Ca2+ currents, the concentration of EGTA in the intracellular solution was increased to 5 mM, with Ca2+ added at a concentration of 1 mM (as CaCl2). Ca2+ currents were generally evoked from a holding potential of −60 mV or a 1-s conditioning hyperpolarization to −100 mV either by voltage ramps (to +40 mV; ramp speed 100 mV/s) or by stepping the membrane potential for 30–150 ms to potentials between −90 and +40 mV. The interpulse interval was 15–20 s. For some measurements of Ca2+ currents, linear leak and capacitative currents were subtracted by a P/N protocol.

Drugs were dissolved in a HEPES-buffered extracellular solution (Hartveit 1996) and applied by pressure from a five- or seven-barrelled pipette complex. The concentrations of the drugs were as follows (obtained from Tocris Cookson, Bristol, UK, unless stated otherwise): 500 μM 3-aminopropyl(dimethyl)phosphonic acid (3-APMA); 200 μM baclofen; 20 or 100 μM bicuculline methochloride; 50 mM 3-(RS)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP); 25 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX); 200 μM γ-aminobutyric acid (GABA); 10 μM nicipidine (Research Biochemicals, Natick, MA); 100 μM nifedipine (Research Biochemicals); 10, 100, 200, or 400 μM nimodipine (Sigma, St. Louis, MO); 500 μM or 1 mM picrotoxin (Research Biochemicals); and 1 μM tetrodotoxin (Research Biochemicals). CNQX, nicipidine, nifedipine, and nimodipine were dissolved in dimethylsulfoxide and diluted to the final concentration in the HEPES-buffered extracellular solution by sonication. In other experiments, nimodipine acid was applied directly in the extracellular solution used to perfuse the slices. Solutions were either made up freshly for each experiment or were prepared from aliquots stored at −25°C.

After each recording, epifluorescent illumination was used to confirm the identity of the cell. Each cell was drawn by hand and for most cells, infrared differential interference contrast video images were digitized (ImageGrabber24, Neotech, Hampshire, UK) and stored on computer. In addition, some cells were photographed at a series of focal planes on high speed film (Kodak Tmax 400) and the negatives subsequently were scanned and used to construct a montage (Adobe Photoshop, Adobe Systems, Mountain View, CA). For details, see Hartveit (1997a).

General data analysis

Recordings were analyzed off-line with the use of the computer programs PulseFit (HEKA elektronik), A xoGraph (Axon Instruments), Igor Pro (Wavemetrics, Lake Oswego, OR), and GB-Stat (Dynamic Microsystems, Silver Spring, MD). Curve-fitting with polynomial functions employed a singular value decomposition algorithm (Igor Pro). Curve-fitting with exponential functions employed a Lev- enberg-Marquardt algorithm (Igor Pro) or a Chebyshev algorithm (A xoGraph) with iterative sum-of-squares minimization. Equilibrium potentials were calculated according to the Nernst equation with no
correction for ionic activities. Statistical analyses were performed with the use of Student’s two-tailed or paired t-test with a level of significance of $P < 0.05$. Data are presented as means ± SE ($n =$ number of cells) and percentages are presented as percentage of control. For illustration purposes, most records were low-pass filtered at 0.5 or 1 kHz (-3 dB; digital nonlagging Gaussian filter).

Detection of synaptic events

The occurrence of spontaneous postsynaptic currents (PSCs) was detected by a computer program (AxoGraph) employing a variable amplitude event detection algorithm (Clements and Bekkers 1997). A simulated transient with the width and time course of a typical synaptic event, was moved along the recorded data trace one point at a time. At each position, this transient (template) was scaled optimally and offset to fit the data and a detection criterion (the template scaling factor divided by the goodness-of-fit at each position) was calculated. An event was detected when this criterion exceeded a threshold and reached a sharp maximum. The template function used to approximate the time course of spontaneous synaptic events consisted of a flat baseline region followed by an exponential rise and decay,

$$\text{TEMPLATE}(t) = \begin{cases} 0 & (t \leq 0) \\ [1 - \exp(-(t/\text{RISE})]\exp(-t/\text{DECAY}) & (t > 0) \end{cases}$$

where

- $\text{RISE}$ = the time constant of the rising phase,
- $\text{DECAY}$ = the time constant of the falling phase of the template,
- $t =$ time from onset of idealized synaptic event.

Standard values for $\text{RISE}$ and $\text{DECAY}$ were 0.5 and 5 ms, respectively. Because the reliability of the detection algorithm falls as the distribution of rise and decay times varies (Clements and Bekkers 1997), the threshold was set to a low value (~1.5 times the noise level). This resulted in many incorrectly identified events which then were detected by eye and deleted during manual inspection of all captured events.

Results

A total of 179 rod bipolar cells are included in this study. They were all investigated electrophysiologically and were identified morphologically as rod bipolar cells after the recording by including Lucifer yellow in the recording pipette. An example of a rod bipolar cell with characteristic large, knob-shaped swellings at the axon terminal in the proximal part of the inner plexiform layer is shown in Fig. 1.

Voltage-gated $\text{Ca}^{2+}$ currents in rod bipolar cells

A depolarizing voltage ramp command to +40 mV from a 1-s conditioning potential of -100 mV evoked a low-amplitude, low-threshold inward current at about -70 mV (Fig. 2A). An additional, high-amplitude inward current was evoked at about -50 mV (Fig. 2A). To plot the current-voltage ($I$-$V$) relationship of the voltage-activated currents, the linear leak current first was subtracted by fitting a line to the initial linear segment of the current trace (typically 0-0.3 s). After extrapolation, the linear curve was subtracted from the raw current trace and the difference current was plotted against the corresponding voltage for each point in time (Fig. 2B). The activation threshold for the current was defined as the intersection of a line representing the average -2 SDs of the baseline at hyperpolarized potentials with a fifth-order polynomial fit of the relevant segment of the $I$-$V$ curve. The low-threshold current activated at $-66.2 \pm 1.3$ mV ($n =$ 15). The high-threshold current was studied in isolation by starting the voltage ramp command from a holding potential of -60 or -55 mV, thereby inactivating the low-threshold current (Fig. 2, A and B). The high-threshold current activated between -50 and -40 mV (-47.7 ± 1.4 mV) and reached maximum amplitude between -30 and -20 mV (-22.3 ± 0.9 mV; $n =$ 10; Fig. 2, A and B). The difference in time course of the low- and high-threshold current components was studied with discrete depolarizing voltage pulses (Fig. 2C). Depolarization to -20 mV from a 1-s conditioning potential of -100 mV evoked both a transient and a sustained component. Depolarization to -20 mV from a holding potential of -60 mV inactivated the transient component and left intact a more sustained component. Subtracting the two current traces from each other displayed the transient current in isolation (Fig. 2C). Corresponding depolarizations from conditioning potentials up to -50 mV did not further alter the time course (not shown). Similar results were seen for six other cells tested. The transient, low-threshold current usually ran down faster than the sustained, high-threshold current. The $I$-$V$ relationships generated from currents evoked by discrete depolarizing voltage steps were very similar to those generated by voltage ramps ($n =$ 3; data not shown).

During these recordings, $\text{K}^+$ currents were blocked by $\text{Cs}^+$ and $\text{K}^+$ currents were blocked by $\text{Cs}^+$. The occurrence of spontaneous postsynaptic currents (PSCs) was detected by a computer program (AxoGraph) employing a variable amplitude event detection algorithm (Clements and Bekkers 1997). A simulated transient with the width and time course of a typical synaptic event, was moved along the recorded data trace one point at a time. At each position, this transient (template) was scaled optimally and offset to fit the data and a detection criterion (the template scaling factor divided by the goodness-of-fit at each position) was calculated. An event was detected when this criterion exceeded a threshold and reached a sharp maximum. The template function used to approximate the time course of spontaneous synaptic events consisted of a flat baseline region followed by an exponential rise and decay,
identified as Ca\(^{2+}\) currents because they were blocked completely by adding Co\(^{2+}\) (3 mM) to the extracellular solution (Fig. 2B; \(n = 10\)). The block was reversible on washout of Co\(^{2+}\) (not shown). The Na\(^{+}\) channel blocker tetrodotoxin (1

and TEA\(^{+}\) in the intracellular solution and by TEA\(^{+}\) in the extracellular solution. The voltage-gated, inward currents were identified as Ca\(^{2+}\) currents because they were blocked completely by adding Co\(^{2+}\) (3 mM) to the extracellular solution (Fig. 2B; \(n = 10\)). The block was reversible on washout of Co\(^{2+}\) (not shown). The Na\(^{+}\) channel blocker tetrodotoxin (1
μM) did not suppress either the transient low-threshold component (Fig. 2D; n = 5) or the sustained high-threshold component (Fig. 2E; n = 3). Application of the dihydropyridine Ca$^{2+}$ channel antagonists nicardipine (10 μM) and nifedipine (100 μM) incompletely blocked the Ca$^{2+}$ current evoked from a holding potential of −60 mV (Fig. 2F). The average suppression was 71 ± 3% (n = 6). In two of these cells, a weak recovery was observed after washout of antagonist and before complete rundown of the Ca$^{2+}$ current (Fig. 2F). Little effect of the dihydropyridine antagonists was observed on Ca$^{2+}$ currents evoked by depolarizations from hyperpolarized potentials (−100 mV). These results suggest that rod bipolar cells express (at least) two types of Ca$^{2+}$ currents, a transient low-threshold T-type current of relatively low amplitude and a sustained high-threshold L-type current of larger amplitude.

For cells with cut axons, most likely severed during cutting of the slices (Hartveit 1996, 1997a), depolarization (ramps or voltage steps) from −100 mV evoked the low-threshold but not the high-threshold Ca$^{2+}$ current (Fig. 2G). The low-threshold current activated at −70.3 ± 1.1 mV (n = 5), not significantly different from the corresponding values for intact cells (P = 0.09). It was blocked completely and reversibly by 3 mM Co$^{2+}$ (not shown). Depolarization from −60 mV evoked no Ca$^{2+}$ current at all (n = 17; Fig. 2G). These results suggest that the high-threshold L-type current is expressed exclusively in the axon terminal region, whereas the low-threshold T-type current is expressed predominantly, possibly exclusively, in the soma-dendritic region.

For the high-threshold Ca$^{2+}$ current, the threshold and maximum amplitude were located at more negative membrane potentials than found for isolated large-terminal goldfish bipolar cells (Burrone and Lagnado 1997; Heidelberger and Matthews 1992; Tachibana et al. 1993). This could be a genuine species difference, but the series resistance imposed by the axon in the present somatic end recordings also may be expected to cause a measurable voltage error during activation of currents in the axon terminal (Mennerick et al. 1997; Protti and Llano 1998). A low-threshold Ca$^{2+}$ current has not been reported in goldfish bipolar cells.

**Reciprocal postsynaptic currents are evoked by depolarization of single rod bipolar cells**

To test whether depolarization of a rod bipolar cell might evoke a feedback response to the same cell, 25- to 140-ms-long (typically 50 ms), 10- to 90-mV depolarizing voltage steps from a holding potential of −70 mV were applied to single rod bipolar cells. With the standard (high) intracellular chloride concentration, the chloride equilibrium potential (E$_{Cl}^\text{c}$) was close to 0 mV (+2.1 mV). At negative holding potentials, this creates a strong, outwardly directed driving force for chloride ions. A representative example of the responses evoked by applying a series of depolarizing voltage steps to a rod bipolar cell is shown in Fig. 3. At a command potential of −60 mV, there was no response during or after the voltage step. At command potentials greater than or equal to −40 mV, an inward current occurred during the voltage step and a long-lasting inward tail current immediately followed the termination of the voltage step. The tail current was accompanied by a transient increase in the frequency of discrete, as well as partially overlapping, postsynaptic currents (PSCs; Fig. 3, A and B). The discrete PSCs were characterized by a rapid rise and a slower decay. The threshold for the response suggested that it depended on activation of the L-type Ca$^{2+}$ current. Similar responses were evoked in the large majority of cells tested (103/116).

Depolarization of rod bipolar cells from a conditioning hyperpolarization of −100 mV (n = 7) did not alter the threshold for evoked PSCs, suggesting that the T-type Ca$^{2+}$ current is not involved in mediating transmitter release. Application of Co$^{2+}$-containing extracellular solution blocked both the tail current and the depolarization-evoked PSCs. A clear increase
in the frequency of PSCs after a depolarizing voltage step rarely was observed >10–12 min after establishing the whole cell recording configuration. In general, the rundown occurred after 6–8 min. The voltage-gated Ca\(^{2+}\) current was also subject to rundown, but it ran down more slowly than the synaptic transmission. A difference in the time course of rundown between voltage-gated Ca\(^{2+}\) current and transmitter release or capacitance increase has also been observed in goldfish bipolar cells (Sakaba et al. 1997; von Gersdorff and Matthews 1994).

The baseline frequency of PSCs was always low enough that the depolarization-evoked increase in frequency could be detected clearly. Under the standard recording conditions, spontaneous PSCs were detected in 11 of 12 cells during observation periods that ranged from 30 to 180 s. To estimate the frequency of spontaneous PSCs, they were detected and captured by a variable amplitude event detection algorithm (see METHODS). The frequency of PSCs, averaged over the entire observation period, varied from 0.03 to 0.8 Hz (0.27 \(\pm\) 0.08 Hz; \(n = 11\)).

To quantify the magnitude of the depolarization-evoked response, a series of points first were positioned by eye on presumed baseline segments of the long-lasting tail current, from 0.0025 to 1 s after return of the membrane potential to \(-70\) mV after a depolarizing voltage-step (Fig. 4). Next a double exponential function was fit to the series of points, thereby constituting a hypothetical baseline from which the PSCs arose. Finally the double exponential function was subtracted from the raw current trace to yield an estimate of the postsynaptic current (Fig. 4). Integration of the postsynaptic current over the 1-s period yielded the charge flowing across the cell membrane. This method of quantification, which will be called the “fit-and-subtract” method, excluded any contribution from a Ca\(^{2+}\)-activated chloride current (see following text). Figure 5A shows the relation between the command potential and the averaged synaptic feedback response, measured as charge, for nine rod bipolar cells. The responses in each cell first were normalized to the cell’s maximum response and error bars indicate \(\pm\) SE (A and B).

![Figure 4](image-url)  
**FIG. 4.** Fit-and-subtract method used to measure the postsynaptic current evoked at \(-70\) mV after a depolarizing voltage step. Series of points (empty boxes) were positioned by eye on presumed baseline segments of the current trace (thin line), between 0.0025 and 1 s after return of the membrane potential to \(-70\) mV (top). A double exponential was fit to the series of points, thereby constituting a hypothetical baseline from which the PSCs arose (thick line). Subtraction of the double exponential function from the raw current trace yielded the postsynaptic current (bottom). Horizontal dashed lines indicate the average holding current during a 260-ms period preceding the depolarizing voltage step. Current traces start immediately after terminating the depolarizing voltage step.
goldfish retina (Okada et al. 1995). When the total current was integrated during a period of \( \sim 1 \text{s} \) (Fig. 3A, 1), the measurements were dominated by the putative \( \text{Ca}^{2+} \)-activated chloride current. Nevertheless, the shape of the corresponding charge-voltage relation was similar to that for the postsynaptic current (Fig. 5B). Unfortunately, niflumic acid, which has been reported to block \( \text{Ca}^{2+} \)-activated chloride currents in other preparations (e.g., Okada et al. 1995), suppressed not only the long-lasting tail current, but the voltage-activated \( \text{Ca}^{2+} \) current as well (10 – 400 \( \mu \text{M} \); \( n = 17 \)). No further attempts were made to isolate pharmacologically the postsynaptic response from other \( \text{Ca}^{2+} \)-activated processes.

Reversal potential of evoked PSCs

For some cells, evoked PSCs could be discriminated clearly during several of the depolarizing voltage steps, allowing a reversal of current close to 0 mV to be observed (Fig. 6A). This is the value expected for a GABA-evoked current mediated by chloride ions (\( E_{\text{Cl}} = +2.1 \text{mV} \)). The \( E_{\text{rev}} \) of GABA-evoked currents in rod bipolar cells was confirmed by drug application at different holding potentials (Fig. 6B) or during voltage ramps (−100 to +40 mV; 100 mV/s). The average \( E_{\text{rev}} \) was −6.5 ± 0.7 mV (\( n = 5 \)). When \( E_{\text{Cl}} \) was changed to −46.9 mV (\( n = 3 \)) by recording with a low intracellular chloride concentration, outwardly directed PSCs could be observed during (longer-lasting) voltage steps to 0 mV (Fig. 6C). This indicated that \( E_{\text{rev}} \) was more negative than 0 mV and thus followed the change in \( E_{\text{Cl}} \), suggesting that the depolarization-evoked PSCs were mediated by chloride ions.

Depolarization-evoked PSCs are GABAergic inhibitory PSCs (IPSCs)

The frequency of depolarization-evoked PSCs was strongly suppressed, and in some cases even blocked, by ionotropic \( \text{GABA}_A \) and/or \( \text{GABA}_C \) receptor antagonists. An example of...
the effect of the competitive GABA$_A$ receptor antagonist bicuculline (100 μM) on the response of a rod bipolar cell to a depolarizing voltage step (to ~30 mV) is shown in Fig. 7A. In this case, the response clearly recovered after bicuculline was washed away. For some cells it was difficult, however, to observe a clear recovery, due to the rapid rundown of the synaptic response. This also made it difficult to test more than one drug on each cell. To quantify the suppression, the response was measured by the fit-and-subtract method during the 1-s time period after return of the membrane potential to ~70 mV after a depolarizing voltage step (see above). The response was measured for each sweep in the control condition, during drug application and after washout. The degree of suppression was calculated as (CONTROL − DRUG)/CONTROL × 100% where CONTROL is the response in the control condition and DRUG is the response during drug application. To correct for drug-independent rundown of the response, a theoretical control value was estimated by fitting a line through the response values before and after drug application. For a few cells, a single exponential gave a better fit. The effect of bicuculline was variable from one cell to another (range of suppression 10–96%), and for one cell the postsynaptic charge actually increased almost 100%. A possible explanation for this result is that bicuculline primarily disinhibited the A17 amacrine cell(s) (cf. Zhang et al. 1997) and that most of the GABAergic input to this rod bipolar cell was mediated by GABA$_C$ receptors. When this cell was excluded from the analysis, the average suppression was 63 ± 15% (n = 6).

GABA$_C$ receptors in rat retina are characterized by insensitivity to bicuculline (Feigenspan et al. 1993) as well as being relatively insensitive to concentrations of picrotoxin that will block GABA$_C$ receptors in other species (Qian and Dowling 1993). They can be antagonized by 3-APMPA (Pan and Lipton 1995). An example of strong, reversible suppression of the depolarization-evoked PSCs by 500 μM 3-APMPA is shown in Fig. 7B. As for bicuculline, the degree of suppression by 3-APMPA varied from one cell to another (range 38–100%), suggesting that the proportion of GABA$_A$ and GABA$_C$ receptors varies between the rod bipolar cells recorded here (cf. Euler and Wässle 1998; Feigenspan et al. 1993; Qian and Dowling 1995; Yeh et al. 1996). Overall, 3-APMPA reduced the evoked response, measured as integrated synaptic current, by 65 ± 6% (n = 9). Due to rapid rundown of the evoked response, it was not possible to compare the relative suppression evoked by application of GABA$_A$ and GABA$_C$ receptor antagonists for individual cells. However, when bicuculline and 3-APMPA were co-applied, the depolarization-evoked PSCs were strongly suppressed in most cells tested (range of suppression 66–100%). An example of a reversible block is shown in Fig. 8A. The average suppression by co-application of bicuculline and 3-APMPA was 90 ± 5% (n = 10). Furthermore, application of a high concentration of picrotoxin (1 mM), presumably antagonizing both GABA$_A$ and GABA$_C$ receptors, also was able to strongly suppress the depolarization-evoked PSCs (Fig. 8B). In the case of picrotoxin, the synaptic response only partially recovered (before rundown) in one cell. On average, picrotoxin reduced the evoked response, measured as integrated synaptic current by 83 ± 3% (n = 5; range 80–95%). These results indicate that the evoked PSCs are GABAergic IPSCs mediated by a variable combination of GABA$_A$ and GABA$_C$ receptors on the rod bipolar cells.

![Fig. 8. Suppression of depolarization-evoked IPSCs by antagonists of GABA$_A$ and GABA$_C$ receptors. A: suppression of depolarization-evoked IPSCs by co-application of bicuculline (100 μM) and 3-APMPA (500 μM). Panels show response in control condition (top), during application of bicuculline and 3-APMPA (middle), and after washout (bottom). B: suppression of depolarization-evoked IPSCs by application of picrotoxin (1 mM). Panels show response in control condition (top) and during application of picrotoxin (bottom). Response did not recover to control levels before rundown. -- -- --, average holding current during a 260-ms period preceding the 50-ms depolarizing voltage step (A and B). Current traces start immediately after terminating the depolarizing voltage step (A and B).](http://jn.physiology.org/)

Because 3-APMPA is also a potent GABA$_B$ receptor agonist (Seabrook et al. 1990), the inhibition of the depolarization-evoked IPSCs observed with this drug might have been mediated by the activation of putative GABA$_B$ receptors on the rod bipolar axon terminal (which could suppress the voltage-gated Ca$^{2+}$ current and depolarization-evoked release of glutamate) (cf. Maguire et al. 1989). There was no significant difference, however, in the peak amplitude of voltage-dependent Ca$^{2+}$ currents between control recordings (~49.2 ± 3.8 pA) and recordings with application of the selective GABA$_B$ agonist baclofen (~50.0 ± 2.9 pA; n = 4; P = 0.70; paired t-test). This is consistent with previous evidence that GABA$_B$ receptors are not expressed by rod bipolar cells (Koulen et al. 1998b; Pan and Lipton 1995; Yeh et al. 1990). Alternatively, 3-APMPA might act on putative presynaptic GABA$_B$ receptors on the A17 amacrine cell(s) with consequent reduction of GABA release. The effect of baclofen was tested on depolarization-evoked IPSCs on four rod bipolar cells. For three of the cells, there was a small, reversible reduction in the response (15 ± 5%). For the last cell, baclofen evoked a reversible increase of the response (25%; data not shown). This suggests that release of GABA from the amacrine cells can be modulated by pre-synaptic GABA$_B$ receptors, but that this mechanism is not sufficient to account for the inhibitory effect of 3-APMPA on depolarization-evoked IPSCs in these experiments.

**Depolarization-evoked IPSCs depend on glutamate release from rod bipolar cells**

If the transmission from rod bipolar cells to A17 amacrine cells is mediated by glutamate, it should be possible to block the depolarization-evoked IPSCs by antagonists of glutamate
receptors. This was examined by application of the competitive non-NMDA receptor antagonist CNQX. As predicted, CNQX evoked a strong but reversible suppression of the depolarization-evoked IPSCs (Fig. 9A). CNQX reduced the average response, measured as integrated synaptic current, by 88 ± 5% (n = 10; range 62–93%). These results strongly suggest that activation of non-NMDA receptors on A17 amacrine cells evokes the GABAergic feedback onto rod bipolar cells. Functional (i.e., conductance increasing) ionotropic non-NMDA receptors are not expressed by rod bipolar cells (Hartveit 1996), excluding the possibility that CNQX acts directly on these cells.

To examine whether NMDA receptors also are involved in the generation of the feedback response, depolarizing voltage steps were applied in the presence of the competitive NMDA receptor antagonist CPP. There was no consistent effect of CPP on the feedback response. The effect ranged from a 30% decrease to a 10% increase of the response relative to control (0.5 ± 6%; n = 8; Fig. 9B). Although it is difficult to exclude any involvement of NMDA receptors, it seems safe to conclude that under the present conditions, the GABAergic feedback mainly is driven by activation of non-NMDA receptors on the A17 amacrine cells.

In some of the pharmacological tests (Figs. 8B and 9A), it cannot be excluded that the Ca\(^{2+}\)-activated chloride current was suppressed weakly by some of the receptor antagonists used. An alternative explanation is that there was overlap of IPSCs in the control recordings (e.g., Fig. 9A; Control, Wash). For responses with such overlap, the fit-and-subtract method would underestimate the synaptic current integral. This would only weakly influence the quantitative estimate of the degree of suppression, except in cases of moderate suppression where it would be underestimated.

**Time course of the feedback response**

The period of increased frequency of IPSCs after 50- to 150-ms depolarizing voltage steps seemed to last <1 s for most cells (Figs. 3 and 4). This was analyzed in more detail by extending the sampling period to 10 s after the voltage step. The postsynaptic current was measured by the fit-and-subtract method. A single epoch of recording from a representative cell is shown in Fig. 10A. The curve in Fig. 10B displays the average time course of the postsynaptic response. It was calculated by averaging 17 individual epochs of depolarization-evoked postsynaptic currents from nine cells. The response peaked immediately after the end of the voltage step and then decayed to a relatively constant level in <2 s. The decay could be fit well with a single exponential (τ = 215 ms; Fig. 10B).

It is not clear whether the time course of the feedback response is determined primarily by the rod bipolar cell or the A17 amacrine cell(s). If the decay of the synaptic output from the rod bipolar cell follows a time course similar to that of the GABAergic feedback response, it means that transmitter release must continue for some time after closure of the Ca\(^{2+}\) channels in the rod bipolar cell. In isolated goldfish bipolar cells, Tachibana and Okada (1991) observed a maintained release of transmitter that continued for some seconds after closure of Ca\(^{2+}\) channels (their Fig. 7, see also Burrone and Lagnado 1997). If this is also the case for rod bipolar cells, one would expect the decay of the free Ca\(^{2+}\) concentration in the synaptic terminal to follow an equally slow time course on repolarization to −70 mV. Interestingly, for seven of the nine cells tested with a 10-s sampling period, sweeps sampled after rundown of synaptic transmission contained a robust long-lasting tail-current (most likely a Ca\(^{2+}\)-activated chloride current; see preceding text). The time course of the tail current could be fit well with the sum of two exponentials. The average time constant of the slow component was 620 ± 350 ms (n = 7), in the same range as the time constant of decay of the postsynaptic response in the rod bipolar cell (Fig. 10B). This is consistent with a hypothesis that both transmitter release as well as the tail current in the rod bipolar cell could follow the decay of the cytosolic Ca\(^{2+}\) concentration with similar affinity.

Alternatively, the time course of the feedback response in the rod bipolar cell could be determined primarily by the time course of transmitter release from the A17 amacrine cell(s). A long-lasting decay has been suggested for synaptic transmission between chick amacrine cells in culture (Gleason et al. 1993, 1994). To further examine the release properties of rod bipolar cells and A17 amacrine cells, in particular, whether they are capable of sustained release during prolonged depolarization of a rod bipolar cell, 20-s-long depolarizing voltage steps to either −30 or −20 mV were applied (n = 11). To compensate for the reduced driving force for chloride ions at the higher command potential compared with −70 mV, the extracellular chloride concentration was reduced to 20.5 mM by replacing 114 mM NaCl with an equivalent amount of sodium isethionate, changing the E\(_{Cl}^t\) to +49.6 mV (+47.8 mV when cesium glutamate partially replaced CsCl intracellularly). In this condition, a sustained feedback response with a markedly enhanced frequency of IPSCs was observed for the
estimated by the fit-and-subtract method, for 17 epochs of stimulation (as in A).

For the depolarizing voltage step. - - - , average holding current during a 260-ms period preceding the voltage step.

duration of the depolarizing voltage step for all cells examined (Fig. 10C). This suggests that both rod bipolar cells and A17 amacrine cells are capable of sustained release (for periods up to \( \approx 20 \) s). To verify that \( \text{Ca}^{2+} \)-influx in rod bipolar cells could be sustained for correspondingly long periods, cells were tested with similar depolarizing pulses (to \(-30\) or \(-20\) mV for 20 s) in the presence of 1 mM picrotoxin (\( n = 5 \)). The example illustrated in Fig. 10D indicates that although there is a clear reduction in the current amplitude, the voltage-gated \( \text{Ca}^{2+} \) current in rod bipolar cells can last \( \approx 20 \) s. Similar results were seen for two cells. In the remaining two cells, the \( \text{Ca}^{2+} \) current decayed more or less completely within the 20-s period.

**IPSCs are evoked in the inner plexiform layer**

From the results reported so far, it cannot be concluded unequivocally that the IPSCs are evoked in the inner plexiform layer by reciprocal connections from A17 amacrine cells onto rod bipolar cell axon terminals. Alternatively, they could be evoked in the outer plexiform layer from horizontal cell contacts with rod bipolar cell dendrites (Yang and Wu 1991). Two sets of observations argue against this interpretation. First, the depolarizing voltage steps evoked discrete IPSCs, strongly suggesting vesicular release, whereas transmitter release from horizontal cells is thought to be nonvesicular (Schwartz 1987). Second, in recordings from 12 cells with cut axons, all of which presumably were rod bipolar cells (based on the location of the somata in close approximation to the outer plexiform layer), a depolarization-evoked feedback response was never observed (Fig. 11). This indicates that the response is mediated in the inner plexiform layer.

**Release of GABA does not require activation of voltage-gated Na\(^+\)-channels**

The experiments described so far suggest that release of GABA from A17 amacrine cells onto rod bipolar terminals depends on a vesicular release mechanism. To examine whether the release is influenced by voltage-gated Na\(^+\) currents, possibly mediating spiking behavior in the A17 amacrine cells, tetrodotoxin, a specific blocker of voltage-gated Na\(^+\) channels, was applied during the depolarizing voltage step protocol. Tetrodotoxin had no or only moderate effects on the synaptic transmission in 5/8 rod bipolar cells. Compared with the control condition, the change in response varied from a 10% increase to a 25% reduction (7 ± 5%; \( n = 5 \)). For the
In this study, I have examined the functional characteristics of the reciprocal synaptic interaction between rod bipolar cells and amacrine cells in the rat retina. The major findings are that depolarization of a single rod bipolar cell evokes feedback PSCs in the same cell, that the PSCs are GABAergic IPSCs mediated by chloride ions, that both GABA_A and GABA_C receptors can participate in the feedback response, that the threshold for the feedback response is approximately $-50$ mV, indistinguishable from the threshold for the voltage-gated L-type Ca^{2+} current in rod bipolar cells, that this Ca^{2+} current is localized to the axon terminal, that the feedforward excitation of the amacrine cells is mediated primarily by CNQX-sensitive non-NMDA receptors, and finally that activation of voltage-gated Na^+ channels is not essential for, but can possibly contribute to, release of GABA from the amacrine cells.

It is well established that the reciprocal synapses do not account for all of the synaptic inputs to rod bipolar axon terminals (Kim et al. 1998; Strettoi et al. 1990) and that some of the nonreciprocal input might be GABAergic (Kim et al. 1998). Accordingly, it might be argued that some of the depolarization-evoked IPSCs originated via a hypothetical synaptic circuit: rod bipolar $\rightarrow$ AII amacrine $\rightarrow$ on-cone bipolar $\rightarrow$ unknown amacrine $\rightarrow$ rod bipolar via nonreciprocal synapses (Strettoi 1990, 1992, 1994). While it is not possible to exclude a contribution of such a circuit to the evoked responses, it is likely to have been unimportant in the present study, given its multisynaptic complexity. With varying patterns of convergence and divergence at each stage, it is hard to see that an output signal from a single rod bipolar cell would be transmitted reliably through this circuit. In contrast, the depolarization-evoked response was clearly detected in the large majority of cells tested.

**Voltage-gated Ca^{2+} current and transmitter release in rod bipolar cells**

Two components of a voltage-gated Ca^{2+} current were identified in rat rod bipolar cells. A low-threshold, transient, T-type component activated at about $-70$ mV and a high-threshold, sustained component activated at about $-50$ mV. Both currents were blocked reversibly by Co^{2+}. The high-threshold component was blocked (incompletely) by dihydropyridine antagonists (nicardipine and nifedipine), suggesting an L-type pharmacology (cf. De la Villa et al. 1998; Heidelberger and Matthews 1992; Pan and Lipton 1995; Protti and Llano 1998; Tachibana et al. 1993). However, it should be noted that the incomplete block leaves open the possibility that other types of Ca^{2+} currents are present in rod bipolar cells (N, P/Q, or R type). Cells with cut axons displayed the low-threshold current but not the high-threshold current. This suggests that the L-type Ca^{2+} channels are located at the (presynaptic) axon terminal (consistent with previous findings) (De la Villa et al. 1998; Pan and Lipton 1995; Protti and Llano 1998) and that they are involved directly in mediating transmitter release. A similar role for an L-type Ca^{2+} current has been found in goldfish bipolar cells (Tachibana et al. 1993) where the dynamics of the Ca^{2+} response at the axon terminals correlates with exocytotic release of neurotransmitters (Tachibana and Okada 1991; von Gersdorff and Matthews 1994). It remains to be determined, however, how well the operating
range of the voltage-gated L-type Ca\(^{2+}\) current is matched to the voltage excursion produced by a visual stimulus in a rod bipolar cell.

The threshold for the synaptic feedback response was indistinguishable from the threshold for the voltage-gated L-type Ca\(^{2+}\) current in rod bipolar cells. This indicates that the release properties of rod bipolar cells and A17 amacrine cells are well matched to each other and that depolarization-evoked transmitter release from a rod bipolar cell immediately gives rise to an inhibitory feedback response. In recordings with brief (~50 ms) depolarizations of a rod bipolar cell, the frequency of feedback IPSCs decayed to the baseline level with a time constant of ~200 ms. Because the Ca\(^{2+}\) current in the rod bipolar cell would terminate rapidly, the decay may correspond to a maintained component of release, employing a release sensor with high Ca\(^{2+}\) affinity. In goldfish bipolar cells, the presence of asynchronous transmitter release after the termination of Ca\(^{2+}\) current has not yet been settled (cf. Burrone and Lagnado 1997; Lagnado et al. 1996; Sakaba et al. 1997; Tachibana and Okada 1991; von Gersdorff and Matthews 1997). Alternatively, the time course of decay of IPSC frequency may be a property of the A17 amacrine cell(s), consistent with the release properties of chick amacrine cells, most likely involving a high-affinity Ca\(^{2+}\) sensor for exocytosis (Gleason et al. 1993, 1994).

In recordings with longer-lasting depolarization of rod bipolar cells, it was demonstrated that both rod bipolar cells and A17 amacrine cells are capable of sustained transmitter release. This again could reflect a role for a release sensor with high Ca\(^{2+}\) affinity in rod bipolar cells, responding to an increase in the bulk Ca\(^{2+}\) concentration in the terminal (Lagnado et al. 1996; Sakaba et al. 1997; von Gersdorff and Matthews 1997). However, at least in some rod bipolar cells, maintained depolarization evoked a sustained Ca\(^{2+}\) current (Fig. 10D), presumably giving rise to strongly elevated Ca\(^{2+}\) concentrations close to open Ca\(^{2+}\) channels. In that case, a release sensor with low Ca\(^{2+}\) affinity (Heidelberger et al. 1994; von Gersdorff and Matthews 1994) could be involved in mediating sustained release from the rod bipolar cell. It is possible that paired recordings from a rod bipolar cell and an A17 (or an AII) amacrine cell can provide more information concerning the mechanism.

A transient, T-type Ca\(^{2+}\) current has also been reported in mouse bipolar cells of unspecified type (De la Villa et al. 1998; Kaneko et al. 1989). In contrast, a recent investigation of rat rod bipolar cells found a complete absence of a T-type current and it was suggested that only cone bipolar cells express such currents (Protti and Llano 1998). The present results indicate that rod bipolar cells do indeed express a T-type current and suggest that the absence of this current in the study of Protti and Llano (1998) could be due to its relatively rapid rundown. Alternatively, the absence of a T-type current, as well as the low frequency of cells with intact synaptic responses and the extremely low probability of recording from cone bipolar cells (as reported by the authors) could suggest that methodological differences in the preparation of the slices may have physiological consequences.

The T-type Ca\(^{2+}\) current in rod bipolar cells does not seem to be directly involved in transmitter release from the axon terminal. However, the functional role and precise cellular localization of the corresponding ion channels remains to be determined. Although the majority of the channels most likely have a soma-dendritic location, mouse bipolar cells seem to have T-type channels both at the axon terminal and at the soma (Kaneko et al. 1989).

**Neurotransmitter receptors involved in reciprocal synaptic transmission**

Previous morphological studies indicate that subunits of both GABA\(_A\) and GABA\(_C\) receptors are localized on the axon terminals of rod bipolar cells, but it has been difficult to demonstrate a synaptic localization of the individual subunits (Greferath et al. 1994; Koulen et al. 1998a). The results presented here suggest that the transmitter released from A17 amacrine cells is GABA and that it activates both GABA\(_A\) and GABA\(_C\) receptors on the axon terminals of rod bipolar cells. This result is well in accord with recent findings for bipolar-amacrine interactions in the tiger salamander retina (Dong and Werblin 1998). It will be important to determine whether release of a single synaptic vesicle from an A17 amacrine cell can activate both GABA\(_A\) and GABA\(_C\) receptors on rod bipolar terminals (corresponding to a dual-component IPSC) or whether the receptors are strictly segregated to individual synapses (Koulen et al. 1998a).

Because glycine receptors were blocked pharmacologically, this study leaves open the possible existence of glycineric A17 amacrine cells. Interestingly, however, Kim et al. (1998) found that all amacrine cell processes with reciprocal connections to rod bipolar cells in rat retina show immunoreactivity for glutamic acid decarboxylase (cf. Freed et al. 1987). Given that GABA and glycine are not colocalized in the mammalian retina, only some of the presynaptic, nonreciprocal amacrine cell processes are candidates for glycineric input to rod bipolar cells.

The sensitivity of single-cell-evoked feedback IPSCs to the non-NMDA antagonist CNQX indicates that A17 amacrine cells express ionotropic non-NMDA receptors and consequently are depolarized by glutamatergic output from rod bipolar cells (cf. Dong and Werblin 1998). This supports previous evidence that the synaptic transmission from rod bipolar cells to A17 amacrine cells is sign-conserving (Raviola and Dacheux 1987). The evidence for sustained signal transmission between rod bipolar cells and A17 amacrine cells (Fig. 10C) suggests either that non-NMDA receptors on A17 amacrine cells undergo little desensitization or that partially desensitized receptors are sufficient for transmission. Alternatively, long-lasting depolarization of rod bipolar cells might activate NMDA receptors on A17 amacrine cells (cf. Matsui et al. 1998).

**Functional characteristics of reciprocal amacrine cells**

The present results indirectly reveal some properties of reciprocal (A17) amacrine cells. First, these cells most likely employ a vesicular release mechanism, as evidenced by discrete, depolarization-evoked IPSCs in rod bipolar cells. It is difficult, however, to entirely eliminate the possibility that a nonvesicular release mechanism could generate similar IPSCs. One could imagine that discrete IPSCs might be induced by rapidly desensitizing GABA receptors in rod bipolar cells or by transient depolarization of A17 amacrine cells mediated by...
non-NMDA receptors. Another potential mechanism for generation of discrete IPSCs, Na\(^+\) spike-induced GABA release from A17 amacrine cells, is less likely, given the resistance of some IPSCs to tetrodotoxin.

Second, the threshold for transmitter release from A17 amacrine cells is low enough that it does not require spiking mediated by tetrodotoxin-sensitive voltage-gated Na\(^+\) channels. However, tetrodotoxin reversibly suppressed feedback IPSCs in some rod bipolar cells, suggesting that under some conditions activation of voltage-gated Na\(^+\) channels can enhance transmitter release. This might occur when an excitatory postsynaptic potential in an A17 varicosity or spine head exceeds a putative threshold for initiation of regenerative currents (cf. Miller et al. 1985). The evidence for action potentials in A17 amacrine cells is conflicting (Bloomfield 1992, 1996; Nelson and Kolb 1985; Raviola and Dacheux 1987). Alternatively, A17 amacrine cells in rat retina might be a heterogeneous group of cells, with different dependence on spiking (mediated by voltage-gated Na\(^+\) channels) for signal transmission.

Comparison between retina and olfactory bulb

The results obtained here for rod bipolar cells and reciprocal amacrine cells are remarkably similar to those obtained previously for the mitral cell–granule cell interaction in the olfactory bulb (Wellis and Kauer 1993; see also Jahr and Nicoll 1982). Granule cells are axonless GABAergic inhibitory interneurons that form dendrodendritic synapses with glutamatergic mitral cells. Application of depolarizing voltage steps to a mitral cell evokes a prolonged increase in the frequency of GABAergic IPSCs in the same cell, and the reciprocal synaptic interaction is sensitive to NMDA and non-NMDA receptor antagonists (mitral cell → granule cell) and to GABA\(_A\) antagonists (granule cell → mitral cell) (Wellis and Kauer 1993). Jahr and Nicoll (1982) found that reciprocal inhibition in the olfactory bulb persists despite the presence of tetrodotoxin, implying that the contribution of voltage-gated Na\(^+\) channels to the potentials in granule dendritic spines (Wellis and Kauer 1994) is not essential for release of transmitter.

Self-inhibition versus lateral inhibition

Although this study has demonstrated that depolarization of a rod bipolar cell evokes a GABAergic feedback response in itself (self-inhibition), it is unknown whether there is also output to other rod bipolar cells in contact with the same amacrine cell (lateral inhibition). Morphological data indicate that the axon terminal of a single rod bipolar cell is in contact with the dendrites of several reciprocal amacrine cells (Chun et al. 1993; Strettoi et al. 1990), and in cat retina it has been estimated that a single A17 cell makes reciprocal contacts with \(\sim 1,000\) rod bipolar axon terminals (Nelson and Kolb 1985). Lateral inhibition requires that depolarization spreads from an activated region of an amacrine cell into the neighboring dendritic tree. This might occur solely by passive, electrotonic spread of depolarization, but it also could be enhanced by any active or regenerative properties of the dendritic membrane of the amacrine cell (cf. Bloomfield 1996). Even regenerative depolarization of a nonpropagating nature could facilitate the spread of depolarization in the amacrine dendritic tree. An alternative view is that the dendritic varicosities of reciprocal amacrine cells are isolated electrically (Ellias and Stevens 1980; Masland 1988), implying that each reciprocal synapse is a locally operating microcircuit. For A17 amacrine cells, however, Nelson and Kolb (1985) and Bloomfield (1992) reported that receptive-field measurements do not support the notion of isolated dendritic regions. The present study found that spontaneous IPSCs in rod bipolar cells occur at a low frequency, suggesting that there is little spontaneous transmitter release from A17 amacrine cells. This may be because there is little excitatory drive from rod bipolar cells. Alternatively, an A17 amacrine cell may continuously receive excitatory input in several regions of its dendritic tree, except from the rod bipolar cell under study, which is voltage-clamped at \(-70\) mV and therefore does not release glutamate. The latter interpretation implies that there is little or no integration in the dendritic tree of A17 amacrine cells and that release of GABA is controlled locally. It will be interesting to see whether dual recordings from pairs of rod bipolar cells can be used to examine whether lateral inhibition, as well as self-inhibition, occurs in the synaptic transmission between rod bipolar cells and A17 amacrine cells.

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