Light-Evoked Excitatory Synaptic Currents of X-Type Retinal Ganglion Cells

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Cohen, Ethan D. Light-evoked excitatory synaptic currents of X-type retinal ganglion cells. J Neurophysiol 83: 3217–3229, 2000. The excitatory amino acid receptor (EAAR) types involved in the generation of light-evoked excitatory postsynaptic currents (EPSCs) were examined in X-type retinal ganglion cells. Using isolated and sliced preparations of cat and ferret retina, the light-evoked EPSCs of X cells were isolated by adding picrotoxin and strychnine to the bath to remove synaptic inhibition. N-methyl-D-aspartate (NMDA) receptors contribute significantly to the light-evoked EPSCs of ON- and OFF-X cells at many different holding potentials. An NMDA receptor contribution to the EPSCs was observable when retinal synaptic inhibition was either normally present or pharmacologically blocked. NMDA receptors formed 80% of the peak light-evoked EPSC at a holding potential of −40 mV; however, even at −80 mV, 20% of the light-evoked EPSC was NMDA-mediated. An α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor-mediated component to the light-evoked EPSCs predominated at a holding potential of −80 mV. The light-evoked EPSC was blocked by the AMPA receptor-selective antagonist GYKI52466 (50–100 μM). The AMPA receptor–mediated EPSC component had a linear current-voltage relation. AMPA receptors form the main non-NMDA EAAR current on both ON- and OFF-X ganglion cell dendrites. When synaptic transmission was blocked by the addition of Cd2+ to the Ringer, application of kainate directly to ganglion cells evoked excitatory currents that were strongly blocked by GYKI52466. Experiments using selective EAAR modulators showed the AMPA receptor–selective modulator cyclothiazide potentiated glutamate-evoked currents on X cells, while the kainate receptor–selective modulator concanavalin A (ConA) had no effect on kainate-evoked currents. Whereas the present study confirms the general notion that AMPA EAAR-mediated currents are transient and NMDA receptor–mediated currents are sustained, current-voltage relations of the light-evoked EPSC at different time points showed the contributions of these two receptor types significantly overlap. Both NMDA and AMPA EAARs can transmit transient and sustained visual signals in X ganglion cells, suggesting that much signal shaping occurs presynaptically in bipolar cells.

INTRODUCTION

In the vertebrate retina, the principle neurotransmitter used to encode the excitatory visual signal to ganglion cells is the excitatory amino acid (EAA) glutamate. Photoreceptors release glutamate onto bipolar cells (Copenhagen and Jahr 1989; Slaughter and Miller 1981, 1983a). Bipolar cells in turn release glutamate onto the dendrites of ganglion cells (Slaughter and Miller 1983b; Tachibana and Okada 1991). Thus using glutamate, light-evoked excitation to the ganglion cell is transduced through a vertical pathway of EAA receptor (EAAR)–gated currents.

Glutamate, released by bipolar cells can potentially bind to a wide variety of EAAR subunits known to be present on the retinal ganglion cell. In situ hybridization studies of ionotropic EAARs in mammalian retinae show that ganglion cells express the N-methyl-D-aspartate (NMDA) receptor types, NR1, and NR2A-C, the α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor types, GluR1, 2, and 4, and kainate receptor types KA1 and GluR7 (Brandstatter et al. 1994; Hammasaki-Britto et al. 1993; Mishina et al. 1994). Metabotropic G-protein–gated EAARs are also present on ganglion cells (Brandstatter et al. 1994; Rothe et al. 1994). Thus a number of potentially different glutamate receptor complexes could be involved in mediating the light-evoked current (LEC) signal at individual synapses on the ganglion cell dendrite (e.g., Puchalski et al. 1994; Takumi et al. 1999).

Physiological studies of mammalian ganglion cells in dissociated and sliced retinal preparations have shown that most cells respond to exogenous application of several different EAAR agonists, including NMDA, AMPA, and kainic acid (KA) (Aizenmann et al. 1988; Cohen et al. 1994; Karschin et al. 1988); however, little is known how the individual EAAR–gated currents contribute to the light-evoked EPSC in mammalian ganglion cells (Cohen 1998). In addition, many early pharmacological studies used the EAA agonist KA. KA also binds to AMPA receptors, so it has been unclear what proportions of AMPA or kainate type EAARs are involved in generating these ligand-gated currents. Research on ON-OFF and ON-center ganglion cells in amphibians has shown that both NMDA and AMPA/KA EAARs contribute to their light-evoked EPSCs (Diamond and Copenhagen 1993; Mittman et al. 1990). However, the EAARs involved in generating the light-evoked EPSCs on the dendrites of OFF-center ganglion cells remains unexamined in any species to date (Cohen 1998).

The “X” or “β” ganglion cell is a well-known model cell type in the mammalian visual system whose physiologic properties have been extensively studied using extracellular recording (for review, see Boycott and Wassle 1999). X-type ganglion cells respond with sustained firing to light, display linear receptive field summation, have medium-large somas, and display a narrow bushy dendritic arborization termed β. ON- and OFF-X ganglion cells receive synaptic input from multiple cone bipolar cell types (Cohen and Sterling 1991, 1992; Kolb 1979; Kolb and Nelson 1993; McGuire et al. 1986). These cone bipolar synaptic inputs to X ganglion cell dendrites could potentially use different EAARs.

This paper examines the pharmacology of the light-evoked currents.
EAAR-gated synaptic current components driving ON- and OFF-X ganglion cell types in cat and ferret retinae. The results of this study show that in mammals, both AMPA and NMDA EAARs strongly contribute to the time course of the light-evoked excitatory postsynaptic current (EPSC) of ON- and OFF-sustained (X) type ganglion cells. In contrast, kainate receptors contribute little direct synaptic current to the light-evoked EPSC of X-type ganglion cells.

Methods

The preparation of slices and isolated retina has been described in detail in a previous paper (Cohen 1998) and will only be summarized here. The eyes of cats and ferrets were obtained using university approved protocols. Both adult and young cats were used for these experiments. Some adult cats (age >1 yr) were used in the experiments using pentobarbital sodium anesthesia (25 mg/kg, n = 20). The remaining 70% of the retina experiments used kittens or young cats (averaging 74 days of age), whose eyes were typically obtained after a series of physiology experiments using halothane anesthesia. The animals were euthanized after eye removal using pentobarbital. Ferret eyes were obtained from animals 2–4 mo of age, anesthetized with intraperitoneal ketamine/pentobarbital, following decapitation. No significant differences were noted between the light-evoked conductance mechanisms among the animal ages used in this study.

The EAARs involved in the LECs of cat and ferret ganglion cells were studied using thick retinal slice and isolated retina preparations. However, results using these two preparations were found to be virtually identical and have been pooled in this study (see Cohen 1998). Using retinal slice preparations, the LECs of 73 X ganglion cells were examined using whole cell recording. Most recordings were from cat retinal ganglion cells; however, a limited number of ferret X ganglion cells (10) have been included (e.g., Cohen 1998, Fig. 1D). In the isolated retina, the LEC pharmacology of a series of 81 X ganglion cells were recorded, mainly from cats but also including 7 ferret retinal units.

Retinal slice preparation

Retinal slices were prepared similar to the methods of (Cohen 1998; Cohen et al. 1994). The retina was isolated in the dark using dim far red wavelength lamps for dissection purposes. Small pieces of central retina were placed on nitrocellulose filter paper disks and cut into slices (300 μm thickness) in a chilled low sodium Ringer. The slices were placed in a covered incubation chamber containing oxygenated (95% O2-5% CO2) Ames Ringer solution that contained (in mM) 120 NaCl, 3.1 KCl, 0.5 KH2PO4, 23 NaHCO3, 1.2 Mg2SO4, 1.15 CaCl2, were photographed, fixed in 4% paraformaldehyde in 0.12 M phosphate buffer containing 0.05% Lucifer yellow and neurobiotin/biocytin (0.5%; Mountain View, CA) and then processed for immunohistochemistry. The cells were examined using whole cell recording. Most recordings were obtained using depolarizing current stimuli to dissect off the nerve fiber layer and Muller cell endfeet. For cell identification, internal solutions using thinly tapered patch electrodes to disengage the cell body were exposed by a chloride wire or agar bridge in the bath located out of the light path. Holding potentials were corrected for liquid junction potentials (Barry 1994; Neher 1992).

Table 1. Composition of internal Ringer

<table>
<thead>
<tr>
<th>Ringer</th>
<th>A</th>
<th>B</th>
<th>C</th>
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<tbody>
<tr>
<td>KMnSO4</td>
<td>120</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CsMnSO4</td>
<td>0</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>KCl</td>
<td>4*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MgCl2</td>
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<td>0</td>
<td>2</td>
</tr>
<tr>
<td>HEPES</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>EGTA</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>CaCl2</td>
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<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>TEA-Cl</td>
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<td>0</td>
<td>5</td>
</tr>
<tr>
<td>TBA-Cl</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>4-AP</td>
<td>0</td>
<td>0</td>
<td>1</td>
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All above Ringers contained 1 mM glutathione, 1 mM ATP, and 0.5 mM GTP, and 0.05% Lucifer yellow (Li+ or methoxy carbonyl salt), pH 7.2.

* Some early studies used 2 mM KCl.

Recording

Patch electrodes (3–5 MΩ) were drawn from borosilicate glass (1.5 mm OD, 0.86 mm ID) on a Brown Flaming P-87 electrode puller (Sutter Instruments, San Rafael, CA). Cells were voltage clamped using a DAGON 3900A amplifier (DAGON Corp., Minneapolis, MN) and PCLAMP software (Axon Instruments, Foster City, CA) and held at the standard holding potential of −80 mV. Current records were Bessel filtered at 2 kHz and voltage records at 10 kHz. All data were simultaneously recorded on VCR tape at a sampling rate of 18.5 kHz using a VR10B data recorder (Instrutech, Great Neck, NY). On formation of a gigaseal with the patch electrode, the seal resistance before rupture was typically around 3–6 GΩ. The center light-evoked response of the ganglion cell was examined from the cell-attached capacitative spike currents. On rupture of the patch, cellular charging currents to a 5-ms calibration pulse were compensated using the DAGON 3911A whole cell expander controls. The settings for series resistance compensation, and cell capacitance were noted for each cell, and series resistance compensation was used for all whole cell recordings (typically 40–60%, fast setting). The standard recording pipette (internal) solutions were methanesulphonate salt-based and contained low concentrations of Cl− to distinguish inhibitory postsynaptic currents (IPSCs) from EPSCs. The calculated ECl of the internal solutions was −67 to −70 mV (Table 1). The reference electrode was a chlorided wire or agar bridge in the bath located out of the light path. Holding potentials were corrected for liquid junction potentials (Barr 1994; Neher 1992).

Light-evoked currents of X ganglion cells were examined at holding potentials between −100 mV and +40 mV in 20-mV steps, and were leak subtracted. For isolation of the light-evoked EPSCs, 120 μm picrotoxin (40–80 μM in a few early experiments) and 1–2 μM strychnine were added to the tetrodotoxin (TTX) Ringer (termed “PST Ringer”). The average holding current during the 100 ms prior to onset/offset of the stimulus was subtracted from the light-evoked response components, depending on the polarity of the ganglion cell.

Drugs and perfusion

Light-evoked currents were studied in retinal slices and isolated retina superfused with Ames Ringer. The Ringer flowed by gravity at a rate of 4–5 ml/min., and was heated to 35–37°C by an in line heater just before entering the recording chamber. Drugs were stored as pH 7.4 stocks at −10°C. NS102 (6,7,8,9 tetrahydro-5-nitro-1Hbenz(g)indole-2,3-dione-3-oxime), GYKI 52466 (1,4-Aminophenyl-4-methyl-7,8-methylendioxy-2,3-benzodiazepine), and cyclothiazide were purchased from Research Biochemicals (Natick, MA). DAPS (N-amino-5-phosphono-heptanoic acid) and AP7 (N-amino-7-phosphono heptanoic acid) were purchased from Tocris Cookson (St. Louis, MO). ATP Na+ salt was purchased from P-L Biochemicals (Milwaukee, WI). All other drugs were purchased from Sigma (St. Louis, MO). Drug solutions were held in Ames Ringer at 37°C in a series of continuously gassed wells, and exchanged with the bath Ringer under manual control.
receptive field center. For displaying cumulative data on a sample of ganglion cells, normalized current-voltage (I-V) curves were employed (see Cohen 1998 for details). The magnitude of the current at each holding potential reflects the average value of the conductance estimate for all cells in the sample.

After recording, the Lucifer yellow–stained cells were examined with epifluorescence illumination, and the position and branching pattern of the stained cell’s dendritic arborization was observed with transmitted illumination in the slice (for examples, see Cohen 1998; Cohen et al. 1994). In the isolated retina, the LEC response polarities transmitted illumination in the slice (for examples, see Cohen 1998; pattern of the stained cell’s dendritic arborization was observed with epifluorescence illumination, and the position and branching estimate for all cells in the sample.

Each holding potential reflects the average value of the conductance employed (see Cohen 1998 for details). The magnitude of the current at light onset, that decay into a smaller sustained portion by spot termination. I

**Light stimuli**

The retina was viewed for recording using a fixed stage Zeiss Axioskop microscope (Carl Zeiss, Thornwood, NY) equipped with a ×40 fluor water immersion lens, and a high numerical aperture condenser whose iris was held fixed in position. Retinal slices and isolated retina were manipulated and viewed under dim red light (>650 nm filter) or using infrared (IR) illumination and a charge-coupled device (CCD) camera. White light stimulus spots, imaged on the slice through the microscope condenser, were used to stimulate the ganglion cell receptive field center. The unattenuated illumination at the ganglion cells was 140 lux. In most experiments in the isolated retina, a dim white background light was used in the optical path of the ganglion cell receptive field center. For displaying cumulative data on a sample of ganglion cells, normalized current-voltage (I-V) curves were employed (see Cohen 1998 for details). The magnitude of the current at each holding potential reflects the average value of the conductance estimate for all cells in the sample.

**RESULTS**

Using K+-based electrodes, current-clamp recordings monitored the light-evoked firing of X ganglion cells in the intact retina. The cells were then switched into voltage-clamp mode to examine the corresponding light-evoked generator currents. Figure 1 shows examples of this experiment on cat ON- and OFF-center X ganglion cells recorded in Ames Ringer (right and left panel, respectively). Figure 1, A and C, shows examples of the change in action potentials elicited by a spot stimulus. ON-X ganglion cells are depolarized by center spot stimulation, while OFF-X ganglion cells are hyperpolarized. Excluding voltage spikes to form action potentials, the ganglion cell’s membrane

**Direct effects of EAA agonists**

The effects of AMPA and kainate receptor agonists were studied on ganglion cells in retinal slices using a Cd²⁺-containing Ringer to block synaptic transmission. Recordings were made at room temperature. Slices were perfused with an oxygenated HEPES-buffered Ringer containing 200 µM Cd²⁺, 120 µM picrotoxin, 1 µM strychnine, 100 µM DAP5, and 200 nM TTX. The Ringer contained the following salts (in mM): 135 NaCl, 4.3 KCl, 1.2 MgCl₂, 0.1 CaCl₂, 10 HEPES, and 15 glucose, pH 7.4. Glucose was reduced to 6 mM in some experiments using Concanavalin A (ConA). An EAA agonist-containing puffer pipette (1 µm diam) was positioned next to the IPL directly above the recorded ganglion cell. Under computer control, a series of short-duration puffs (typically 40–80 ms duration) were applied to the IPL, in the control condition, and in the presence of the test agent in the bath (Picospritzer, General Valve, Fairfield, NJ).

**FIG. 1.** Comparison of the light-evoked depolarizations of ON- and OFF-X ganglion cells in the isolated retina in current clamp (A and C) and their corresponding light-evoked generator currents in voltage clamp (B and D). A: light-evoked response of an ON-X cell in current-clamp mode. Center spot stimulation caused a sustained train of action potentials. While the spot stimulus elicits a robust increase in the firing rate, the cell is only depolarized ~12 mV from its resting potential. Spot I = −1, 225 µm diam. r.p. −73 mV. Internal Ringer A. B: corresponding light-evoked currents of the same ON-X cell above. Note the light-evoked inward current at spot-on displays large transients at light onset, that decay into a smaller sustained portion by spot termination. I = −1. C: light-evoked spot stimulation of an OFF-X cell causes a small hyperpolarization, while at spot offset a small depolarization is evoked. Spot stimulus I = −1.0, 650 µm diam. r.p. −58 mV. Internal Ringer A. D: light-evoked currents of the same OFF-X cell above at −80 mV holding potential. Spot stimulation evokes an outward current at light-on, while in inward current is seen at light-off that decays with time. I = −0.5. Dimmer stimuli gave a similar pattern of currents on this cell.
potential is limited to an excursion of about 12 mV even at high sustained firing rates (see also Baylor and Fettiplace 1979; Diamond and Copenhagen 1995). Figure 1, B and D, shows the LECs generating these firing patterns at a holding potential of −80 mV. For the ON-X cell, center spot stimulation evoked a large transient inward current with a smaller sustained component that declined at spot offset. For the OFF-X cell, center spot stimulation evoked a small net outward current (see also Cohen 1998, Fig. 1, C and D). At spot offset an inward current was evoked with larger transient and smaller sustained kinetic components.

Figure 2 shows the voltage dependance of the light-evoked conductances involved in exciting ON- and OFF-X ganglion cells in the presence of normal retinal synaptic inhibition. Center spot stimulation of the receptive field center of ON-center X ganglion cells activated a prominent transient-sustained excitatory conductance at light-ON that reversed positive to −20 mV, while at light-OFF a smaller inhibitory conductance was activated on many cells (Fig. 2, top right) (see Belgum et al. 1982; Cohen 1998; Freed and Nelson 1994). This inhibitory conductance often declined with continued perfusion of TTX Ringer, suggesting that it may be amacrine mediated (see also Cohen 1998; Cook et al. 1998). Center spot stimulation of the receptive field center of OFF-center X ganglion cells activated a transient-sustained inhibitory conductance. This conductance reversed near or slightly negative to the calculated ECl of the internal solution. At light-OFF, an excitatory conductance predominated; reversing positive to −20 mV (Fig. 2, bottom right). In this fashion, the LECs of X ganglion cells are composed of both inhibitory and excitatory current components. These light-evoked currents are superimposed over a resting level of glutamate release in the dark (Cohen 1998).

**NMDA receptors contribute to X cell light-evoked currents in the presence of synaptic inhibition**

To compare the relative synaptic contributions from NMDA and AMPA/KA EAARs in the presence of synaptic inhibition, the peak excitatory LECs of individual ON- and OFF-X ganglion cells were measured at holding potentials of −80 and −40 mV. The ratio of the peak LEC at −40 to −80 mV in TTX Ringer was calculated for 10 ON- and OFF-X ganglion cells. In Ringer containing physiological levels of Mg2+, most studies indicate that NMDA receptor conduc-
tances are largely blocked at a holding potential of −80 mV (e.g., Ascher and Nowak 1987; Cohen et al. 1994; Mittman et al. 1990). However, at −40 mV, NMDA receptors begin to enter their Mg$^{2+}$ ion unblocked current-voltage region, and their conductance increases. A purely NMDA receptor–mediated light-evoked conductance would give a −40 mV/−80 mV ratio around 3.0 (see also Fig. 4B). In contrast AMPA/KA EAARs have a linear I-V relation, reversing near 0 mV. The current contribution from a purely AMPA/KA receptor–mediated conductance at −40 mV would be half of its value at −80 mV, giving a ratio of 0.5. The distribution histogram of the −40 mV/−80 mV ratios for the ten on- and off-X cells measured is shown in the left panels of Fig. 2. For on-X ganglion cells, the ratios averaged 0.76 ± 0.26 (mean ± SD), while the average ratio for off-center X cells was higher: 1.15 ± 0.54. These values were both significantly >0.5. This implies that on average, light-evoked NMDA receptor–mediated conductance components were present on cat X ganglion cell dendrites under near normal physiological conditions.

Light-evoked excitatory current properties of X cells in the absence of synaptic inhibition

The light-evoked EPSCs of X ganglion cells were pharmacologically isolated by adding picrotoxin and strychnine to the bath Ringer to remove synaptic inhibition. In the absence of synaptic inhibition, center spot stimulation of the ganglion cell evoked an EPSC with a prominent fast initial transient and smaller sustained current components similar to those seen in TTX Ringer alone. Figure 3A shows an example of the raw light-evoked EPSCs of an ON-X ganglion cell taken at a series of different holding potentials. At negative holding potentials, the sustained component of the light-evoked EPSC was noisier and smaller in magnitude when compared with the corresponding sustained EPSC component at positive potentials (n = 8 cells).

When synaptic inhibition was removed, I-V relations of the light-evoked EPSCs of both on- and off-X ganglion cells showed a prominent L-shape at negative potentials. An example of the raw EPSCs of an ON-X cell is shown in Fig. 3A at different holding potentials. At both positive and negative holding potentials, the light-evoked EPSC to the spot stimulus had a peak and a sustained component. I-V relations of this cell, taken at the peak current showed an L-shaped relation. Similar L-shaped curves could be seen on individual ON-X cells taken at their peak currents (Fig. 3B). The L-shape is indicative of a “mixed” receptor synapse where both NMDA and AMPA/KA EAARs contribute to the light-evoked synaptic current (see Cohen 1998; Diamond and Copenhagen 1995; Edmonds et al. 1995). However, the light-evoked EPSCs of the ON-X ganglion cell did not decline to zero at holding potentials of −80 to −100 mV, a region where NMDA receptors are thought to be normally inactive. This implies that an AMPA/KA EAAR–mediated EPSC component is also present. Furthermore, at nearly all time points during the sustained and also at the peak portion of the EPSC, the EPSC showed significant current components at these negative holding potentials (Fig. 3A). Figure 3C shows the I-V relation of the light-evoked EPSCs of an ON-X ganglion cell. An L-shaped I-V relationship was also observed at all times during the EPSC. L-shaped I-V relations could be seen on the peak light-evoked EPSCs of many off-X cells examined (Fig. 3D).

Examination of the −40 to −80 mV ratio of the peak light-evoked EPSCs of on- and off-X ganglion cells in PST Ringer gave ratios averaging significantly above 0.5 (0.94 ± 0.42, n = 8 cells and 1.56 ± 1.03, n = 6 cells), respectively. These ratios were similar to the values found in TTX Ringer alone, again with the ratio for off-X cells averaging slightly larger than their on-X cell counterparts. Thus NMDA receptors contribute significantly to the LECs of on- and off-X ganglion cells either in the presence or absence of retinal synaptic inhibition and at many time points overlap with their non-NMDA receptor counterparts.

Effects of NMDA receptor antagonists on X cell light-evoked currents

The contributions of NMDA EAAR receptors to forming the X ganglion cell light-evoked EPSC were examined on 21 X ganglion cells by bath applying a series of selective NMDA receptor antagonists. The antagonists DAP5 (100 μM), and AP7 (200 μM) were both used at concentrations that strongly blocked exogenously applied NMDA (see Cohen et al. 1994). Using extracellular recording techniques, previous EAAR studies of mammalian ganglion cells have reported that NMDA antagonists had moderate depressant effects on their light-evoked firing rates (Boos et al. 1990; Cohen and Miller 1994; Massey and Miller 1990). The results of this study indicate that NMDA receptors contribute a significant fraction of the light-evoked EPSC at the X ganglion cell.

Application of NMDA receptor antagonists had significant depressant effects on the light-evoked EPSCs and noise of most ganglion cells even at a holding potentials of −80 mV. On average, the peak EPSC of both on- and off-center X ganglion cells were reduced 20–30% in NMDA antagonists at −80 mV, and averaged of 81 ± 18% and 67 ± 45%, of control values (n = 7 and n = 14 cells, respectively). However, the contributions of NMDA receptor–mediated EPSCs are better observed at more positive holding potentials where their conductance increases.

At more positive holding potentials, NMDA receptor antagonists selectively reduced a slow rise time, long duration kinetic component of the light-evoked EPSC of ON-X ganglion cells. Figure 4A shows detailed comparisons of the light-evoked EPSCs of an ON-X ganglion cell in the control condition and in the presence of the NMDA receptor antagonist DAP5 at different holding potentials (left panels). Subtraction of the two curves yields the EPSC components reduced in DAP5 (right panels). In the presence of NMDA antagonists, slower sustained kinetic components of the light-evoked EPSC were preferentially blocked. What remained in DAP5 were fast onset light-evoked EPSCs. These EPSCs are typical of those thought to be mediated by AMPA/KA receptors (Forsythe and Westbrook 1988; Mittman et al. 1990). However, even in the presence of the NMDA antagonist DAP5, small sustained current components of the EPSC persisted during the spot stimulus.

I-V relations of the light-evoked EPSC in the presence of NMDA antagonists showed a linear I-V relation for a series of seven ON- and OFF-X ganglion cells (Fig. 4B). In the control condition (— line), an L-shaped I-V relation was observed.
When NMDA receptors were blocked, the I-V relation became linear in form (--- line), as expected for the remaining AMPA/KA receptor–mediated currents (Forsythe and Westbrook 1988; Mittman et al. 1990). The inset graph shows the I-V relation of the EPSC component blocked in NMDA antagonists, obtained by subtracting the two curves. This difference current showed a characteristic J-shaped I-V relation, as expected for NMDA receptors. At -60 mV, near the normal resting potential of X ganglion cells, the NMDA receptor conductance contribution to the light-evoked EPSC of X cells averaged ~35% of the total conductance, and increased with more positive holding potentials.

Reductions in the LEC by NMDA receptor antagonists could also be observed in the presence of normal retinal synaptic inhibition. In TTX Ringer, the light-evoked excitatory currents of ON- and OFF-X cells averaged, respectively, 85 and 68% of their peak control LECs at -80 mV (n = 7, 4 cells). Figure 4C shows the effects of an NMDA antagonist on an ON-X ganglion cell at -80 mV in TTX Ringer. In the presence of DAP5, the light-evoked inward current changed, becoming more transient at spot onset with a smaller sustained component that lasted for the spot duration. The current blocked by DAP5 had a slow onset and long duration typical of NMDA receptor–mediated processes (bottom trace). However, in DAP5, the light-evoked EPSC remaining still shows a sustained current component. This explains how ON-sustained ganglion cells recorded extra-
cellularly in NMDA antagonists retained their sustained firing patterns in the absence of NMDA receptor input (e.g., Cohen and Miller 1994; Massey and Miller 1990).

**Effects of AMPA and kainate receptor antagonists on X cell light-evoked currents**

The effects of selective AMPA or kainate receptor antagonists were tested on the light-evoked EPSCs of X ganglion cells. The benzodiazepine antagonist GYKI52466 has been reported to be a selective antagonist for AMPA EAARs (Donevan and Rowgawski 1993; Paternain et al. 1995). The antagonist NS102 (Verdoorn et al. 1994) has been reported to block kainate receptor-mediated release of GABA in hippocampal interneurons (Chittajallu et al. 1996; Cunha et al. 1997). To examine these EAAR contributions to the light-evoked EPSC, X ganglion cells were held at −80 mV, where the conductance contributions of these non-NMDA receptor types predominate.

**AMPA receptor antagonists**

ON-Center ganglion cells possess both NMDA and AMPA/KA EAARs on their dendrites (Cohen 1998; Cohen et al. 1994). Since the LECs of their presynaptic ON-bipolar cell inputs use a metabotropic glutamate receptor, one would expect that the changes observed in the LECs of ON-center X ganglion cells in AMPA receptor antagonists would predominately reflect effects of these agents at receptors located directly on the ganglion cell.

Application of the AMPA-selective antagonist GYKI52466 (50–100 μM) produced massive reductions in the magnitude of the light-evoked EPSC.
of the light-evoked EPSC at −80 mV. The effects of GYKI were measured in the presence of 100 μM DAP5 to block NMDA responses and reveal any small residual kainate receptor–mediated components to the light-evoked EPSC. GYKI strongly reduced the light-evoked EPSC of ON-X cells, and EPSCs averaged 8.2 ± 7.3% of the control response, \( n = 5 \) cells (Fig. 5A). Similar strong LEC reductions were observed with GYKI alone for ON-X cells in PST Ringer (13 ± 18% control, \( n = 7 \) cells), or in TTX Ringer (\( n = 5 \) cells). These results for ON-X cells all imply that a large component of the non-NMDA receptor–mediated light-evoked EPSC at the ganglion cell was mediated by AMPA receptors.

OFF-Center X ganglion cells are excited by EAARs operating through a disynaptic ligand-gated EAAR-mediated circuit. Cones release glutamate onto OFF-center cone bipolar cells activating non-NMDA type EAARs (Sasaki and Kaneko 1996; Slaughter and Miller 1983a; Vardi et al. 1998), which in some species appear to be kainate receptors (DeVries and Schwarz 1999). OFF-Center cone bipolar cells in turn release glutamate onto OFF-center ganglion cells (Cohen 1998; Cohen et al. 1994). This makes localization of the effects of AMPA or KA receptor antagonists on OFF-X cell light-evoked EPSCs more complex to interpret.

Similar to ON-X cells, the light-evoked EPSCs of most OFF-X cells were either blocked, or strongly reduced by the AMPA receptor antagonist GYKI52466 in PST Ringer. An example of GYKI’s effects on the EPSC of an OFF-center X cell is shown in Fig. 5B. Addition of GYKI strongly reduced the light-evoked EPSC at light-OFF. Light-evoked EPSCs of OFF-X cells in GYKI (50–100 μM, all in the presence of DAP5) averaged 8.2 ± 5.8% of control values (\( n = 6 \) cells). These results also imply that AMPA-type EAARs form the main non-NMDA receptor–mediated component of the light-evoked EPSC of OFF-X ganglion cells. However, it was also important to examine the effects of kainate receptor–selective antagonists on the LECs of X ganglion cells.

**Kainate receptor antagonists**

NS-102 (5–10 μM), a reported kainate receptor selective antagonist, was tested on the LECs of a limited sample of X cells (\( n = 12 \) cells) at a holding potential of −80 mV (Chit-jajallu et al. 1996; Cunha et al. 1996). Application of NS102 had only small effects on their LECs. An example of NS102’s effects is shown on an ON-X cell in the inset of Fig. 5. For ON-cells, LECs in NS102 averaged near control levels (96.8 ± 17.5%, \( n = 5 \) cells; TTX or PST Ringer). The LECs of OFF-X ganglion cells showed only a small reduction in NS102, averaging 84.9 ± 32.1% of controls in PST Ringer (\( n = 5 \) cells) and 106.3 ± 11.0% of controls (\( n = 3 \) cells) in TTX Ringer.

**FIG. 5.** Kainate receptors play a minor role in generating the light-evoked synaptic current in X ganglion cells, while α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors predominate. A: example of light-evoked EPSCs of an ON-center ganglion cell to spot stimulation (bar) in the control condition in PST Ringer at a holding potential of −80 mV. When the AMPA receptor-selective antagonist GYKI52466 (100 μM, in the presence of 100 μM DAP5) was applied, the EPSCs of this cell were blocked, and little residual kainate-mediated EPSC was observed. I = −1.0, 1,100 μm spot diam. Isolated cat retina preparation. B: the light-evoked EPSCs of OFF-center X ganglion cells were also strongly blocked by AMPA receptor antagonists. In the control condition, the EPSC at −80 mV showed a prominent inward current at spot-offset. When GYKI52466 (50 μM) was applied (in the presence of 100 μM DAP5), the EPSC was strongly reduced. I = −1, spot diam. 900 μm (Internal Ringer B, isolated cat retina). Inset: effects of the kainate antagonist NS102 (5 μM) on the light-evoked EPSC of an ON-X cell. In the presence of NS102 (— trace) the EPSC resembled the control response (----- trace). I = −1, 900 μm spot diam. PST Ringer (Internal Ringer A, isolated cat retina).
While this result could imply that off-bipolar cells may have AMPA receptors on their dendrites, the selectivity of NS102 antagonism against all kainate receptor types is currently unclear (see Discussion).

The experimental results above with selective AMPA and KA receptor antagonists suggested that the LECs at cone bipolar to X ganglion cell synapses use AMPA type EAAR-mediated mechanisms. However, as previously stated, AMPA or KA receptor antagonists can potentially also exert presynaptic effects on the retinal network, which could distort the EPSC observed at the ganglion cell (e.g., Cohen and Miller 1999). This question was particularly important given the known synaptic circuitry for off-center X ganglion cells. Therefore it was necessary to reexamine the pharmacology of these non-NMDA EAARs directly on the X ganglion cell dendrite.

Tests of direct effects of AMPA and kainate receptor antagonists on X cells

Previous EAAR studies in mammalian ganglion cells have not distinguished whether distinct AMPA or KA receptor-mediated conductances were present on ganglion cell dendrites (e.g., Aizenmann et al. 1988; Cohen et al. 1994). To examine this question, synaptic transmission was blocked in the retinal network using a Cd$^{2+}$-containing Ringer, and the nonselective agonist KA was applied to six X ganglion cells in retinal slices (2 ON, 4 OFF) using a puffer pipette (see Methods for details). Short-duration puffs of kainate were applied directly to ganglion cell dendrites at a holding potential of ~80 mV. These puffs evoked a series of large, rapidly rising inward currents in X cells that slowly decayed, as shown in Fig. 6A. The kainate puff currents were strongly reduced by the addition of the

FIG. 6. When synaptic transmission is blocked, AMPA- but not kainate-selective excitatory amino acid receptors (EAARs) form the main non-NMDA EAAR conductance on X-type ganglion cell dendrites. A: current record of an OFF-X ganglion cell to exogenous puffs of KA applied in the presence of increasing doses of the AMPA-selective antagonist GYKI52466. Cadmium was added to the Ringer to block synaptic transmission (see METHODS). During the KA puff (bar) a rapid inward current is observed. The AMPA receptor-selective antagonist GYKI 52466, at doses $>$30 μM, blocked the KA-induced current. Holding pot. ~80 mV. Internal Ringer C. B: inhibition curve of the AMPA-selective antagonist GYKI52466 to the kainate puffs. Line indicates least-squares fit to data, while error bars indicate SE. Data taken from a group of 8 ON- and OFF-X ganglion cells, with the exception of the values at 0.1 and 1,000 μM ($n = 4$ cells, *). C: effects of the selective AMPA receptor modulator cyclothiazide (100 μM) on puff-evoked glutamate currents recorded in an ON-X ganglion cell in the presence of 100 μM DAPS in Cd$^{2+}$ Ringer. Note the glutamate-induced current was potentiated 7-fold in the presence of cyclothiazide. Recovery was observed after 5 min wash out. D: removal of desensitization of kainate receptors by ConA pretreatment had did not potentiate kainate-induced puff currents recorded on X ganglion cells in Cd$^{2+}$ Ringer. Left panel: the kainate-selective antagonist NS-102 10 μM had only minor effects on the puff-evoked current. In contrast, the AMPA-selective antagonist GYKI 52466 (100 μM) blocked nearly all of the current. Right panel: summary histograms of the effects of the 2 antagonists on ON- and OFF-X ganglion cells to kainate puffs in ConA. Error bars indicate mean ± SD.
selective AMPA receptor antagonist GYKI 52466 to the Ringer. Dose-response curves to KA puffs with GYKI revealed an IC50 of 4.5 μM (Fig. 6B). Thus using concentrations of GYKI 52466 > 30 μM, the inward current evoked by kainate application was strongly blocked. These results imply that AMPA type non-NMDA EAAR conductances predominate directly on X ganglion cell dendrites.

**Tests of selective AMPA and kainate receptor modulators**

This finding was reinforced by a series of experiments examining the effects of AMPA- and kainate-selective receptor modulators on X ganglion cells. Several different drugs can selectively potentiate the action of AMPA or kainate receptors, by preventing receptor desensitization. For AMPA receptors, these drugs include aniracetam, cyclothiazide, and its derivatives (Wong and Mayer 1993; Yamada and Tang 1993) Cyclothiazide (30 or 100 μM) was tested on X ganglion cell EAAR currents (4 ON-, 5 OFF-X cells) elicited by short puffs of L-glutamate (500 μM) in the Cd2+-containing Ringer. Glutamate application normally evokes a rapidly desensitizing inward current at AMPA receptors (Fig. 6C). Bath application of cyclothiazide (30 μM) potentiated the peak glutamate current on all cells tested by an average of 5.9 ± 2.7 fold (n = 5 cells). Thus AMPA receptors are present on X ganglion cell dendrites.

Cloned kainate receptors rapidly desensitize to application of glutamate or its agonists. Kainate receptor desensitization can be slowed irreversibly by preapplication of the high molecular weight lectin ConA (Huettner 1990). To examine the role of kainate receptor desensitization at the ganglion cell dendrite, retinal slices were preincubated in the ConA for >1 h prior to recording (see METHODS for details). Kainate (500 μM) was applied to X ganglion cell dendrites using a puffer pipette in the same Cd2+-based Ringer. In ConA treated slices, this resulted in a series of transient inward currents (Fig. 6D). However, the kainate-selective antagonist NS-102 (10 μM) still caused only a slight reduction in the kainate-evoked puff current. In contrast, the AMPA-selective EAAR antagonist GYKI 52466 (100 μM) blocked nearly all of the kainate-evoked current under these conditions. Similar results were seen with the two antagonists on all ON- and OFF-X ganglion cells tested (Fig. 6D, left panel histogram). ConA pretreatment did not potentiate a kainate receptor–mediated current component on X ganglion cells. These results reinforce the conclusion that while kainate EAARs contribute at best to only a minor fraction of the kainate-evoked current, AMPA-type EAARs account for the bulk of the kainate-induced current tested directly on X ganglion cell dendrites.

**DISCUSSION**

I have examined the EAAR pharmacology of the LECs of X ganglion cells in voltage clamp using isolated and sliced preparations of the cat and ferret retina. Previous EAAR studies on the light responses of mammalian ganglion cells have relied mainly on extracellular recording techniques (e.g., Boos et al. 1990; Cohen and Miller 1994; Massey and Miller 1988). The results of these pharmacology studies in the intact retinal network have been difficult to interpret in relation to direct effects on EAARs localized directly on the ganglion cell. This was particularly true for the light responses of OFF-center ganglion cells in some retinas. In the presence of the quinoxalines or kynurenic acid, OFF-center rabbit ganglion cells often paradoxically increased their firing rates (e.g., Cohen and Miller 1999; Massey and Miller 1988).

The light-evoked EPSCs of ON- and OFF-X ganglion cells showed a significant NMDA-mediated EPSC component in addition to those mediated by AMPA receptors. These results for ON- and particularly for the lesser known OFF-center ganglion cells resemble pharmacological studies of the light-evoked EPSCs of ON- and OFF–center ganglion cells in the retinas of the larval tiger salamander (Diamond and Copenhagen 1993, 1995; Hensley et al. 1993; Mittman et al. 1990; Velte et al. 1997). Although the light-evoked EPSCs of X ganglion cells were often increased in the presence of PST Ringer (Cohen 1998), NMDA receptor–mediated EPSC components could still be observed on X cells in TTX Ringer, where many forms of retinal synaptic inhibition persist. This was evident by the finding that the −40 to −80 mV LEC ratios of ON- and particularly OFF-X ganglion cells were on average significantly >0.5 in TTX Ringer. Thus the LECs on X ganglion cells are normally composed of NMDA EAAR-mediated components that appear to summate with their AMPA receptor counterparts to excite X-type ganglion cells.

The light-evoked EPSC kinetics of sustained X ganglion cell types to bright spot stimuli show that they are composed of a fast initial transient current followed by slower sustained current components. Their kinetics, recorded under light-adapted conditions, differ from the slower sustained light-evoked EPSCs found in ON-sustained larval tiger salamander ganglion cells under dark-adapted conditions (Diamond and Copenhagen 1993). The I-V relation of the X cell light-evoked EPSCs showed a prominent L-shaped relation (Cohen 1998). The form of this I-V relation is typical for cells receiving mixed excitatory amino acid receptor synaptic input (Diamond and Copenhagen 1993, 1995; Edmonds et al. 1995; McBain and Mayer 1994). Other potential excitatory neurotransmitter contributors to ganglion cell LECs, such as from cholinergic neurons could also produce rectifying I-V relations. However, these neurons appear to play only a minor role in the light-evoked EPSCs of X ganglion cells in the presence of PST Ringer, as application of the nicotinic antagonist dihydro-β-erythroidine had little effect on the light-evoked EPSC (n = 3 cells) (unpublished observations).

Given that AMPA and KA receptor–mediated currents have a faster rise time than those of NMDA receptors, it might be expected that these receptors would play a more critical role in the generation of the initial peak current transients of the light-evoked EPSCs of X (sustained type) ganglion cells. However, careful examination of the I-V relations of the light-evoked currents of X ganglion cells at many different time points showed this was only partially true. Even at the peak of the light-evoked EPSC, the NMDA and AMPA EAAR contributions to the light-evoked EPSC appear to significantly overlap (Fig. 4) (see also Cohen 1998, Fig. 7). The light-evoked EPSC of X cells also show an initial transient to the EPSC at −100 mV; a potential where NMDA receptor EPSC contributions are largely absent, and the same initial transient persists at +40 mV, a potential where the NMDA receptor–mediated EPSC contribution becomes especially large. Conversely, the faster AMPA EAARs can mediate a portion of the sustained...
Mennerick and Matthews (1996). Several different synaptic vesicle stores in the bipolar cell (e.g., Matsui et al. 1998). The time course of glutamate release could reflect kinetic contributions from bipolar cell inputs (e.g., Matsui et al. 1998). The time course of ganglion cells at both positive and negative holding potentials suggests that the EPSC form strongly reflects in part the time relationship where the light-evoked EAAR-mediated conductances operate so that both receptor types can excite the ganglion cell (see also Cohen 1998; Diamond and Copenhagen 1995). This result explains why extracellular recordings of sustained firing ganglion cell types in rabbit and primate retinas had persistent sustained firing patterns in the presence of NMDA antagonists (Cohen and Miller 1994; Massey and Miller 1990). In addition, the similarity of the waveform of the light-evoked EPSC at X ganglion cells at both positive and negative holding potentials suggests that the EPSC form strongly reflects in part the time course of glutamate release by an X cell’s presynaptic cone bipolar cell inputs (e.g., Matsui et al. 1998). The time course of this glutamate release could reflect kinetic contributions from several different synaptic vesicle stores in the bipolar cell (e.g., Mennerick and Matthews 1996).

In the presence of blockers of NMDA receptors, the principal LEC kinetic components lost at negative holding potentials were slow onset components of long duration. In NMDA antagonists, I-V relations of the light-evoked EPSC of ON- and OFF- X ganglion cells lost their normal L-shaped relation and became linear, reversing near 0 mV in P Staples Ringer. These linear I-V relations reflect the properties of the remaining AMPA/KA EAAR-mediated EPSCs (Forsythe and Westbrook 1988; Mittman et al. 1990). Similar losses of slow components could be seen by NMDA antagonists on the LECs of X cells in the presence of normal synaptic inhibition. Since cat X ganglion cells, like many other ganglion cells, possess direct conductances for NMDA receptors (Aizenmann et al. 1988; Cohen et al. 1994; Mittman et al. 1990). The above data suggest that these NMDA receptors appear to be located at or near bipolar synapses (Matsui et al. 1998) and actively participate in forming part of the transient, and a large fraction of the sustained LEC in X ganglion cells under normal physiological conditions.

AMPA type glutamate receptors appear to mediate the major non-NMDA EAAR component of the LEC on X ganglion cell dendrites. At a holding potential of −80 mV, where NMDA receptors are largely inactive, the AMPA-selective antagonist GYKI52466 blocked the light-evoked EPSCs of both ON- and OFF- X cells. A similar pattern of pharmacology was observed in a series of experiments applying EAA agonists directly at the ganglion cell. When synaptic transmission was blocked in the presence of Cd2+, bath application of GYKI52466 blocked virtually all of the kainate or glutamate puff-evoked currents at the ganglion cell. Glutamate puff-evoked currents could be strongly potentiated by the selective AMPA receptor modulator cyclothiazide. In contrast, application of a reported kainate receptor selective antagonist, NS-102 (Chittajallu et al. 1996) had little effect on kainate puff-evoked currents. Finally, application of a kainate receptor–selective modulator ConA did not potentiate the kainate-evoked currents, even with prolonged preincubation of the retinal slice in the lectin. These results all suggest a model where AMPA receptors predominantly mediate the non-NMDA receptor LEC component on both ON- and OFF-center X type ganglion cells (Fig. 7). These results are similar to the conclusions of a previous study of ON- and OFF-type retinal ganglion cells in the larval tiger salamander by Lukasiewicz et al. (1997).

What function is conferred by the kainate receptors thought to be present on retinal ganglion cells is unclear. In situ hybridization and immunocytochemical studies for these receptors show they are clearly present in the ganglion cells of mammals (Brandstatter et al. 1994; Hammersaki-Brito et al. 1993; Qin and Pourcho 1996; Vardi et al. 1998). While kainate receptors could conceivably be transported to the axon terminals of X cells in the lateral geniculate nucleus, kainate receptors could also play several other roles in the ganglion cell. Physiological evidence shows that kainate receptors may act by either metabotropic or ionotropic mechanisms. In the hippocampus, kainate receptors act indirectly by a pertussis toxin–sensitive G-protein–mediated mechanism; presynaptically inhibiting GABAergic synaptic release onto pyramidal cells (Rodriguez-Moreno and Lerm 1998). Kainate receptors also appear to be involved in the generation of the slow excitatory currents activated by multiple tetanic stimuli on pyramidal cells (e.g., Vignes et al. 1997). For X-type retinal ganglion cells, the residual LECs and agonist-induced currents observed in the noncompetitive AMPA antagonist GYKI52466 (in the presence of DAP5) were quite small. This suggests that while kainate receptors contribute at best to only a minor current fraction at cone bipolar-X ganglion cell synapses, they could influence ganglion cell firing through metabotropic receptor–mediated mechanisms.

However, the currently developed kainate receptor antagonists, such as NS102 can exhibit poor selectivity in some brain regions (Paternain et al. 1996), making isolation of AMPA receptor–mediated EPSC components more difficult. In the ground squirrel retina, it has been proposed that cone to OFF-bipolar cell neurotransmission uses kainate receptors exclusively (DeVries and Schwartz 1999). However, it remains to be
determined whether kainate receptors play a similar role on off-bipolar cells in the retinae of other species, such as the cat. Further insights into the role of kainate receptors will require the development of more selective and potent kainate receptor antagonists.

Thus in summary, the light-evoked synaptic currents on on- and off-“X/sustained” type mammalian ganglion cell dendrites show overlapping contributions from NMDA and AMPA EAAR types. The contribution of NMDA receptors to the light-evoked EPSC of X ganglion cells is substantial. Given the slow nature of the light transduction mechanisms in retinal networks, an NMDA receptor contribution to the “faster” components of the LEC at the ganglion cell would not be unexpected. The polysynaptic delay from photoreceptor stimulation to generation of the LEC in retinal ganglion cells is much longer than the monosynaptic delay of most single synapses that have been previously studied (i.e., Forsythe and Westbrook 1988; Lester et al. 1990; Rossi et al. 1995). The time constant of activation of mature NMDA receptors (~10 ms) is entirely within the response rise time of the peak EPSC of a ganglion cell to a light stimulus (~35 ms) (see Mc Bain and Mayer 1994 for review). Thus this study supports a substantial contribution from NMDA receptors to the light-evoked EPSCs of sustained ganglion cell types. However, during periods of sustained spiking in ganglion cells, activation of voltage-dependent Na+ channels and subsequent activation of delayed rectifier K+ channels will limit the NMDA receptor contributions to more positive potentials (Diamond and Copenhagen 1995) (Fig. 1, this paper). In addition, AMPA EAAR-mediated mechanisms also play a significant role in generating X cell LECs; as some 60–70% of the light-evoked EPSC at the ganglion cell’s resting potential (approximately ~60 mV) is mediated by AMPA receptors.

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