AMPAN-Preferring Receptors Mediate Excitatory Synaptic Inputs to Retinal Ganglion Cells

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Lukasiewicz, Peter D., James A. Wilson, and Jean E. Lawrence. AMPA-preferring receptors mediate excitatory synaptic inputs to retinal ganglion cells. J. Neurophysiol. 77: 57 ± 64, 1997. Pharmacological studies were performed to determine whether α-amino-3-hydroxy-5-methyl-4-isoazoleprionic acid (AMPA)- and or kainate (KA)-preferring receptors mediate excitatory synaptic inputs to tiger salamander retinal ganglion cells. Excitatory postsynaptic currents (EPSCs), evoked either by light or by stimulating bipolar cells with puffs of K⁺, were measured using whole cell recording techniques in the tiger salamander retinal slice. The AMPA/KA component of the EPSCs was isolated by including antagonists of glycine-, γ-aminobutyric acid (GABA) - and NMDA-receptors in the bath. The AMPA-preferring receptor antagonists, 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine hydrochloride (GYKI-52466) and 1-(4-aminophenyl)-3-methylcarbamyl-4-methyl-7,8-methylenedioxy-3,4-dihydro-5H-2,3-benzodiazepine (GYKI-53665), reduced light-evoked EPSCs and K⁺ puff-evoked EPSCs amplitudes in a concentration-dependent manner. The IC₅₀ values for GYKI-52466 were 3.6 and 4.2 μM for the light- and puff-evoked responses, respectively. The more potent GYKI-53665 had IC₅₀ values of 0.7 μM for both the light- and puff evoked responses. KA activates both KA- and AMPA-preferring receptors. KA-evoked currents were completely blocked by 10–40 μM GYKI-52466, indicating that little or no excitatory synaptic current was mediated by KA-preferring receptors. Concanavalin A, a compound that preferentially potentiates responses mediated by KA-preferring receptors, did not enhance either EPSCs or glutamate-evoked responses. By contrast, cyclothiazide, which selectively enhances AMPA-preferring receptor mediated responses, was found to enhance both EPSCs and glutamate-evoked currents. Our results indicate that the non-NMDA component of ganglion cell EPSCs is mediated by AMPA-preferring receptors and not significantly by KA-preferring receptors.

INTRODUCTION

Glutamate is the major excitatory transmitter in the vertebrate retina. It is the transmitter used by bipolar cells (Ehinger et al. 1988; Marc et al. 1990), the cells that provide the major excitatory input to retinal ganglion cells. The excitatory synaptic inputs to ganglion cells have been shown to be mediated by both α-amino-3-hydroxy-5-methyl-4-isoazoleprionic acid/kainate (AMPA/KA) and N-methyl-D-aspartate (NMDA) glutamate receptors in every vertebrate retina examined (Boos et al. 1990; Cohen and Miller 1994; Diamond and Copenhagen 1993; Lukasiewicz and McReynolds 1985; Massey and Miller 1988, 1990; Mittman et al. 1990). It is not known whether AMPA-preferring receptors (Boulter et al. 1990; Keinänen et al. 1990; Partin et al. 1993) and/ or KA-preferring receptors (Bettler et al. 1990, 1992; Egbergh et al. 1991; Partin et al. 1993; Werner et al. 1991) mediate excitatory synaptic inputs to ganglion cells. Recent in situ hybridization (Hamassaki-Brito et al. 1993; Hughes et al. 1992; Muller et al. 1992) and immunocytochemical localization studies (Morigiwa et al. 1995; Peng et al. 1995) have shown that mRNAs and proteins for both AMPA- and KA-preferring receptor subunits are present in the ganglion cell layer of mammalian and teleost retina.

The enhancement by cyclothiazide of synaptic inputs mediated by AMPA/KA receptors suggests that AMPA-preferring receptors mediate a significant portion of the excitatory input to salamander ganglion cells (Lukasiewicz et al. 1995). Cyclothiazide has been shown to selectively enhance responses mediated by AMPA-preferring receptors but not KA-preferring receptors (Partin et al. 1993; Wong and Mayer 1993). Our previous study did not rule out the possibility that both AMPA- and KA-preferring receptors mediated excitatory synaptic inputs to ON-OFF ganglion cells. The present work was undertaken to determine the relative contributions that these two receptor subtypes make to ganglion cell excitatory postsynaptic currents (EPSCs).

Recent work has shown that the 2,3 benzodiazepines GYKI-52466 and GYKI-53665 selectively antagonize responses mediated by AMPA-preferring receptors but not responses mediated by KA-preferring receptors (Paternain et al. 1995; Renard et al. 1995; Wilding and Huetter 1995). These compounds now make it possible to separate the KA-preferring from the AMPA-preferring components of the excitatory responses. Our results indicate that AMPA-preferring receptors underlie most of the AMPA/KA component of ON-OFF ganglion cell excitatory synaptic responses.

METHODS

Whole cell patch recording in tiger salamander retinal slices

Whole cell patch recordings (Hammill et al. 1981) were made primarily from ON-OFF ganglion cells in retinal slice preparations (Werblin 1978). The recording procedures have been described in detail elsewhere (Barnes and Werblin 1986, 1987; Lukasiewicz and Roeder 1995; Lukasiewicz and Werblin 1994). Tiger salamanders were obtained from the Charles D. Sullivan Company (Nashville, TN) and kept in a cold room at 5°C on a 12 h light/12-h dark luminance cycle.

Electrode and bathing solutions

The intracellular electrode solution (Mittman et al. 1990) consisted of (in mM) 90.5 cesium fluoride, 3.4 sodium chloride, 0.4...
onto bipolar cell dendrites (Lukasiewicz and Werblin 1994). The asymptotic minimum and IC\textsubscript{50} is the antagonist concentration.

The bathing medium (salamander Ringer) contained (in mM) 112 sodium chloride, 2 potassium chloride, 2 calcium chloride, 1 magnesium chloride, 5 glucose, and 5 HEPES; adjusted to pH 7.8 with NaOH. Unless otherwise indicated, all chemicals were obtained from Sigma Chemical (St. Louis, MO). AMPA, GYKI-52466 [1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine hydrochloride], 6-cyano-7-nitroquinoxaline-2,3-dione, and the NMDA receptor antagonists d-2-amino-5-phosphono pentanoic acid (d-AP5) and (±) 2-amino-7-phosphono heptanoic acid (AP7), were obtained from Research Biochemicals International (Natick, MA). 6-Nitro-7-sulphamoylbenzo(f)quinoxaline-2,3-dione was obtained from Tocris Cookson (St. Louis, MO). GYKI-53665 [1-(4-aminophenyl)-3-methylcarbamyl-4-methyl-7,8-methylenedioxy-3,4-dihydro-5H-2,3-benzodiazepine] and cyclothiazide were kindly provided by Eli Lilly (Indianapolis, IN). Experiments were performed at room temperature (19 \pm 2°C).

The control bathing solution used in our slice experiments was formulated to pharmacologically isolate the AMPA/KA receptor component of ganglion cell EPSCs. GABAergic and glycinergic inhibitory synaptic inputs were blocked with picrotoxin (150 \mu M) and strychnine (2 \mu M), respectively, and NMDA-receptor-mediated inputs were blocked with either d-AP5 or AP7 (50 \mu M) (Belgum et al. 1984; Diamond and Copenhagen 1993; Mittman et al. 1990). Voltage-gated sodium currents were blocked by including 0.5 \mu M tetrodotoxin in the bath, and voltage-gated potassium currents were blocked by including cesium in the recording electrodes (Lukasiewicz and Werblin 1988). Antagonists and modulators were applied locally over a relatively large area of the slice under study (several millimeters in width) by a gravity-driven superfusion system as described previously (Lukasiewicz and Roeder 1995; Lukasiewicz et al. 1995).

**Puffing agonists or potassium onto ganglion cell and bipolar cell dendrites**

Glutamate agonists were puffed onto the cell bodies and/or dendrites of ganglion cells in the slice preparation with a Picospritzer (General Valve, Fairfield, NJ). Synaptically driven responses in ganglion cell slices were elicited by puffing potassium chloride (KCl) (114 mM) through pipettes with 1 \mu m diameter tips onto bipolar cell dendrites (Lukasiewicz and Werblin 1994). The pipette solution consisted of salamander Ringer where sodium was replaced with potassium. The details of this methodology are described in Lukasiewicz and Roeder (1995).

**Light stimulation**

The light stimulus apparatus and procedures were identical to those described previously (Lukasiewicz and Roeder 1995; Lukasiewicz et al. 1995). Briefly, the light source was a tungsten halogen lamp (20 \text{W}) (Ealing Electro-Optics, Holliston, MA). Full-field, white light stimuli were used. The intensity of the unattenuated light stimulus was equivalent to 3.6 \times 10^{16} quanta/cm\(^2\)/s of a monochromatic light of 500 nm.

**Liquid junction potential correction**

Membrane potential values given in this paper were corrected for junction potentials. Liquid junction potentials were determined as described by Fenwick et al. (1982). They were typically \(-6\) mV for the cesium fluoride (CsF) electrode solution.

**Cell identification**

In the slice preparation, ganglion cells were identified by their characteristic current responses to light stimuli (Mittman et al. 1990). More than 90% of ganglion cells recorded in the slice preparation were ON-OFF cells that responded with transient EPSCs at light onset and offset. The remaining cells were ON cells that responded with a sustained EPSC to the light stimulus. Almost all of the results reported here were obtained from ON-OFF ganglion cells. Some cells were stained by including Lucifer yellow CH (0.25%) (Aldrich Chemicals, Milwaukee, WI) in the electrode solution (Diamond and Copenhagen 1993; Lukasiewicz and Werblin 1994; Mittman et al. 1990). Similar results were obtained when either cesium chloride or cesium gluconate were used as substitutes for cesium fluoride. The bathing medium (salamander preparation were ON-OFF cells that responded with transient EPSCs at light onset and offset. The remaining cells were ON cells that responded with a sustained EPSC to the light stimulus.

The light responses of the stained cells were correlated with the ramification of their dendrites at different depths in the inner plexiform layer (IPL) (Famiglietti et al. 1977; Nelson et al. 1978). Cells and processes were viewed using a Nikon mercury fluorescent epilluminator with an Omega Optical XF15 filter set (Brattleboro, VT).

**Recording system**

The microscope system and patch-clamp apparatus used for this study were described in Lukasiewicz and Roeder (1995). Electrodes were pulled from borosilicate glass (TW150F-4, W.P.I., Sarasota, FL) with a Sachs-Flaming puller (Sutter Instruments, Novato, CA) and had measured resistances of <5 M\Omega. The measured series resistances were typically 15–25 M\Omega. The magnitude of the series resistance compensation, read off of the Dagan 3900A compensation dial, was 5–10 M\Omega. Pclamp software (Axon Instruments, Foster City, CA) was used to generate voltage command outputs, acquire data and trigger the Picospritzer. The data were digitized and stored with a 33-MHz 386-PC using a Labmaster DMA data acquisition board (Scientific Solutions, Solon, OH). Responses were filtered at 2 kHz with the four pole Bessel low pass filter on the Dagan 3900A and sampled at 100 Hz. Data were analyzed using Clampfit (Axon Instruments). The experimental concentration-response curves were fitted by the Sigma Plot (San Rafael, CA) nonlinear regression algorithm to Eq. I

\[
\frac{I}{I_{\text{max}}} = \frac{a - c}{1 + \left(\frac{[\text{antagonist}]}{IC_{50}}\right)} + c
\]

where \(a\) is the asymptotic maximum, \(b\) is the slope parameter, \(c\) is the asymptotic minimum and \(IC_{50}\) is the antagonist concentration that reduced \(I\) to one-half of \(I_{\text{max}}\). Results are expressed as means \pm SD.

**RESULTS**

To determine the effects of the 2,3 benzodiazepines on ganglion cell EPSCs, excitatory synaptic inputs were evoked by puffing potassium onto bipolar cells. Because AMPA/KA receptors also are present in the outer plexiform layer, these receptors were bypassed by depolarizing bipolar cells directly with potassium puffs (Lukasiewicz et al. 1995). Similar responses were observed in ganglion cells by depolarizing bipolar cell somas directly with extracellular stimulating electrodes (data not shown).

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ampA receptors on retinal ganglion cells

Figure 1A shows a family of AMPA/KA receptor-mediated excitatory synaptic currents recorded in the absence and presence of increasing concentrations of GYKI-52466. NMDA, GABA and glycine receptors were blocked pharmacologically as described in Methods. GYKI-52466 decreased the amplitude of the EPSCs in a concentration-dependent manner. The response was blocked by 30 μM GYKI-52466 in this cell. Similar results were obtained with the more potent GYKI-53665.

The results obtained with GYKI-52466 and GYKI-53665 are summarized in the concentration-response curves illustrated in Fig. 1B. The mean peak currents are plotted as a function of log concentration of either GYKI-53665 (○) or GYKI-52466 (●). Both compounds reduced the peak amplitude of the EPSCs in a concentration-dependent manner. The solid lines are the best nonlinear regression fits to Eq. 1. The mean IC₅₀ value for GYKI-52466 was 4.2 μM whereas the mean IC₅₀ value for the more potent GYKI-53665 was 0.7 μM. These values are similar to those reported by Wilding and Huettner (1995) for AMPA-prefering receptors on dissociated rat cortical neurons.

Light-evoked, ON EPSCs were blocked by GYKI compounds

To determine whether or not AMPA-prefering receptors mediated the AMPA/KA component of the light-evoked EPSCs, we determined the GYKI compounds’ effectiveness in inhibiting the ON component of the ON-OFF ganglion cell light responses. Because AMPA/KA receptors are present on OFF, but not ON, bipolar cell dendrites (Nawy and Jahr 1990, 1991; Slaughter and Miller 1983a,b), the actions of the GYKI compounds were assessed only for the ganglion cell ON, light-evoked EPSCs.

Figure 2A shows that the amplitude of the AMPA/KA component of the ON, light-evoked EPSC was reduced by GYKI-53665 in a concentration-dependent manner. The NMDA-receptor-mediated component of the response and the inhibitory synaptic inputs were blocked as described above. The light stimuli used were relatively bright and were adjusted to elicit the largest light-evoked EPSCs without causing the attenuation of subsequent responses. The amplitude of the response was reduced by >95% by 10 μM and completely blocked by 40 μM GYKI-53665. The mean amplitudes for a population of ganglion cells are plotted versus log GYKI concentration in Fig. 2B. The solid lines are the best non-linear regression fits to Eq. 1. The IC₅₀ values determined from the fits were 0.7 μM for GYKI-53665 and 3.6 μM for the less-potent GYKI-52466. The effectiveness of the GYKI compounds in reducing the light-evoked ON EPSCs was almost identical to their effectiveness in reducing the potassium puff-evoked EPSCs.

Spontaneous, AMPA/KA receptor-mediated EPSCs also were blocked by GYKI-53665. Figure 2C shows an example of spontaneous EPSCs recorded from an ON, ganglion cell. The spontaneous EPSCs were blocked completely and reversibly by 40 μM GYKI-53665, indicating that they were mediated primarily by AMPA-prefering receptors.

KA-evoked currents were reduced by GYKI-53665

The actions of the potent and selective GYKI compound 53665 on the potassium puff-evoked, light-evoked and spontaneous ganglion cell EPSCs indicate that AMPA-prefering receptors mediated most of the AMPA/KA receptor component of the responses. To determine whether KA-prefering receptors played some role in mediating the AMPA/KA receptor component of the ganglion cell responses, we tested the ability of GYKI-53665 to reduce KA-evoked currents in ganglion cells. Because KA activates both KA- and AMPA-prefering receptors, any GYKI-resistant current would indicate the presence of KA-prefering receptors. To rule out any indirect effects of KA, synaptic transmission was blocked by including 100 μM cadmium chloride in the bath.

Responses to KA (500 μM pipette concentration) puffed at the inner plexiform layer near the ganglion cell dendrites are illustrated in Fig. 3A. When the cell was clamped to −70 mV, the KA puffs elicited large inward currents. The amplitude of the kainate currents was reduced by GYKI-53665 in a concentration-dependent manner. Similar to Wilding and Huettner (1995), we found that 10 μM GYKI-53665 produced >90% block. The KA current in this cell was blocked almost completely by 40 μM GYKI-53665 indicating that the response was mediated almost exclusively by AMPA-prefering receptors. Similarly, both AMPA- (n = 5) and glutamate-evoked (n = 6) currents (1-mM pipette concentrations) were completely suppressed by 40 μM GYKI-53665 (not shown). NMDA receptors were blocked by 50 μM D-AP5 when glutamate was pulsed. In Fig. 3B a concentration-response curve was generated by plotting the mean EPSC amplitude versus the log concentra

FIG. 1. α-amino-3-hydroxy-5-methyl-4-isozoleprionic acid/kainate (AMPA/KA) component of K⁺ puff-evoked excitatory postsynaptic currents (EPSCs) was inhibited by GYKI compounds. A: ganglion cell EPSCs recorded in absence and presence of indicated concentrations (in micromolar) of 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-2,3-benzodiazepine hydrochloride (GYKI-52466). Amplitudes of the EPSCs were reduced by GYKI in a concentration-dependent manner; maximal inhibition was observed with 30 or 100 μM GYKI. Cell was voltage clamped to −75 mV. AMPA/KA component of EPSCs was isolated by including 2 μM strychnine, 150 μM picrotoxin, 0.5 μM tetrodotoxin, and 50 μM D-AP5 when glutamate was applied. B: concentration-response curves for GYKI-52466 and 1-(4-aminophenyl)-3-methylcarbamyl-4-methyl-7,8-methylenedioxy-3,4-dihydro-2,3-benzodiazepine (GYKI-53665). Points are normalized, peak amplitudes plotted vs. log [GYKI]. GYKI-53665 (n = 6) was ~6 times more effective than GYKI-52466 (n = 4). As described in Methods, solid lines are best, nonlinear regression fits to Eq. 1, in this and subsequent figures. IC₅₀ values were 4.2 and 0.7 μM for GYKI-52466 and GYKI-53665, respectively.
voltage clamped to 0 by 40 receptor-mediated spontaneous EPSCs (Control) were blocked completely.

Inhibition of the KA-evoked currents was 0.6 m 0.7 m 0. Å which blocks KA-preferring receptor desensitization, did not responses to puffs of kainate (500 current. Pretreatment (10 ± 30 min) with concanavalin A, FIG. 3. KA-evoked currents were reduced by GYKI-53665. The IC50 value for GYKI-53665 was added to the bath, the KA-evoked currents were blocked at both negative and positive holding potentials (Fig. 4, B and C, ■). After washing out the GYKI-53665, the cell’s responsiveness to KA recovered (Fig. 4C, ○). Similar results were seen in five additional cells. These data indicate that inhibition of ganglion cell AMPA-preferring receptors by GYKI-53665 is independent of holding potential, in agreement with similar studies on isolated cortical neurons (Wilding and Huettner 1995).

K+ puff- and light-evoked EPSCs were enhanced by cycllothiazide, but not concanavalin A

Concanavalin A has been shown to be more effective in potentiating responses mediated by KA-preferring receptors than in potentiating responses mediated by AMPA-preferring receptors (Huettner 1990; Partin et al. 1993; Wong and Mayer 1993). Cyclothiazide, which selectively potentiates responses mediated by AMPA-preferring receptors (Partin et al. 1993; Wong and Mayer 1993) has been shown to enhance ganglion cell EPSCs (Lukasiewicz et al. 1995). We compared the ability of concanavalin A and cyclothiazide to enhance the AMPA/KA components of EPSCs in the same ganglion cell.

An example of the effects of concanavalin A (300 μg/ml) on ganglion cell EPSCs is shown in Fig. 5. The current traces in Fig. 5A illustrate responses to puffs of potassium onto bipolar cells whereas the current traces in Fig. 5B illustrate light-elicited EPSCs at light onset and at light offset.

centration of GYKI-53665. The IC50 value for GYKI-53665 inhibition of the KA-evoked currents was 0.6 μM, which is similar to the values reported above for the GYKI-53665 inhibition of AMPA/KA component of the ganglion cell EPSCs.

Desensitization of KA-preferring receptors (Huettner 1990) during KA puffs probably did not account for our inability to observe GYKI-resistant, KA-receptor-mediated currents. Pretreatment (10–30 min) with concanavalin A, which blocks KA-preferring receptor desensitization, did not reveal a GYKI-resistant, response component. KA-evoked currents were blocked completely by 40 μM GYKI-53665 in the presence of concanavalin A (1.2 ± 1.4% of control, n = 6), indicating that these currents were mediated primarily by AMPA-preferring receptors.

We demonstrated potent inhibition by GYKI-53665 when ganglion cells were voltage clamped to −70 mV. For GYKI-53665 to be an effective tool to study ganglion cell voltage responses and other aspects of retinal function, its inhibitory actions should be independent of membrane potential. The voltage dependency of GYKI-53665 inhibition was tested as illustrated in Fig. 4. A family of KA-evoked currents obtained when the cell was clamped to a series of holding potentials, ranging from −60 to +30 mV, is shown in Fig. 4A. The peak current responses plotted as a function of holding potential in Fig. 4C (●) indicate that the KA-evoked currents reversed polarity near 0 mV. When 40 μM GYKI-53665 was added to the bath, the KA-evoked currents were blocked at both negative and positive holding potentials (Fig. 4, B and C, ■). After washing out the GYKI-53665, the cell’s responsiveness to KA recovered (Fig. 4C, ○). Similar results were seen in five additional cells. These data indicate that inhibition of ganglion cell AMPA-preferring receptors by GYKI-53665 is independent of holding potential, in agreement with similar studies on isolated cortical neurons (Wilding and Huettner 1995).

FIG. 2. AMPA/KA component of light-evoked and spontaneous EPSCs was inhibited by GYKI compounds. A: ganglion cell, light-evoked EPSCs recorded in absence and presence of the indicated concentrations (in micromolar) of GYKI-53665. Amplitudes of EPSCs were reduced by 40 μM GYKI-53665 in a concentration-dependent manner; maximal inhibition was observed with 10 or 40 μM GYKI. Cell was voltage clamped to −75 mV. Time course of light stimulus is indicated by bar above current traces. Intensity of light stimulus was attenuated by 3.4 log units.

B: concentration-response curves for GYKI-52466 and GYKI-53665. Points are normalized, peak amplitudes plotted vs. log [GYKI]. GYKI-53665 (n = 4) was ~5 times more effective than GYKI-52466 (n = 7). IC50 values were 3.6 and 0.7 μM for GYKI-52466 and GYKI-53665, respectively. C: AMPA/KA-receptor-mediated spontaneous EPSCs (Control) were blocked completely by 40 μM GYKI-53665 (labeled GYKI-53665). Each set of responses represents 7, consecutively recorded, superimposed current traces that were filtered at 5 kHz and sampled at 2.5 kHz. This was an ON cell that was voltage clamped to −75 mV.

FIG. 3. KA-evoked currents were reduced by GYKI-53665. A: current responses to puffs of kainate (500 μM pipette concentration) were reduced by GYKI-53665. Concentrations present when each response was recorded are indicated (in micromolar) near each trace. Synaptic transmission was blocked by including 100 μM cadmium chloride in bath. Cell was voltage clamped to −70 mV. B: concentration-response function for peak KA response amplitudes plotted vs. log [GYKI] (n = 7). IC50 value was 0.6 μM. Little or no GYKI-resistant kainate current was seen with 10 and 40 μM.

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An example of the effects of concanavalin A (300 μg/ml) on ganglion cell EPSCs is shown in Fig. 5. The current traces in Fig. 5A illustrate responses to puffs of potassium onto bipolar cells whereas the current traces in Fig. 5B illustrate light-elicited EPSCs at light onset and at light offset.
Lack of a significant enhancement of the EPSCs by concanavalin A in 15 of 17 cells, suggests that KA-preferring receptors play little role in mediating the EPSCs.

**Glutamate-evoked currents were not enhanced by concanavalin A**

Concanavalin A did not enhance ganglion cell EPSCs. This may have been because there were few KA-preferring receptors present or because concanavalin A acted presynaptically to reduce synaptic transmission (Mayer and Vyklicky 1989; Shinozaki and Ishida 1979). To control for the latter possibility, we tested the effectiveness of concanavalin A when synaptic transmission was bypassed by puffing glutamate directly onto ganglion cell dendrites. Figure 6A shows that concanavalin A did not enhance the glutamate current recorded in this ganglion cell. In contrast, cyclothiazide did enhance the glutamate current recorded in the same cell. Figure 6B shows that amplitude of the glutamate current recorded in the presence of concanavalin A was not significantly different from the control current amplitude. Cyclothiazide, however, enhanced the amplitude of the glutamate currents on average fourfold. These data indicate that little of the glutamate-evoked current was mediated by KA-preferring receptors.

**DISCUSSION**

Our results indicate that the AMPA/KA receptor component of ganglion cell EPSCs is mediated primarily by}

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**FIG. 4.** Inhibition by GYKI-53665 is not voltage dependent. A: currents evoked by puffing KA (500 μM pipette concentration) at inner plexiform layer when ganglion cell was clamped to −60, −45, −30, −15, 0, 15, and 30 mV (bottom to top traces). Synaptic transmission was blocked by including 100 μM cadmium chloride in bath. B: KA currents were blocked at all holding potentials by 40 μM GYKI-53665. Ganglion cell was clamped to same potentials as in A. C: current-voltage relationships in absence (●) and presence (■) of 40 μM GYKI-53665. GYKI blocked KA-evoked currents at all holding potentials. After washing, cell’s responsiveness to KA recovered (○).

The AMPA/KA receptor components of the responses were isolated as described above. Pyruvate was substituted for glucose in the bath because glucose can bind to concanavalin A, reducing its effectiveness (Goldstein et al. 1965; Mayer and Vlklicky 1989; Thio et al. 1993). It was found that the area of the EPSC waveform (the integral of the EPSC over time) was a more sensitive measure of the effect of concanavalin A than was the peak amplitude of the waveform. In the cells illustrated, concanavalin A slightly reduced the integrals of the EPSCs for both the potassium puff- and light-evoked EPSCs. On average, concanavalin A did not significantly affect the integral values of the K⁺ puff evoked EPSCs (98 ± 13% of control values, n = 7). Concanavalin A reduced the integrals of the light-evoked EPSCs to 83 ± 9% of control values (n = 10). By contrast, cyclothiazide (30 μM) always enhanced the integral values for the puff-evoked EPSCs (4 ± 1.6-fold, n = 5) and the on, light-evoked EPSCs (4 ± 1.4-fold, n = 8). The small reduction of the EPSCs by concanavalin A may be due to a presynaptic

**FIG. 5.** K⁺ puff- and light-evoked EPSCs were enhanced by cyclothiazide, but not concanavalin A. A: EPSCs evoked by a K⁺ puff in absence and presence of concanavalin A or cyclothiazide. Concanavalin A (300 μg/ml) caused a small suppression of ganglion cell EPSC. Cyclothiazide (30 μM), in contrast, enhanced K⁺ puff-evoked EPSC. B: light-evoked EPSCs recorded from a different ganglion cell in absence and presence of concanavalin A or cyclothiazide. Light-evoked EPSCs were increased slightly by concanavalin A but augmented by cyclothiazide. Intensity of light stimulus was attenuated by 3 log units. Cells were voltage clamped to −75 mV.
AMP receptor subunits; AMPA-preferring receptor subunits include GluR1-7 was seen in the GCL. Although ganglion cells and displaced amacrine cells were not distinguished in these studies, the possibility exists that subunits

Our studies with the GYKI compounds indicate that KA-preferring receptors play little or no role in mediating ganglion cell EPSCs. The GYKI compounds have been shown to be very selective for AMPA-preferring receptors (Paternain et al. 1995; Renard et al. 1995; Wilding and Huettner 1995). In rat cerebellar Purkinje (Renard et al. 1995) and hippocampal (Paternain et al. 1995) neurons, blockade of AMPA-preferring receptors unmasked a small component of KA-preferring receptors. The GYKI-resistant, KA-preferring receptors accounted for ~10 and 30% of the agonist-elicted response in hippocampal and cerebellar neurons, respectively. Excitatory synaptic responses, however, were completely suppressed by GYKI-53665 in hippocampal neurons (Paternain et al. 1995), indicating that KA-preferring receptors did not participate in synaptic transmission and were located extrasynaptically. We found that in the ON-OFF ganglion cells both the KA-evoked currents and the AMPA/KA component of the EPSCs were suppressed completely by GYKI-53665. This indicates that, at most, only a very small percentage of the ganglion cell EPSC is mediated by KA-preferring receptors.

Our results with concanavalin A and cyclothiazide also support the idea that AMPA-preferring receptors mediate most of the ganglion cell synaptic responses. In most cells, concanavalin A slightly decreased the responses. Others have reported that concanavalin A reduced the amplitudes of evoked excitatory responses in hippocampal neurons (Mayer and Vylický 1989) and at crayfish neuromuscular junction (Shinozaki and Ishida 1979). It has been suggested that concanavalin A may reduce transmitter release at hippocampal excitatory synapses (Mayer and Vylický 1989). This effect could account for the reductions in ganglion cell synaptic responses that we observed. Thio et al. (1993) showed that concanavalin A increased the mean EPSC amplitude by 30% in rat hippocampal neurons. In two ganglion cells, concanavalin A produced about a 10% enhancement of the integrated synaptic current responses. These enhancements may have been due to the weak potentiating effects of concanavalin A on AMPA-preferring receptors (Partin et al. 1993; Wong and Mayer 1993). In addition to its postsynaptic effects on AMPA-preferring receptors, cyclothiazide also has been shown to enhance transmitter release from dissociated CA1 hippocampal neurons (Diamond and Jahr 1995). Although cyclothiazide has a potent postsynaptic effect on retinal ganglion cells (Lukasiewicz et al. 1995), we cannot rule out an additional presynaptic component of cyclothiazide action because its effect on transmitter release from retinal neurons is presently unknown.

Recent molecular cloning studies have demonstrated a variety of non-NMDA, ionotropic, glutamate receptor subunits; AMPA-preferring receptor subunits include GluR1-4, whereas kainate-preferring subunits include GluR5-7 (Seeburg 1993). The distribution of glutamate receptor subunits in salamander retinal ganglion cells is presently unknown. However, several studies have shown the expression patterns for ionotropic glutamate receptor subunits in mammalian retinal ganglion cell layer (GCL) (Hamasaki-Brito et al. 1993; Hughes et al. 1992; Muller et al. 1992). Labeling by probes for GluR1-7 was seen in the GCL. Although ganglion cells and displaced amacrine cells were not distinguished in these studies, the possibility exists that subunits
for AMPA- and KA-prefering could exist in the same ganglion cell. The coexpression of both AMPA- and KA-subunit mRNA also has been found in single hippocampal neurons (Craig et al. 1993). The presence of mRNA for a subunit in the somata does not necessarily mean that these subunits mediate synaptic inputs at the dendrites. It is possible that some subunits are extrasynaptic or are transported to the axon terminals. For example, in the hippocampal neurons where AMPA-prefering receptors mediate excitatory inputs, KA-prefering receptors on axon terminals may regulate glutamate release (Chittajallu et al. 1996).

The presence of ionotropic GluR mRNA does not indicate the location of the receptor protein within the neuron nor does it necessarily mean that the protein is even expressed. Peng et al. (1995) used immunocytochemistry to identify the protein distribution of GluR subunits in rat and goldfish retina. Antibodies to AMPA-prefering subunits GluR1 and GluR2/3, as well as antibodies to kainate preferring subunits GluR6 and -7 labeled somata in the rat GCL. In goldfish, antibodies to GluR2/3 and GluR6/7 both labeled somata in the GCL, as well as the IPL. Immunoreactivity to GluR4 was not detected in ganglion cells in either rat or goldfish (but see Morigwa et al. 1995). The colocalization of AMPA- and KA-subunit immunoreactivity has also been found in single cortical neurons (Vickers et al. 1993).

It is not known if AMPA- and KA-prefering subunits combine to form functional receptors in retinal ganglion cells (or other neurons), but evidence from several studies suggests that this is not the case. When AMPA- and KA-prefering subunits were expressed in Xenopus oocytes, physiological and pharmacological studies indicated that these subunits assembled into independent AMPA- and KA-prefering receptors (Partin et al. 1995). Analysis of the glutamate receptor-mediated currents in glial cells that have been shown to express mRNAs for both AMPA- and KA-prefering subunits also suggests that native KA and AMPA receptors are assembled independently (Patneau et al. 1994). Finally, immunoprecipitation experiments with antibodies specific for AMPA- or KA-subunits also suggest that individual native glutamate receptors are not assembled with both AMPA- and KA-prefering subunits (Pulchalski et al. 1994; Wenthold et al. 1994).

Our results predict that salamander, ON-off ganglion cells should be immunoreactive to AMPA-prefering subunit antibodies and possibly also to KA-prefering subunit antibodies. Nevertheless, our study indicates that AMPA-prefering receptors play the primary role and that KA-prefering receptors play little, if any, role in excitatory synaptic transmission.

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