AMPAG-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-aminoo-3-hydroxy-5-methyl-4-isooazoleprionic acid (AMPA) - and/or kainate (KA)-preferring receptors mediate excitatory synaptic inputs to retinal ganglion cells. Excitatory postsynaptic currents (EPSCs), evoked either by light or by stimulating bipolar cells with puffs of K<sup>+</sup>, were measured using whole cell recording techniques in the tiger salamander retina 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-methylcarbamil-4-methyl-7,8-methylenedioxy-3,4-dihydro-5H-2,3-benzodiazepine (GYKI-53665), reduced light-evoked EPSCs and K<sup>+</sup> puff-evoked EPSCs amplitudes in a concentration-dependent manner. The IC<sub>50</sub> 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 IC<sub>50</sub> 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-53665, 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-aminoo-3-hydroxy-5-methyl-4-isooazoleprionic 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; Ege-
onto bipolar cell dendrites (Lukasiewicz and Werblin 1994). The IC\textsubscript{50} is the antagonist concentration.

**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 MΩ. The measured series resistances were typically 15–25 MΩ. The magnitude of the series resistance compensation, read off the Dagan 3900A compensation dial, was 5–10 MΩ. 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

$$\frac{I}{I_{\text{max}}} = \frac{a - c}{1 + \frac{\text{IC}_{50}}{[\text{antagonist}]/b}} + c$$

where \(a\) is the asymptotic maximum, \(b\) is the slope parameter, \(c\) is the asymptotic minimum and \(\text{IC}_{50}\) is the antagonist concentration that reduced \(I\) to one-half of \(I_{\text{max}}\). Results are expressed as means ± 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).
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 IC$_{50}$ value for GYKI-52466 was 4.2 μM whereas the mean IC$_{50}$ 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 GKI concentration in Fig. 2B. The solid lines are the best non-linear regression fits to Eq. 1. The IC$_{50}$ 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 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-nM pipette concentrations) were completely suppressed by 40 μM GYKI-53665 (not shown). NMDA receptors were blocked by 50 μM D-AP5 when glutamate was puffed. In Fig. 3B a concentration-response curve was generated by plotting the mean EPSC amplitude versus the log con-
We demonstrated potent inhibition by GYKI-53665 when ganglion cells were voltage clamped to $-70 \text{ mV}$. 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 $+30 \text{ mV}$, is shown in Fig. 4A. The peak current responses plotted as a function of holding potential in Fig. 4C ($\bullet$) indicate that the KA-evoked currents reversed polarity near $0 \text{ mV}$. When $40 \mu M$ 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-prefering receptors by GYKI-53665 is independent of holding potential, in agreement with similar studies on isolated cortical neurons (Wilding and Huettner 1995).

$K^+ \text{ puff- and light-evoked EPSCs were enhanced by cyclothiazide, but not concanavalin A}$

Concanavalin A has been shown to be more effective in potentiating responses mediated by KA-prefering receptors than in potentiating responses mediated by AMPA-prefering receptors (Huettner 1990; Partin et al. 1993; Wong and Mayer 1993). Cyclothiazide, which selectively potentiates responses mediated by AMPA-prefering 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 ($300 \mu g/ml$) 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.

![Concentration of GYKI-53665](image1)

**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 GYKI-53665 in a concentration-dependent manner; maximal inhibition was observed with 10 or $40 \mu M$ GYKI. Cell was voltage clamped to $-75 \text{ mV}$. 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 $[\text{GYKI}].$ GYKI-53665 ($n = 4$) was $\sim 5$ times more effective than GYKI-52466 ($n = 7$). IC$_{50}$ values were 3.6 and $0.7 \mu M$ for GYKI-52466 and GYKI-53665, respectively. C: AMPA/KA-receptor-mediated spontaneous EPSCs (Control) were blocked completely by $40 \mu M$ GYKI-53665 (labeled GYKI-53665). Each set of responses represents 7, consecutively recorded, superimposed current traces that were filtered at $5 \text{ kHz}$ and sampled at $2.5 \text{ kHz}$. This was an ON cell that was voltage clamped to $-75 \text{ mV}$.

![Concentration of GYKI-53665](image2)

Concentration of GYKI-53665 inhibition of the KA-evoked currents was $0.6 \mu M$, 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-prefering receptors (Huettner 1990) during KA puffs probably did not account for our inability to observe GYKI-resistant, KA-receptor-mediated currents. Pretreatment ($10-30 \text{ min}$) with concanavalin A, which blocks KA-prefering receptor desensitization, did not reveal a GYKI-resistant, response component. KA-evoked currents were blocked completely by $40 \mu M$ GYKI-53665 in the presence of concanavalin A ($1.2 \pm 1.4\% \text{ of control, } n = 6$), indicating that these currents were mediated primarily by AMPA-prefering receptors.

![Concentration of GYKI-53665](image3)

**FIG. 3.** KA-evoked currents were reduced by GYKI-53665. A: current responses to puffs of kainate ($500 \mu M$ 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 $100 \mu M$ cadmium chloride in bath. Cell was voltage clamped to $-70 \text{ mV}$. B: concentration-response function for peak KA response amplitudes plotted vs. log $[\text{GYKI}].$ IC$_{50}$ value was $0.6 \mu M$. Little or no GYKI-resistant kainate current was seen with 10 and $40 \mu M$. 

![Concentration of GYKI-53665](image4)
action (Mayer and Vyklicky 1989; Shinozaki and Ishida 1979). Lack of a significant enhancement of the EPSCs by concanavalin A in 15 of 17 cells, suggests that KA-prefering 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-prefering 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-prefering receptors.

DISCUSSION

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

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
AMPAs. We have shown that AMPA-evoked (Massey and Miller 1988) responses are mainly due to the AMPA-preferring receptors, and before it was known that K+ activates both AMPA- and KA-preferring receptors, and in hippocampal neurons, which have predominantly AMPA-preferring receptors (Evans et al. 1987; Huettner 1990; Robinson et al. 1984).

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 unmasks a small component of KA-preferring receptors. The GYKI-resistant, KA-preferring receptors accounted for ~10% of the agonist-evoked current 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 Vyklicky 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 Vyklicky 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. 1995; Thio 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 layer (GCL) (Hamassaki-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 Morigiwa 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.

We thank Drs. P. B. Cook, J. S. McReynolds, and C. Romano for comments on the manuscript and Eli Lily and Co. for the gifts of GYKI-53665 and cyclothiazide.

This work was supported by Research to Prevent Blindness and National Eye Institute Grants EY-08922 and EY-02687 to P. D. Lukasiewicz, a Core Grant to the Dept. of Ophthalmology, and a Mr. and Mrs. Spencer T. Olin Fellowship to J. E. Lawrence.

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Received 5 April 1996; accepted in final form 5 September 1996.

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