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Differential modulation of retinal ganglion cell light responses by orthosteric and allosteric metabotropic glutamate receptor 8 compounds

Brian T. Reed^{a,*}, Catherine W. Morgans^{a,b}, and Robert M. Duvoisin^{a,b,†}

^aDepartment of Physiology & Pharmacology, Oregon Health & Science University, Portland, OR 97239, USA

^bCasey Eye Institute, Oregon Health & Science University, Portland, OR 97239, USA

Abstract

To investigate the role of mGluR8 in modulating the synaptic responses of retinal ganglion cells, we used a recently identified positive allosteric modulator of mGluR8, AZ12216052 (AZ) and the mGluR8-specific orthosteric agonist (*S*)-3,4-dicarboxyphenylglycine (DCPG). These agents were applied to whole-cell voltage-clamped ganglion cells from an isolated, superfused mouse retina preparation. DCPG reduced OFF-ganglion cell excitatory currents, whereas AZ enhanced the peak excitatory currents in ON-, OFF-, and ON-OFF-ganglion cells. The effects on ganglion cell inhibitory currents were more varied. The effects of the allosteric modulator were stronger for bright stimuli than for dim stimuli, consistent with receptor stimulation by endogenous glutamate being stronger during bright light stimulation and with mGluR8 receptors mainly being localized away from glutamate release sites, immuno-labeled with VGLUT1. The differential sensitivity of ganglion cell light responses to DCPG and AZ supports multiple sites where mGluR8 modulates the light responses of ganglion cells.

Keywords

metabotropic glutamate receptors; mGluR8; retina; positive allosteric modulators

1. Introduction

As in the rest of the central nervous system, glutamate is the main excitatory neurotransmitter in the vertebrate retina. It mediates synaptic transmission from photoreceptors to bipolar cells, and from bipolar cells to amacrine and ganglion cells. The fast actions of glutamate are mediated by ionotropic glutamate-gated channels, whereas metabotropic glutamate receptors (mGluRs) activate G protein-mediated intracellular second messenger cascades that elicit diverse effects on neuronal function. mGluRs are classified into three groups based on amino acid sequence and pharmacology. Group-III mGluRs,

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[†]Corresponding author. Department of Physiology and Pharmacology, Mail code: L-334, Oregon Health & Science University, 3181 SW Sam Jackson Park Rd., Portland, OR 97239, USA, Tel.: +1 503 418 2665; Fax: +1 503 494 4352; duvoisin@ohsu.edu.

^{*}present address: Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA 99164, USA.

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comprising the mGluR4, -R6, -R7, and -R8 subtypes, are selectively activated by L-2-amino-4-phosphonobutyric acid (L-AP4, also abbreviated APB). In the brain, they are generally localized at presynaptic sites where they regulate neurotransmitter release (Schoepp, 2001).

In the retina, with the exception of mGluR6, the functions of group-III mGluRs are not well defined. mGluR6 is expressed in the outer plexiform layer (OPL) in ON-bipolar cell dendrites and controls these cells' depolarizing response to light. By contrast, the roles of mGluR4, -R7, and -R8 in visual processing and the cell types that express them remain unclear. Immunohistochemical studies have shown that they are all present in the inner plexiform layer (IPL) of the retina, where they could modulate neurotransmitter release from bipolar and amacrine cells and directly or indirectly affect post-synaptic ganglion cells (Brandstätter et al., 1996; Koulen et al., 1996; Quraishi et al., 2007). Indeed, Awatramani and Slaughter (2001) found that L-AP4 regulated glutamate release from OFF-bipolar cells in the salamander retina. Further, Higgs et al. (2002) showed that L-AP4 modulated light-evoked OFF responses in ganglion cells, primarily by an effect on bipolar cell terminals. Finally, Quraishi et al. (2010) found that DCPG reduced OFF ganglion cell light responses in mouse, suggesting that the effects observed by Awatramani and Slaughter (2001) and Higgs et al. (2002) are at least partially mediated by mGluR8.

An obstacle to understanding group-III mGluR function has been the lack of subtype-specific pharmacological agents. One approach to obtain drugs selective for specific receptor subtypes is to identify allosteric modulators (Conn et al., 2009; Urwyler, 2011). In contrast to competitive agonists and antagonists which interact with the orthosteric glutamate binding site, allosteric modulators bind to sites that are usually less conserved. In the case of mGluRs for example, allosteric sites are generally located in the 7-helical transmembrane domain. Further, allosteric modulators have no intrinsic activity. Instead, positive allosteric modulators (PAMs) increase the efficacy of the agonist and consequently the activity of the receptor for which they are selective, whereas negative allosteric modulators (NAMs) decrease the efficacy of the agonist and thus receptor activity.

Here we examined the effects of the recently described mGluR8 PAM, AZ12216052 (AZ; Duvoisin et al., 2010), and that of the mGluR8-specific orthosteric agonist, (*S*)-3,4-dicarboxyphenylglycine (DCPG; Thomas et al., 2001), on the light responses of mouse retinal ganglion cells. Since orthosteric agonists stimulate all cognate receptors regardless of their location and activity, whereas PAMs will only affect receptors that are simultaneously stimulated by the endogenous agonist, we varied the intensity of the light stimulus and thus the amount of glutamate released at bipolar cell terminals, and then compared the effects of AZ and DCPG on the light evoked currents.

2. Materials and methods

2.1. Animals and tissue preparation

All animal maintenance and handling was performed in accordance with NIH guidelines and approved by the Institutional Animal Care and Use Committee at OHSU. C57Bl/6 mice (The Jackson Laboratory, Bar Harbor, ME) were fed and housed under a 12 h light/dark cycle. For histological experiments, mice were euthanized by CO₂ asphyxiation and enucleated following cervical dislocation. For electrophysiological analyses, mice were dark-adapted for at least 1 h prior to experimentation and all subsequent animal handling and experimental recordings were carried out in dim red light to maintain the retina in a dark-adapted state. Mice were deeply anesthetized with an i.p. injection of sodium pentobarbital (300 mg/kg; Ovation Pharmaceuticals, Deerfield, IL) and enucleated following cervical dislocation. The cornea, lens, and vitreous body were excised and the resulting

posterior eyecup was submerged in bicarbonate-buffered Ames medium (Sigma-Aldrich, St. Louis, MO) equilibrated with 95% O₂ and 5% CO₂ (carbogen). The retina was dissected from the pigment epithelium, flattened by making three radial cuts at its outer edge, then placed with ganglion cells facing up onto a nitrocellulose filter (8 μ m, 13 mm, SCWP; Millipore, Billerica, MA) whose center was punched out leaving a 2 mm diameter hole in the center. The retina was placed in a recording chamber (World Precision Instruments, Sarasota, FL) and held down with a U-shaped piece of platinum wire that had an array of parallel nylon filaments glued across it. The second retina was also removed and placed in Ames medium for later use. The tissue was maintained in a healthy state by continual perfusion with 35°C oxygenated and bicarbonate-buffered Ames medium for the duration of the experiment. Perfusion solutions were maintained in carbogen-bubbled reservoirs located above the recording chamber and gravity fed over the retina at a rate of 3 ml/min.

2.2. Electrophysiology

The recording chamber was placed under the 40 $\times$ water-immersion objective of an upright microscope (Zeiss Axioskop 2 FS). Cells were located and recording electrodes positioned while viewing the preparation via an infrared-sensitive video camera with Dodt phase optics. The cell soma was exposed by micro-dissecting a hole in the inner limiting membrane, which overlies the ganglion cell layer. Once access to the cell membrane was achieved, the recording electrode was applied, and light-evoked responses were recorded.

Extracellular recordings were made by pushing the microelectrode against a ganglion cell soma and recording in a loose-patch configuration. Voltage-clamp recordings were obtained using whole-cell patch electrode techniques. We examined the effects of exogenous mGluR compounds on the magnitude of the currents evoked by a light stimulus. The AZ compound was provided by Drs. Vijay Chhajlani and Edwin Johnson at Astra-Zeneca (Wilmington, DE) and used at 10 μ M. Its EC₅₀ is $\sim$ 1 μ M (Duvoisin et al., 2010). DCPG was obtained from Tocris Bioscience (Ellisville, MO) and used at 1 μ M, as previously (Quraishi et al., 2010). Its EC₅₀ is $\sim$ 30 nM (Thomas et al., 2001).

Light-evoked synaptic currents were recorded in the whole-cell configuration as follows. The membrane potential was adjusted by -10 mV to account for the electrode liquid junction potential. The series resistance was not routinely compensated for, as it was generally less than 30 M Ω . Signals were recorded with an Axon Instruments Multiclamp 700A amplifier connected to an Axon Instruments Digidata 1321A 16 bit A-D converter (Molecular Devices, Sunnyvale, CA) and a Dell Windows PC. To reduce noise, signals were low-pass filtered offline at 0.2 – 2 kHz with an 8-pole Bessel software filter. Data were analyzed offline with pClamp (Molecular Devices) and Axograph X (Axograph Scientific, Sydney, Australia) software.

2.3. Whole-cell recording electrodes

Two types of recording electrodes were used, differing only in the composition of their filling solutions. Patch electrodes were pulled from borosilicate glass (Sutter Instrument Co., Novato, CA; 1.5 mm O.D., 0.86 mm I.D.) and filled with either the extracellular Ames solution for extracellular recording or with an intracellular solution for whole-cell patch-clamp recording. Filled electrodes had a tip resistance ranging from 5–7 M Ω .

2.4. Solutions and drug application

Except where indicated elsewhere in the text, all of the chemicals that we used were obtained from Sigma-Aldrich (St Louis, MO). For whole-cell recordings, the filling solution for the electrodes was as follows (in mM): 110 Cs-gluconate, 10 NaCl, 5 Na-HEPES, 1 Cs-EGTA, 1 Na-ATP, 0.1 Na-GTP, and 10 QX-314. Cesium was used in place of potassium to

block voltage-gated potassium currents and thereby improve the quality of the voltage clamp at positive potentials. QX-314 was included to block voltage-dependent sodium channels and abolished all spiking activity within 1–2 minutes of establishing the whole-cell configuration. A fluorescent dye (Alexa Fluor 488 hydrazide) was added to the internal electrode solution to allow visualization of the cell by epifluorescence following the recordings.

2.5. Visual displays and responses

Stimuli were generated using custom software incorporated into Vision Egg (visionegg.org) running on a Windows XP (32 bit) PC. The images were displayed on a monochrome OLED microdisplay (eMagin Corporation, Bellevue, WA) and focused via the microscope objective onto the photoreceptors. Stimuli were adjusted in both size (between 200 and 300 μm in diameter) and position to optimally activate the center of the receptive field. In each experiment, ganglion cells were usually stimulated at varying contrasts. Contrast is defined as $\text{contrast} = 100\% (F - B)/B$, where F and B represent foreground and background illumination, respectively. Stimuli were given on a constant gray background of $B = 50 \text{ cd/m}^2$. The background light level was sufficient to ensure that the retina was operating in the low photopic range. Bright stimulus intensity (F) was adjusted to either +80 or –80% contrast and dim stimuli to either +20 or –20% contrast. During most experiments, each contrast was presented 4 times in pseudorandom order for each holding potential. Light responses were measured before, during, and after administration of drugs. Drugs were bath applied and allowed to wash out completely. Typical drug treatments lasted 5–10 min, and the wash out period generally followed for 5–10 min.

We identified ganglion cell subtypes by extracellular recording of spiking to light responses, visually by their appearance under the microscope, and by examining their current traces and matching them with previous electrophysiological and morphological data (Sun et al., 2002; Pang et al., 2003; van Wyk et al., 2009). When possible, a fluorescence micrograph was taken to document the morphology of the cell at the conclusion of the recordings.

2.6. Immunohistochemistry

The posterior eyecups were fixed in 4% (w/v) paraformaldehyde in 0.1 M phosphate-buffer (PB; pH 7.4) for 10–15 minutes at 4°C, washed in PB, cryoprotected, and 16–18 μm transversal cryostat sections were prepared as described previously (Quraishi et al., 2007; Jeffrey et al., 2010). Sections were washed in PB, pre-incubated for 10 minutes, and incubated overnight with primary antibodies as described previously. The guinea pig anti-VGLUT1 antiserum was obtained from Chemicon (Temecula, CA) and used at 1:10,000 dilution. The specificity of this antiserum was tested previously by Western blotting and the same distribution of expression was observed using antisera produced in two different host species (Sherry et al., 2003). Our VGLUT1 staining pattern was the same as published by Sherry et al. (2003). We generated the mouse monoclonal antibody against mGluR8 and used it as described (Quraishi et al., 2007). Its specificity was verified by the absence of immunolabeling on retina sections from mGluR8-deficient mice (Quraishi et al., 2007). Secondary antibodies conjugated to Cy3 and Alexa Fluor 488 (Jackson ImmunoResearch, West Grove, PA; Molecular Probes, Eugene, OR) were used to visualize binding of the primary antibodies. Confocal images were acquired with a LSM510 confocal microscope (Zeiss, Oberkochen, Germany) with a focal plane 1.0 μm . Images were pseudocolored and merged using Pixelmator (Pixelmator Team, London, UK).

3. Results

To examine the role of mGluR8 in the modulation of synaptic inputs to retinal ganglion cells, we measured the light-evoked responses of these cells in the presence of the mGluR8 PAM, AZ, and the orthosteric agonist, DCPG. This study was based on whole-cell recordings from 75 ganglion cells using 70 C57Bl/6 mice. Each cell filled with a fluorescent dye while recording had an identifiable axon, which emanated from the cell body and extended toward the optic nerve layer of the retina, confirming their identification as ganglion cells. We targeted cells with the largest cell bodies ($>20\ \mu\text{m}$) and obtained records from 4 broad physiological groups based on their light responses: ON-transient ($n=16$), ON-sustained ($n=8$), OFF-transient ($n=13$), and ON-OFF-transient ($n=9$). For the filled cells, the physiological responses correlated with the stratification of their dendrites in the inner plexiform layer. The first 3 groups had monostратified dendrites and the last group had a bistratified morphology. Thus, a range of polarities and response types were included in our ganglion cell population.

3.1. ON-ganglion cells

First, we examined the effects of AZ on the responses of ON-transient ganglion cells. Fig. 1A shows the averaged whole-cell current traces recorded from an ON transient ganglion cell responding to a bright spot of light ($90\ \text{cd}/\text{m}^2$) projected onto the excitatory center of its receptive field. Excitatory and inhibitory currents were recorded by clamping the cell at the chloride ($-65\ \text{mV}$) and cation ($0\ \text{mV}$) reversal potentials, respectively. In the control condition, the cell (as typical) responded to stimulus onset with a large, transient inward, excitatory current. When $10\ \mu\text{M}$ AZ was bath applied to the cell, the magnitude of the inward current increased (Fig. 1A; summarized in Fig. 4A, mean increase = $87 \pm 15\%$; SEM). In this cell, the outward, inhibitory current was little affected.

To compare the effects of DCPG on the light responses of ON-transient ganglion cells, after AZ was washed out, $1\ \mu\text{M}$ DCPG was applied to the preparation. In contrast to AZ, the transient peak inward current was unaffected by DCPG (mean decrease = $8\% \pm 6\%$; $n=10$). DCPG also decreased the inhibitory OFF response (mean decrease = 66% ; Fig. 4A).

Next, we tested whether the effects of AZ were dependent on the intensity of the light stimulus and, by extension, the amount of glutamate released by bipolar cells. Fig. 1B shows that the excitatory and inhibitory responses evoked by a dim spot of light ($60\ \text{cd}/\text{m}^2$) were unchanged (mean decrease = $7 \pm 35\%$, Fig. 4D). Evidently, enough glutamate is released by bright stimuli to stimulate PAM-modulated mGluR8, but insufficient glutamate is released by the dim stimuli to reveal an allosteric effect. These results support a scenario in which mGluR8 receptors are located at some distance from the release site and reached through the spillover of relatively large amounts of glutamate or the saturation of glutamate uptake.

3.2. OFF-Ganglion Cells

Fig. 2 shows the averaged current traces of an OFF-transient ganglion cell alternately responding to a bright (left panels) and a dim (right panels) spot of light projected onto the excitatory center of its receptive field. In the control condition (Fig. 2, black traces), the cell responded to stimulus onset with a small outward current (bottom traces) and to stimulus offset with a transient inward current (top traces). For the bright stimulus, when $10\ \mu\text{M}$ AZ was bath applied to the cell, the amplitude of the OFF inward current increased (Fig. 2A, top traces, $n=8/8$, mean increase = $78 \pm 21\%$; summarized in Fig. 4B) while becoming more transient. The ON outward current also tended to increase (bottom traces, mean increase = $84\% \pm 81\%$, $n=6$). In addition, an OFF outward current was observed in this cell following AZ application, but this effect was variable (Fig. 2A, bottom traces). On the other hand, the

responses to dim stimuli were only marginally modulated by 10 μ M AZ, and the effect was not statistically significant compared to the baseline response (Fig. 2B, mean increase = $6\% \pm 17\%$; summarized in Fig. 4E). Both the excitatory OFF and inhibitory OFF responses were significantly different from those under the bright stimulation condition ($p < 0.05$).

After AZ was washed out, 1 μ M DCPG was added to the preparation. The light-evoked responses to both the bright and dim stimuli decreased to the same extent (bright stimuli mean decrease = $68\% \pm 17\%$; dim stimuli mean decrease = $69 \pm 21\%$). The effects of 1 μ M DCPG on the OFF excitatory current were significantly different from that of 10 μ M AZ ($p < 0.05$) for both bright and dim stimuli, whereas the differing effect of these drugs on the outward currents approached statistical significance ($p = 0.06$). This reduction of the amplitude of the excitatory OFF response under DCPG is consistent with previous results (Awatramani and Slaughter, 2001; Higgs et al., 2002; Quraishi et al., 2010) further suggesting that mGluR8 is expressed within the direct OFF excitatory pathway for OFF ganglion cells.

3.3. ON-OFF-Ganglion Cells

Fig. 3 shows the light responses for an ON-OFF-ganglion cell responding to a small spot of light projected onto the center of its excitatory receptive field. In the control condition (black trace), the cell responded both to stimulus onset and offset with transient inward currents. Bath application of 10 μ M AZ to the cell increased the amplitudes of both the ON and OFF inward currents. The ON but not the OFF outward current also increased (mean increase = $87 \pm 16\%$; $n = 6$; summarized in Fig. 4C).

In contrast to AZ, application of 1 μ M DCPG reduced the excitatory and inhibitory ON and OFF currents. On average, the excitatory ON and the excitatory OFF current were decreased by $35 \pm 14\%$ and $57 \pm 10\%$, respectively (Fig. 4C). Similarly, inhibitory ON and OFF currents were decreased by $51 \pm 48\%$ and $80 \pm 71\%$, respectively. The effect of DCPG on both inhibitory ON and OFF currents was significantly different from the effect of AZ ($p < 0.05$). These effects of AZ and DCPG on ON-OFF-ganglion cell light responses are similar to the effects of these drugs on OFF-ganglion cell responses.

In most experiments the effects of DCPG were not dependent on the intensity of the light stimuli, whereas the effects of AZ were only observed with bright stimuli (Fig. 4F). It is possible that the effect of stimulation strength is a reflection of the distance between glutamate release sites and mGluR8 receptors. To examine the relationship between glutamate release sites and mGluR8, we double labeled retinal sections with antibodies against mGluR8 and VGLUT1, the vesicular glutamate transporter expressed in bipolar cell terminals. Some VGLUT1-labelled bipolar cells terminals in sub-laminae 2 and 4 of the IPL were clearly co-labeled with anti-mGluR8 (Fig. 5A and 5B, arrowheads). Overall, though, little co-localization of mGluR8 and VGLUT1 was observed, indicating that mGluR8 is mainly localized postsynaptically, some distance away from sites of glutamate release. This suggests that under normal physiological conditions, mGluR8 may not be recruited during responses to dim light, but possibly only upon bright stimulation.

4. Discussion

To determine the effect of mGluR8 stimulation on the light-evoked responses of ganglion cells, we bath-applied low micromolar concentrations of the orthosteric agonist, DCPG, and the PAM, AZ. The effects of DCPG on the light responses of ganglion cells were consistent with our previous report of a decrease in spiking activity associated with OFF responses (Quraishi et al., 2010). Both ON and OFF excitatory currents in OFF-transient and ON-OFF-ganglion cells were reduced by DCPG. The effect of DCPG on ON-ganglion cells was

an increase in the sustained portion of the inward current. In contrast, AZ enhanced light-evoked currents in ON-, OFF-, and ON-OFF-ganglion cells. These distinct effects reflect the contrasting modes of action of orthosteric agonists and allosteric modulator compounds. While application of DCPG stimulates all mGluR8 receptors in the retina, AZ is only effective on receptors that are also concurrently stimulated by endogenous glutamate.

In the brain, group-III mGluRs are generally presynaptic (reviewed by Cartmell and Schoepp, 2000). For example in the hippocampus, mGluR8 is located on glutamatergic axon terminals of the lateral perforant path input to the dentate gyrus where it provides negative feedback (Cai et al., 2001; Robbins et al., 2007). Presynaptic mGluRs are also present on GABAergic terminals, so called heteroreceptors, where they depress GABA release when stimulated by glutamate released from a nearby terminal (Mitchell and Silver, 2000; Semyanov and Kullmann, 2000). Assuming mGluR8 is also presynaptically localized in the retina, it could provide negative feedback if present on photoreceptors, bipolar cells, or VGLUT3-positive amacrine cells, or it could modulate glycine or GABA release by amacrine cells onto an array of bipolar, amacrine, and ganglion cells. The contrasting effects of DCPG and AZ on light-evoked excitatory currents suggest these compounds differentially activate mGluR8 receptors located at different sites. Our data are consistent with DCPG mainly activating mGluR8 receptors on OFF-bipolar cell terminals (Fig. 5C, a) thereby providing negative feedback on glutamate release. Interestingly, ON-ganglion cell excitatory currents are not reduced by DCPG and AZ application, suggesting that mGluR8 is not expressed in ON-bipolar cell terminals (Fig. 5C, a'). In addition, AZ could modulate mGluR8 heteroreceptors on amacrine cells (Fig. 5C, b and c), thus reducing inhibition onto bipolar cell terminals, and allowing for increased glutamate release and increased ganglion cell excitation. The finding that AZ increases light-ON excitatory currents in ON-ganglion cells (Fig. 4A, a) and light-OFF excitatory currents in OFF-ganglion cells (Fig. 4B, c) indicates that mGluR8 regulates reciprocal feedback inhibition (Fig. 5C, b). In contrast, the effects of AZ on lateral crossover inhibition (Fig. 5C, c), i.e. light-OFF excitatory currents in ON-ganglion cells and light-ON excitatory currents in OFF-ganglion cells are small. The effects of DCPG and AZ on ganglion cell inhibitory currents are more variable suggesting the presence of mGluR8 on a subset of amacrine cell processes presynaptic to ganglion cells (Fig. 5C, d). In addition, mGluR8 could be expressed on ganglion cell dendrites where it could modulate voltage-gated currents, but the experiments presented here cannot address that possibility.

Interestingly, AZ modulated ganglion cell responses only when brightly stimulated. This suggests that glutamate release from bipolar cells evoked by dim stimuli remains too low to activate mGluR8, even when AZ increases glutamate efficacy about 1.8 fold (Duvoisin et al., 2010). Thus mGluR8 modulation of ganglion cell light responses appears to be light intensity dependent. A variable function of mGluR8, and by extension other group-III mGluRs, would be consistent with the observation that while the group-III mGluR agonist L-AP4 depressed synaptic transmission between OFF-bipolar cells and ganglion cells, the antagonist CPPG did not increase OFF excitatory postsynaptic currents in ganglion cells (Higgs et al., 2002).

Our light spots matched the diameter of the recorded cell's excitatory receptive field, typically between 200 and 300 μm across. As such, they were unlikely to activate the large inhibitory surround provided by horizontal and wide-field GABAergic amacrine cells. Thus the source of the inhibitory currents we observed is likely narrow-field amacrine cells of which there are 8 known subtypes, all of them accumulating glycine (Kolb et al., 1981; Menger et al., 1998). Taking this into account, suppression of reciprocal inhibition through the activation of mGluR8 heteroreceptors present on glycinergic amacrine cell processes could be one mechanism responsible for the increase in the excitatory ganglion cell response

that we observed during the application of AZ. Further experiments using GABAergic and glycinergic drugs will be necessary to unravel the inhibitory pathways modulated by mGluR8. Nonetheless, our results indicate that the retina is a good preparation to compare the effects of orthosteric and allosteric drugs.

5. Conclusion

The mGluR8 orthosteric agonist DCPG and the allosteric modulator AZ differentially modulate the light responses of retinal ganglion cells. The effects of AZ are observed under bright, but not dim, light stimuli, whereas those of DCPG are less dependent on stimulus intensity. This is likely due to bright stimuli promoting the synaptic release of higher levels of glutamate that can reach mGluR8 receptors not located close to the release site. Both excitatory and inhibitory currents are modulated by mGluR8 stimulation, likely reflecting the presence of mGluR8 receptors presynaptic to glutamate and glycinergic release sites and their modulation of reciprocal feedback inhibition.

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Highlights

- mGluR8 agonist and allosteric modulator differentially modulate ganglion cell light responses
- Effects of AZ12216052 are observed under bright, but not dim, light stimuli
- Both excitatory and inhibitory currents are modulated by mGluR8 stimulation

Fig. 1.

Effects of AZ and DCPG on the light responses of an ON transient ganglion cell under bright and dim light stimulation. **(A)** The top and bottom panels show the respective excitatory and inhibitory responses to a bright spot of light. For each panel, the black line represents the baseline light-evoked response. The blue line represents the response during exposure to 10 μ M AZ. The amplitude of the excitatory light response increased. The green line represents the response during exposure to 1 μ M DCPG. DCPG did not affect the excitatory but reduced the inhibitory ON component of the response. **(B)** The top and bottom panels show the respective excitatory and inhibitory light responses evoked by a dim spot of light. Both components of the response to the weak stimulus were unchanged under AZ (blue lines). For this cell, 1 μ M DCPG did not significantly affect the amplitude of either the inhibitory or the excitatory ON component of the response (green line).

Fig. 2. Whole-cell recordings from an OFF transient ganglion cell alternately responding to a bright (A) and a dim (B) spot of light applied onto the center of its receptive field. Excitatory (top traces) and inhibitory (bottom traces) currents were recorded by clamping the cell at the chloride and cation reversal potentials, respectively. For the bright stimulus, when 10 μ M AZ (blue line) was bath applied to the cell, the amplitude of the OFF inward current increased while becoming more transient and the amplitudes of the ON and OFF outward currents also increased. The responses to the dim stimuli were not modulated by AZ (right panels). In the presence of 1 μ M DCPG, light-evoked responses to both the bright and dim stimuli were nearly eliminated (green lines).

Fig. 3. Whole-cell recording of the responses an ON-OFF-ganglion cell stimulated by a small spot of light onto the center of its receptive field. Excitatory (top panel) and inhibitory (bottom panel) currents were recorded by clamping the cell at the chloride and cation reversal potentials, respectively. During exposure to 10 μ M AZ, the peak excitatory ON and OFF currents were larger (blue line). The inhibitory current evoked by light onset was also increased.

Fig. 4.

Summary comparisons of the effects of AZ and DCPG on the light-evoked currents in ganglion cells (A–C) and of the light-evoked currents in ganglion cells responding to bright and dim light stimuli during exposure to AZ (D–F). **A:** In ON-transient ganglion cells, 10 μ M AZ (blue bars) enhanced the amplitude of the excitatory peak ON current compared to normalized baseline (100 %, indicated by **a**). 10 μ M AZ did not have a significant effect on the excitatory peak OFF current or on the inhibitory ON and OFF currents. In contrast to AZ, DCPG (green bars) had no significant effects on the excitatory or inhibitory currents of ON-ganglion cells compared to baseline. **B:** In OFF-transient ganglion cells exposed to AZ and DCPG, 10 μ M AZ caused a large increase in OFF excitatory current (**c**) and showed a tendency to increase the OFF inhibitory currents (**d**). In contrast, 1 μ M DCPG decreased ON and OFF excitatory currents (**b**). **C:** Light-evoked currents in ON-OFF-transient ganglion cells exposed to AZ and DCPG. 10 μ M AZ enhanced the peak and ON and OFF excitatory currents (**e**). AZ also enhanced the ON inhibitory current (**g**) and caused a slight enhancement of the OFF current. The effects of 1 μ M DCPG were to decrease ON and OFF excitatory currents (**f**), and had a tendency to decrease ON and OFF inhibitory currents. **D:** In ON-ganglion cells, AZ increased the excitatory ON current for bright stimuli (blue bars; **h**) but had very little effect for dim stimuli (black bars). **E:** In OFF-ganglion cells responding to bright and dim stimuli, AZ increased the OFF excitatory current evoked by

the bright stimulus (i) but had very little effect on the current evoked by the dim stimulus. **F:** In ON-OFF-ganglion cells responding to bright and dim stimuli during exposure to AZ, the average enhancement of the light-evoked response under AZ was greater for brighter stimuli. For dim stimuli, AZ only marginally affected the ON and OFF responses.

Fig. 5.

A: Confocal images of a transversal section through the inner retina, including the outer plexiform layer (OPL) and the 5 sublaminae of the inner plexiform layer (IPL). The section was double-labeled with a guinea pig antiserum against VGLUT1 (left panels) and a mouse monoclonal antibody against mGluR8 (center panels). Merged images, shown in the right panels, show that VGLUT1 and mGluR8 are mostly not co-localized although some VGLUT1-labeled bipolar cell terminals appear co-labeled for mGluR8 in sublaminae 2 and 4. The red labeling in the OPL is from the secondary anti-mouse antibody reacting to endogenous IgG in the retinal vasculature. **B:** IPL sublaminae 2 through 4, immunolabeled as in A, are shown at higher magnification. Arrowheads point to mGluR8 puncta that appear co-localized with VGLUT1-labeled bipolar cell terminals. **C:** Schematic representation of the retina. **6** indicates the localization of mGluR6 in ON (white cell soma)-bipolar cell (ON-BC) dendrites, postsynaptic to photoreceptor (PhR) glutamatergic release sites (blue presynaptic terminals). **a, a'** represent the location of mGluR8 receptors in OFF and ON-bipolar cell terminals where they would provide negative feedback on glutamate (blue) neurotransmitter release. **b, c** indicate possible sites of mGluR8 heteroreceptors at inhibitory (red) synapses, providing reciprocal and lateral feedback inhibition, respectively. **d** indicates mGluR8 locations on amacrine cell processes, where the presence of a nearby active glutamatergic terminal could modulate inhibitory transmission onto ganglion cells. AC, amacrine cells, OFF-GC, ON-GC, OFF- and ON-ganglion cells; INL, inner nuclear layer, GCL, ganglion cell layer.