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

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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.

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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, 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 postsynaptic 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

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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 CO_2 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% O_2 and 5% CO_2 (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 μm, 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× 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 μM. Its EC_{50} is ~1 μM (Duvoisin et al., 2010). DCPG was obtained from Tocris Bioscience (Ellisville, MO) and used at 1 μM, as previously (Quraishi et al., 2010). Its EC_{50} is ~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 MΩ. 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 to 7 MΩ.

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 improves 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 min 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 positioned to optimally activate the center of the receptive field. In each experiment, ganglion cells were usually stimulated at varying contrasts. Contrast is defined as $contrast = 100\% (F - B)/B$, where F and B represent foreground and background illumination, respectively. Stimuli were given on a constant gray background of $B = 50$ 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 min 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 min, 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

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