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Selective activation of mGluR8 receptors modulates retinal ganglion cell light responses

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Abstract

Extracellular and whole-cell light-evoked responses of mouse retinal ganglion cells were recorded in the presence of the mGluR8 selective agonist, *(S)*-3,4-dicarboxy-phenylglycine (DCPG). Off-light responses were reversibly reduced in the presence of DCPG in wild-type but not in mGluR8-deficient retinas. On-responses were only marginally modulated by DCPG. During Off-responses, DCPG suppressed both excitatory and inhibitory synaptic conductances suggesting that mGluR8 receptor activity reduces glutamate release from bipolar cell terminals and possibly also the release of an inhibitory neurotransmitter from amacrine cell processes.

Keywords

Retina; metabotropic glutamate receptor

Glutamate is the main excitatory neurotransmitter in the retina mediating transmission from photoreceptors to bipolar cells, and from bipolar cells to amacrine and ganglion cells. It exerts its effects via ionotropic and metabotropic receptors, thus serving multiple roles in visual signal processing (for reviews see Brandstätter et al., 1998; Thoreson and Witkovsky, 1999). Ionotropic glutamate receptors (NMDA, AMPA, and kainate subtypes) are ligand-gated ion channels that activate non-selective cation channels (Hollmann and Heinemann, 1994). Metabotropic glutamate receptors (mGluRs) can be either pre- or postsynaptic, and modulate G protein-mediated intracellular second messenger cascades that elicit diverse effects on neuronal function (Pin and Duvoisin, 1995).

The mGluRs are classified into three groups based on sequence homology, second messenger coupling and pharmacological selectivity (Conn and Pin, 1997). Group-I receptors (mGluR1 and 5) are coupled to the stimulation of phospholipase C, whereas group-II (mGluR2 and 3) and group-III (mGluR4, 6, 7, and 8) receptors are coupled to the inhibition of adenylyl cyclase

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in transfected cells. Of the eight different mGluR subtypes currently known, all but mGluR3 are expressed in rodent retina (Duvoisin et al., 1995; Hartveit et al., 1995).

The best functionally characterized of the retinal mGluRs is the group-III receptor mGluR6, which occurs postsynaptically at the dendritic tips of On-bipolar cells (Nomura et al., 1994). The mGluR6 receptors transduce the light-evoked hyperpolarization of photoreceptors into a depolarizing response at bipolar cells by gating an excitatory cation channel (Slaughter and Miller, 1981; Shiells and Falk, 1990; Nawy and Jahr, 1991). The compound, *L*-2-amino-4-phosphonobutyrate (*L*-AP4, also *L*-APB), is a selective agonist of group-III mGluRs and its activation of mGluR6 has been widely used to selectively block the On-pathway (Massey et al., 1983; Schiller et al., 1986; Bodnarenko and Chalupa, 1993; Kittila and Massey, 1995). However, *L*-AP4 will activate another retinal group III mGluR receptor, the mGluR8 (Duvoisin et al., 1995), which is expressed at synapses throughout the inner plexiform layer (IPL, Tagawa et al., 1999; Koulen and Brandstätter, 2002; Quraishi et al., 2007). The functional role of mGluR8 receptors in the retina has not been characterized, but they are known to modulate calcium currents in sympathetic neurons (Guo and Ikeda, 2005). In this study we use the selective mGluR8 agonist, *(S)*-3,4-dicarboxy-phenylglycine (DCPG) to examine of the physiological role of mGluR8 in mammalian retina. In artificial expression systems, DCPG is at least 100-fold more potent at cloned mGluR8 receptors (EC_{50}= 30 nM) as compared with other group-III mGluRs (EC_{50} of 8.8, 3.6, and >100 μM for mGluR4, 6 and 7, respectively, Thomas et al., 2001). *In vitro* studies have shown that DCPG depresses glutamate transmission at afferents of various CNS regions, such as spinal cord, superior colliculus, lateral perforant path of the hippocampus, supraoptic nucleus of the hypothalamus, and periaqueductal grey (Cai et al., 2001; Thomas et al., 2001; Pothecary et al., 2002; Zhai et al., 2002; Panatier et al., 2004; Marabese et al., 2005). Here, we report the effects of DCPG on physiologically elicited responses in retinal ganglion cells (RGCs) in the mouse retina.

EXPERIMENTAL PROCEDURES

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 (Charles River, Wilmington, MA) and congenic mGluR8$^{-/-}$ mice (Duvoisin et al., 2005) were fed and housed under a 12 hour light/dark cycle. Experimental animals were dark-adapted for at least 1 hour prior to experimentation. 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 anaesthetized with an intraperitoneal injection of sodium pentobarbital (300 mg/kg) and enucleated following cervical dislocation. The cornea, lens and vitreous body were excised under infrared illumination (900 nm) 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. The retina was dissected from the pigment epithelium, flattened by making 3 radial cuts at its outer edge, then placed, with ganglion cells facing up onto a poly-L-lysine (Sigma-Aldrich) coated coverslip. The coverslip was then mounted into a custom recording chamber and continually perfused with 35°C oxygenated and bicarbonate-buffered Ames' medium (3 ml/min) for the duration of the experiment.

Physiological recordings

Cells were visualized under infrared DIC optics on an upright microscope (Olympus, Center Valley, PA) fitted with a 40x 0.8NA water-immersion objective. Extracellular recordings were made by pushing a glass microelectrode (5–7 MΩ) against a ganglion cell somata in a loose-patch configuration. The electrodes were filled with the bath solution. For intracellular recording, the electrodes were filled with a solution comprising: 110 mM Cs-gluconate or CsCl,

10 mM NaCl, 5 mM Na-HEPES, 1 mM Cs-EGTA, 1 mM Na-ATP, 0.1 mM Na-GTP, and 10 mM QX-314 (Sigma-Aldrich). 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. The 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.

Ganglion cells were stimulated using images displayed on a computer monitor using custom software incorporated into Igor Pro (Wavemetrics, Eugene, OR), and focused via the microscope objective onto the photoreceptors. The 20x objective illuminated an area covering approximately 1mm in diameter on the retina. Two hundred micron diameter dark and bright spots, centered on the cell soma, were flashed on a constant grey background. The background light level was sufficient to ensure that the retina was operating in the mesopic or low-photopic range. Light responses were measured before, during, and after administration of drug *L*-AP4 or *S*-DCPG (Tocris, Ellisville, MO). Drugs were bath applied and allowed to washout completely. Typical drug treatments lasted 3–5 minutes, and the washout period generally followed for 5–10 minutes.

Light evoked synaptic currents were recorded in the whole-cell configuration using the intracellular medium described above. The membrane potential was adjusted by −10mV 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 low-pass filtered at 2 kHz (−3 dB, 4-pole Bessel) and digitized at 5–10 kHz. Light-evoked synaptic conductances were calculated as described in detail elsewhere (Taylor and Vaney, 2002). Briefly, we assumed that the light-evoked synaptic inputs comprised two components: an excitatory component due to activation of non-selective cation channels having a reversal potential, V_e = 0 mV, and an inhibitory component with a reversal potential, V_i, at the chloride equilibrium potential of approximately −60 mV. Current-voltage (*I-V*) relations of the net light-evoked synaptic currents were measure at 9 holding potentials at equal increments between −100 mV and +20 mV every 10 msec for the duration of the synaptic responses. The excitatory and inhibitory components were derived from the slope and reversal potential of the *I-V* relations.

RESULTS

DCPG attenuates Off-responses

DCPG is a member of the pharmacological class of phenylglycines that selectively activates mGluR8 receptors (Thomas et al., 2001). We tested the effects of bath-applied DCPG (1–3 μM) on three broad classes of RGCs: Off-RGCs, which respond to decreases in light intensity, On-RGCs which respond to increases in intensity, and On-Off RGCs, which produce transient spiking responses to both increases and decreases in light intensity.

The cell shown in Fig. 1A had a low background firing rate and a brisk sustained response to the presentation of a dark spot stimulus centered on the receptive field. DCPG (1 μM) increased the baseline firing level, and suppressed the light response. Note that the transient suppression of baseline firing, at the end of the stimulus (the On-response) is not blocked by the DCPG. The effects of DCPG were rapidly reversible upon washout of drug. In fourteen Off-cells, bath-application of 1 μM DCPG reversibly attenuated the peak light responses to 52±15% of the control response (Fig. 1C). Increases in baseline firing observed in this heterogeneous group of cells were not systematic, and are not quantified further. DCPG failed to have any effect in similar recordings from Off-RGCs in mGluR8-deficient mice, (Fig. 1B,C; n=6, 99±4% of control), confirming the specificity of DCPG for mGluR8 receptors. No other physiological or anatomical differences were found to date in the retina of mGluR8$^{-/-}$ mice. In some Off-cells from wild type mice, DCPG treatment appeared to reversibly unmask a late On-burst approximately 800 msec after the end of the Off-stimulus (Fig. 1D, middle panel).

The effects of DCPG on On-RGCs were less systematic. In two On-cells, DCPG reduced a sustained phase of the light response and suppressed background firing (Fig. 1E), while in 5 other cells there were no consistent effects.

Two groups of On-Off RGCs could be distinguished; those that were sensitive to the direction of stimulus motion, On-Off-DSGCs, and those that were not. Both groups of On-Off-GCs responded transiently at the onset and termination of a static spot stimulus (Fig. 2A). In the presence of 3 μM DCPG, the Off-response was reversibly suppressed, while the On-response was largely unaltered (Fig. 2B). In eleven cells, the number of spikes generated by the Off-stimulus was reduced to 14±13% of control (Fig. 2C). Slight increases and decreases in the number of On-response spikes were observed in the presence of DCPG, however, overall there was little effect (89±14% of control, Fig. 2C).

DCPG reduces RGC Off-responses via a presynaptic mechanism

The changes in spiking produced by DCPG presumably result from modulation of synaptic transmission within the IPL where mGluR8 receptors are localized (Quraishi et al., 2007). The spike recordings are consistent with a number of possible synaptic mechanisms. For example, DCPG could suppress Off-excitation or it could potentiate Off-inhibition. To test for presynaptic versus postsynaptic effects we measured synaptic currents over a range of holding potentials, and calculated the excitatory and inhibitory synaptic conductances underlying the responses. Examination of the On-Off RGCs allowed the On- and Off-pathways to be compared within the same cell under the same experimental conditions.

Stimulation of an On-Off RGC with a static dark spot produced transient synaptic currents at the onset and termination of the stimulus (Fig. 3A). During DCPG application (Fig. 3A, center) the synaptic currents associated with the Off-response are reduced, while there was much less effect on the On-responses. There also appeared to be a reduction in the spontaneous synaptic inputs to the cell. The currents and spontaneous activity recovered upon washout. The synaptic responses could be accounted for by a mix of excitatory and inhibitory synaptic conductances (Fig. 3B). DCPG completely suppressed the Off-inhibition, and suppressed the peak Off-excitation to ~30% of control (Fig. 3B, middle panel). In contrast DCPG had little effect on the On-excitation seen at the termination of the dark spot stimulus in Fig. 3B, but did cause a partial suppression of the peak On-inhibition to ~ 57% of control (Fig. 3B, middle panel). In three cells the peak Off excitation and inhibition were reduced to 39±9% and 12±6% of control levels, while the corresponding values for the On-responses were 101±8% and 56±17%. These results show that activation of mGluR8 receptors can suppress both the excitatory and inhibitory synaptic inputs to the RGCs, and that the reduced spiking is at least partially attributable to changes in the synaptic drive to the cells.

Intravitreal injections of *L*-AP4 *in vivo* and superfusion of *L*-AP4 onto retinal explants *in vitro* have been widely used to specifically block the On-pathway. However, *L*-AP4 should also stimulate the group-III mGluR4 and mGluR8 receptors (Wu et al., 1998). Thus similar to the effect of DCPG, *L*-AP4 stimulation of mGluR8 should attenuate RGC Off-response. But low doses of *L*-AP4 produce effects unlike those of DCPG. In the experiment shown in Supplemental Fig. 1, a light spot was flashed onto the cell's excitatory receptive field. As expected, On-responses were inhibited under *L*-AP4. In contrast, the effects on the Off-response varied. Indeed for On-Off-RGCs, we often observed an increase in the Off-response, similar to the findings of Arkin and Miller (1988) and Kittila and Massey (1997). Occasionally at lower doses, we sometimes saw a decrease in the light-Off response. Thus it appears that the effect of *L*-AP4 on the On-response is more predictable than its expected effect on the Off-response, which is mediated by other group-III mGluRs in the inner retina.

DISCUSSION

DCPG, a selective mGluR8 agonist, attenuated the spike activity associated with Off-responses in Off-center and On-Off RGCs, suggesting a functional role for mGluR8 receptors in the Off-pathway of the mouse retina. Extracellular recordings of On-responses were subject to mild modulation, but the effects were variable in magnitude. Voltage-clamp recording of synaptic currents in RGCs indicated that Off-responses were attenuated, due to a decrease in both inhibitory and excitatory conductances during DCPG application. Further pharmacological analyses will be necessary to determine the identity of the inhibitory components that were suppressed by DCPG.

The depression of Off-responses by DCPG is consistent with a presynaptic expression of mGluR8 in Off-bipolar cell terminals, where the receptor could suppress neurotransmitter release by decreasing calcium influx into the synaptic terminals. This suggestion is in line with physiological studies showing a modulation of glutamate release from Off-bipolar cells by the group-III agonist *L*-AP4 (Awatramani and Slaughter, 2001; Higgs et al., 2002). However, although immunohistochemical analyses have shown a uniform distribution of mGluR8 in both On- and Off-sublaminae of the IPL (Tagawa et al., 1999; Quraishi et al., 2007), On-responses were affected in a variable and unpredictable way by DCPG. This suggests that the functional specificity of mGluR8 receptor activity differs in the On- and Off-sublaminae. Interestingly, DCPG unmasked a late On-burst, which was also sometimes seen in control conditions and was initiated between 600–800 msec following the short latency On-response. These late On-responses are reminiscent of the long-latency On-responses observed by Renteria et al. (2006) in mGluR6-null mice. The mGluR8 mediated modulation of the On-pathway will require further analysis in the presence of DCPG and GABAergic drugs to determine whether mGluR8 effects are mediated by polysynaptic interactions.

On-Off ganglion cells

On-Off RGCs have been characterized extensively in rabbit (Barlow and Levick, 1965; Amthor et al., 1984; Taylor et al., 2000) and to a limited extent in mouse (Sun et al., 2002; Weng et al., 2005). On-Off RGCs were targeted for additional analysis because they allow simultaneous examination of the On- and Off-pathways within the same cell. The effects of the mGluR8 agonist in the On-pathway is complicated by the presence of mGluR6 receptors at the photoreceptor to On-bipolar cell synapse. Activation of mGluR6 receptors in the outer plexiform layer results in a complete and reversible blockade of On-response, an effect that has been extensively studied using *L*-AP4 (DeVries and Baylor, 1995; Kittila and Massey, 1995). Although DCPG is reported to be 100-fold more selective for mGluR8 over mGluR6 receptors in artificial expression systems (Thomas et al., 2001), the relative doses for an *in vitro* retinal preparation are unknown, which makes the presence of the On-response an important control when assessing mGluR8 activation. We found that DCPG attenuated Off-responses while On-responses were less affected. However, the analysis of the synaptic conductance during On-responses suggests that changes in the synaptic drive may not always become evident in the spiking responses. It is possible, however, that more subtle changes in the On-pathway inputs would become evident in the spiking responses under specific stimulus conditions.

The effects of DCPG on the On-Off RGCs are difficult to reconcile with some previous work. Kittila and Massey (1995) found that On-responses in rabbit On-Off direction-selective RGCs are completely suppressed by *L*-AP4 with little effect on the Off-responses. These results appear to be inconsistent with the present data because the efficacy of *L*-AP4 is similar on mGluR4, mGluR6, and mGluR8 in pharmacological assays on transfected cells (EC_{50} = 0.32, 0.055, and 0.06 μM for mGluR4, 6, and 8, respectively, Thomas et al., 2001), and therefore application of *L*-AP4 is expected to suppress the Off-responses through activation of the

mGluR8 receptors. How then is the Off-response preserved, and in some cell types even potentiated (Popova et al., 2003), when both mGluR6 and mGluR8 are activated by *L*-AP4? One possibility is that activation of mGluR6 receptors suppresses signaling through the On-pathway, and thus reduces the excitatory drive to amacrine cells that provide cross-over inhibition from the On to the Off-sublaminae of the IPL (Wässle et al., 1986; Cohen, 1998; Zaghloul et al., 2003; Manookin et al., 2008). Perhaps this reduction in cross-over inhibition within the IPL offsets the presynaptic suppression of the Off-pathway that is mediated when the *L*-AP4 simultaneously activates the mGluR8 receptors. Consistent with this explanation are reports of potentiated spontaneous activity in Off-center ganglion cells upon *L*-AP4 application (Massey et al., 1983; Arkin and Miller, 1988; Cohen and Miller, 1994; Popova et al., 2003). Thus, *L*-AP4 appears to produce several physiological effects, likely due to activation of multiple group-III mGluRs (mGluR4, 6, 8, and even 7 at high concentration), and therefore caution must be exercised in interpreting experiments using *L*-AP4 in the retina.

mGluR8 as a heteroreceptor

In the IPL, mGluR8 is both pre- and postsynaptic to bipolar cell terminals (Koulen and Brandstätter, 2002). The presynaptic localization is consistent with our finding of a reduced excitation of ganglion cell light Off-responses upon DCPG application, and with observations that *L*-AP4 reduces glutamate transmission at Off-bipolar terminals (Awatramani and Slaughter, 2001; Higgs et al., 2002). However, our previous immunohistochemical analysis did not indicate a co-localization of mGluR8 with markers of bipolar cell terminal ribbons, suggesting that mGluR8 may be located perisynaptically, slightly away from glutamate release sites (Quraishi et al., 2007).

While it is possible that the postsynaptic localization of mGluR8 receptors corresponds to their presence in ganglion cell dendrites, we did not observe changes in ganglion cell holding currents, following DCPG application. Alternatively, mGluR8 receptors could be present in amacrine cell processes, where they could modulate GABA or glycine release. The finding that ganglion cell inhibitory conductances are depressed by DCPG supports a role for mGluR8 in regulating inhibitory neurotransmitter release by amacrine cells. Though not yet directly observed in the retina, such heterosynaptic modulation of GABAergic neurotransmission by mGluR8 has been observed in the brain (Semyanov and Kullmann, 2000; Kogo et al., 2004; Marabese et al., 2005). In the retina, mGluR8 is in a position to modulate the activity of GABAergic cells, including at reciprocal synapses that could control bipolar cell output (Tachibana and Kaneko, 1987; 1988). In that situation, glutamate release from bipolar cells may be enhanced by an mGluR8-mediated disinhibition of bipolar cell terminals.

In conclusion, our results raise the possibility that mGluR8 receptors are involved in the modulation of synaptic transmission at two sites within the retina; as autoreceptors that are activated by glutamate released from the bipolar cells themselves, and on amacrine cell terminals where they are activated by bipolar cell inputs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

L-AP4	*L*-2-amino-4-phosphonobutyrate
DCPG	*(S)*-3,4-dicarboxy-phenylglycine
IPL	inner plexiform layer
mGluR	metabotropic glutamate receptor
RGC	retinal ganglion cell

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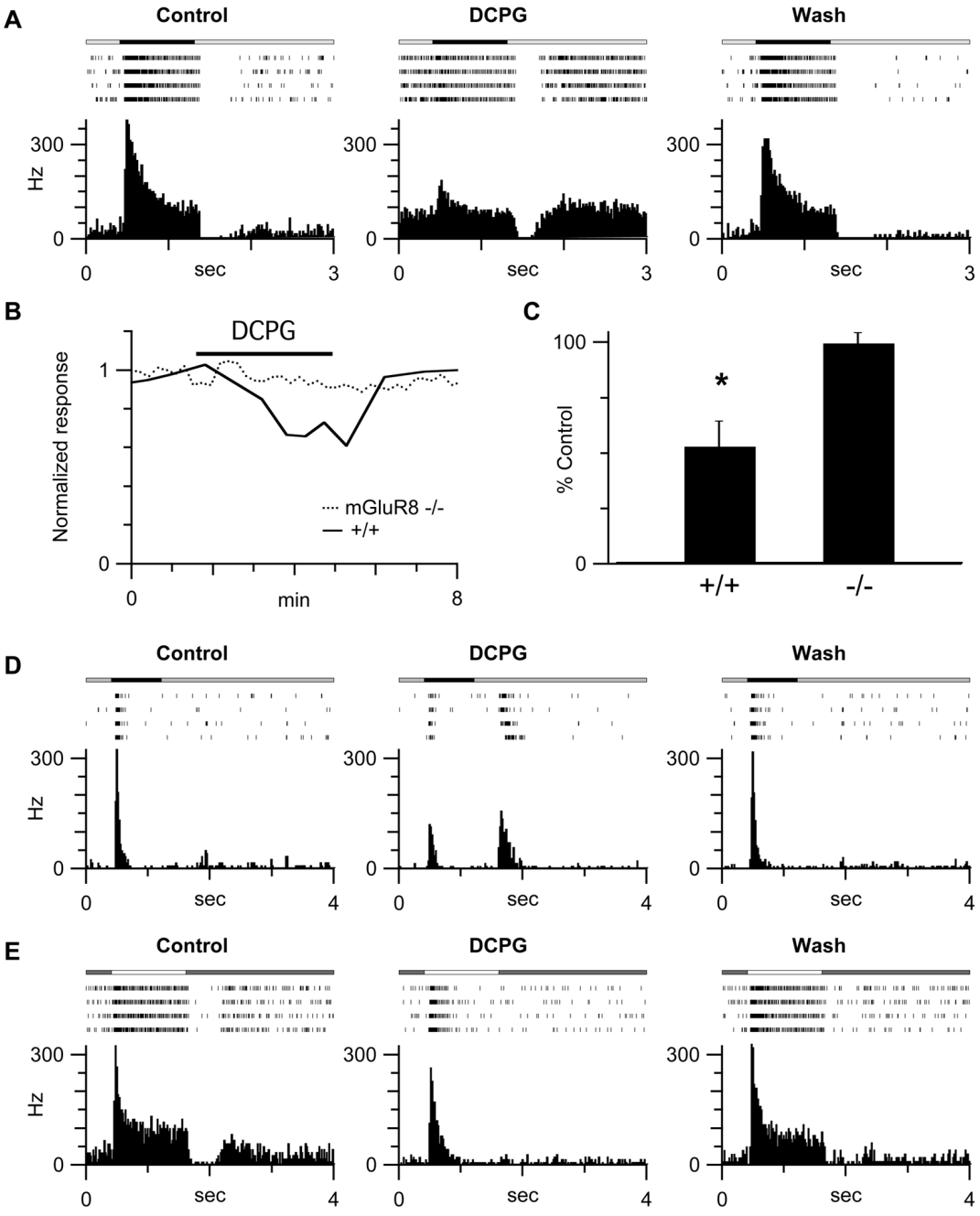

Fig. 1.

DCPG attenuates Off-response in an Off-RGC. (A) Raster plots and average peristimulus-time histograms (7 stimuli) depicting the extracellular spike response of an Off-brisk-sustained ganglion cell during a dark spot. The brisk component is suppressed by 1 μM DCPG. (B) Time-course of DCPG-attenuation of Off-spikes in a wild type (+/+; solid line) and mGluR8-deficient (−/−; dotted line) Off-RGC. The duration of DCPG application is given by the black bar, and only the wild type RGC shows spike attenuation during this period. (C) Population summary of DCPG-induced attenuation of light responses in Off-cells in wild type (+/+; n=14) and mGluR8-deficient (−/−; n=6) retinae. Data is given as percent of control spikes. (D) Raster plots and average peristimulus-time histograms (6 stimuli) depicting the extracellular spike

response of an Off-transient ganglion cell during a dark spot. DCPG depresses the Off-response and unmasks a late On-response. (E) Raster plots and average peristimulus-time histograms (6 stimuli) depicting extracellular spike responses of an On-brisk-sustained ganglion cell during a bright spot. DCPG decreased the sustained component of the light response. In A, D, and E the durations of the stimuli are indicated by the timing bars above raster plots. In each case the raster plots show 4 sequential stimuli.

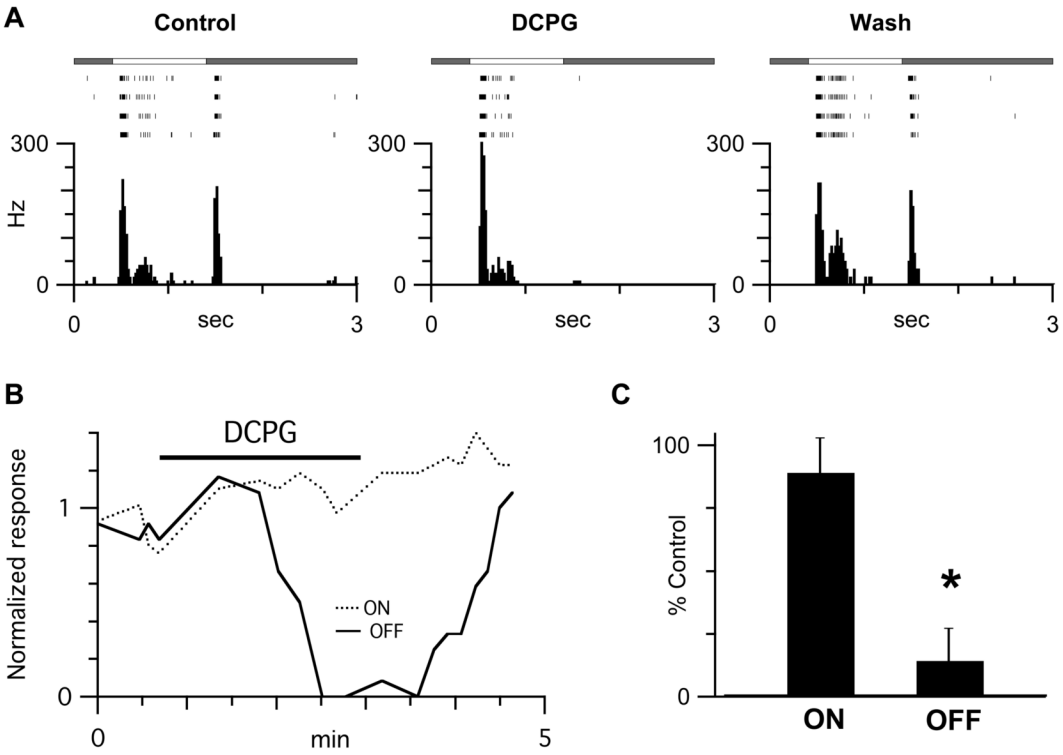

Fig. 2.

DCPG attenuates the Off-response in an On-Off RGC. (A) Extracellular spike activity of an On-Off cell is shown in response to a light stimulus. Conventions are as in Fig. 1. DCPG specifically suppresses the Off-response. (B) Time-course of the DCPG effect on the On-burst (dotted line) and Off-burst (solid line) for the cell in A. (C) Population summary of DCPG-mediated attenuation of On-and Off-responses in On-Off RGCs (n= 11).

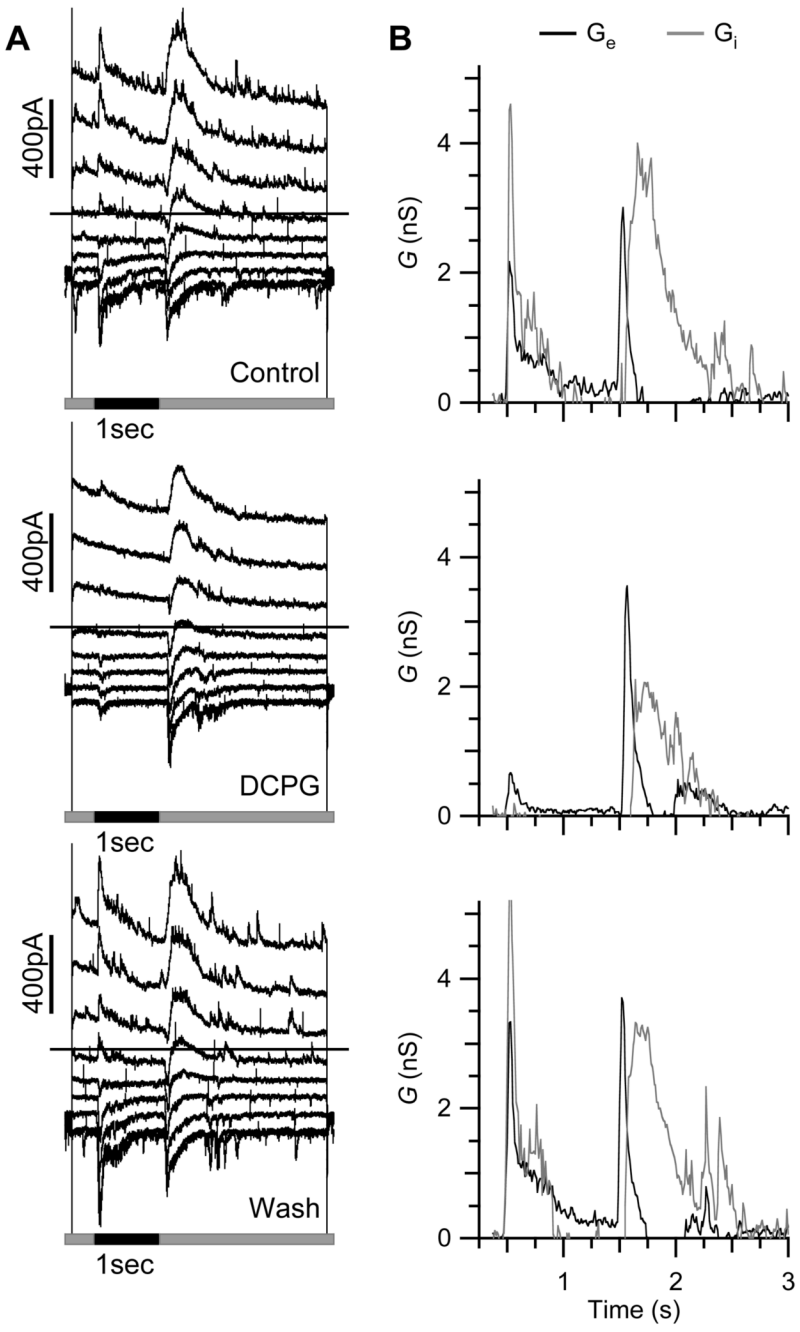

Fig. 3.

Light-evoked synaptic conductances from an On-Off RGC in the presence of DCPG. (A) Currents in response to light stimulation during voltage steps from −100 to +20 mV, in 15 mV increments. The light stimulus was a 200 μm diameter black spot centered on the receptive field of the cell. The light stimulus timing is shown by the bars beneath the traces. (B) Net inhibitory (G_i, grey), and excitatory (G_e, black) conductance components calculated for the data in A.