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Metabotropic glutamate receptor-mediated hetero-synaptic interaction of red- and green-cone inputs to LHC of carp retina

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Abstract

The role of presynaptic metabotropic glutamate receptor (mGluR) on the interaction of red- and green-cone signals was investigated in luminosity-type horizontal cell (LHC) of isolated carp retina. It was found that a dim red background could enhance LHC's light response to green stimulus, and a dim green background was also able to increase the cell's response to red flash. Such mutual color enhancement was eliminated by application of groups II and III mGluR antagonist (*S*)-methyl-4-carboxyphenyl-glycine (MCPG). Furthermore, inhibition of glutamate uptake by using D-aspartate (D-Asp) or DL-threo-β-hydroxy-aspartic acid (THA) completely blocked the mutual enhancement of color signals in LHC. However, the GABAergic feedback pathway in the outer retina was unnecessarily involved.

Keywords: Retina; Luminosity-type horizontal cell; Mutual color enhancement; Metabotropic glutamate receptor; Glutamate transporter

1. Introduction

In teleost fish retinas, luminosity-type horizontal cell receives excitatory glutamatergic synaptic inputs from both red- and green-sensitive cone photoreceptors while provides negative feedback on various cone photoreceptors [11,22]. In the dark, photoreceptors continuously release glutamate, which depolarizes luminosity-type horizontal cell (LHC). Light hyperpolarizes the photoreceptors and reduces their glutamate release, which results in a hyperpolarizing response in LHC [5]. Additionally, it has been shown that glutamate transporter at cone photoreceptors is voltage dependent and robust enough to modulate the concentration of glutamate in the synaptic cleft and thus plays a significant role in adjusting the dynamics of LHC's light response [7].

It has been reported that there existed interaction of red- and green-cone signals in LHC [16], which was described as an increase of LHC's light response to green flash in the presence of a dim red background light, and vice versa. Kamermans et al. [11] suggested that symmetric sign-inverted feedback synapses from LHC to both red- and green-cone photoreceptors could mediate such mutual color enhancement. On the other hand, the engagement of an asymmetric negative feedback from LHC to green cone photoreceptors alone was proposed, based on the finding that only a brief leading green flash could enhance LHC's responsiveness to the successive red flash but not vice versa [22]. However, none of the hypothetical models were experimentally examined. Furthermore, Umino et al. [19] reported that the response enhancement was not affected by application of GABA. Nevertheless, the exact physiological mechanisms underlying this process still remain elusive.

A large number of evidence supported that metabotropic glutamate receptors (mGluRs) found at the presynaptic terminals are important in fine tuning of the synaptic transmission [1]. Activation of mGluRs as an autoreceptor may depress neurotransmitter release from the presynaptic neuron, and this inhibitory action would modulate the synaptic output to the postsynaptic neuron [2]. In addition, glutamate may diffuse or spillover from the synaptic cleft and activate the presynaptic mGluRs at the adjacent synapse, which would mediate the crosstalk of neighboring synapses, for example the hetero-synaptic depression observed in cerebellum [15]. The inhibition may be facilitated by glutamate

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transporter blockers or abolished by mGluR antagonists [18]. Recently, several lines of evidence demonstrated the existence of mGluRs at the terminals of photoreceptors in the vertebrate retina, which was suggested to provide a negative feedback mechanism for regulating glutamate release from photoreceptors [9,12].

In the present study, we initially confirmed that a dim red background light greatly enhanced the response amplitude of LHC to green test flash, and vice versa. We then found that such mutual color enhancement was related to the activation of mGluRs located at the presynaptic terminals of cone photoreceptors. Blockade of glutamate transporter completely eliminated the mutual enhancement of color signals in LHC, as expected. However, the GABAergic feedback pathway in the outer retina seemed unnecessary.

2. Materials and methods

Experiments were performed on adult carp (*Carassius auratus*, body length 15–20 cm) retinas following the procedure similar to the previous report [10]. All procedures strictly conformed to the humane treatment and use of animals as prescribed by the Association for Research in Vision and Ophthalmology. Briefly, the fish was raised in aerated aquarium on a natural light/dark cycle. After 15–25 min of dark adaptation, the fish was decapitated to minimize the animal's pain and discomfort and eyes were enucleated and hemisected. The retina was carefully isolated from the pigment epithelium and placed, with the photoreceptor-side up, on a filter paper. Immediately, the preparation was transferred into a chamber with a volume of 1.4 ml and superfused with oxygenated (95% O_2 + 5% CO_2) Ringer solution which contained (in mM): 116.0 NaCl, 2.4 KCl, 1.0 $MgCl_2$, 1.2 $CaCl_2$, 1.0 NaH_2PO_4, 30.0 $NaHCO_3$, and 10.0 glucose, with a pH value of 7.7 at room temperature. All drugs applied were purchased from Sigma (St. Louis, MO).

The test flashes were originated from two light-emitting diodes (LED) (RS Components Ltd., UK), intensities of which were under computer control via a D/A interface. The flashes were filtered with interference filters of 702/11 and 518/4 nm (peak wavelength/half wavelength width) which were referred to as red and green flash, respectively. The conditioning background light was generated from a 100 W tungsten halogen light source (Osram, Germany). Light with peak wavelengths of 701 and 504 nm, selected by interference filters, were referred to as red and green background, respectively, in this paper. Both the test and conditioning light stimuli were set to project an 8-mm-diameter diffuse light spot onto the retina. The light intensities were calibrated using a photo detector (IL 1400A, International Light, USA). Unattenuated intensity of 702 nm light at the retinal surface was 8.22×10^{13} quanta/(cm^2 s). In this paper, light intensity was given in terms of the log units of attenuation.

Intracellular recordings were made using an MEZ-8300 microelectrode amplifier (Nihon Khoden, Japan). The microelectrode was pulled from glass micropipettes on a PD-30 puller (Narishige, Japan) and had a resistance of 50–100 MΩ when filled with 4 M KAc. An SD-7600 micromanipulator (SD Instrument, USA) was employed to advance the electrode into the cell. Cell's light response was monitored and displayed on an oscilloscope (TDS 210, Tektronix Inc., USA). Data were sampled via an Adlink 8111 A/D interface (Adlink, Taiwan) and stored on an IBM PC-compatible computer for off-line analysis. Student's *t*-test (paired, unless specified) was performed for statistical analysis of data. The statistics in this paper was given in mean ± S.D. form.

3. Results

LHC of fish retina receives excitatory synaptic inputs from both red- and green-sensitive cones, which interact nonlinearly [11,16]. As shown in Fig. 1A, the amplitude of LHC's response to a moderate green test flash (518 nm, $\log I = -2.03$) was 9.2 mV in the dark. In the presence of a dim red background light (701 nm, $\log I = -2.23$), the cell's membrane potential was hyperpolarized by 5.2 mV, and its response to the same green stimulus was conspicuously increased to 14.5 mV. The relative response amplitude (response amplitude under the background versus response amplitude during the dark) was 158%. Similarly, a dim green background illumination (504 nm, $\log I = -4.5$) slightly hyperpolarized the LHC by 2.8 mV and substantially enhanced the cell's responsiveness to a moderate red flash (702 nm, $\log I = -2.04$), from 9.3 to 12.7 mV (Fig. 1B), with the relative response amplitude being 137%. Coincided results were obtained from all the other cells examined. The relative percentages of enhancement induced by the red and green background were 157 ± 16.1% (mean ± S.D., $n = 27$) and 134 ± 12.6% ($n = 30$), respectively, both of which were statistically significant ($P < 0.05$, paired *t*-test), with

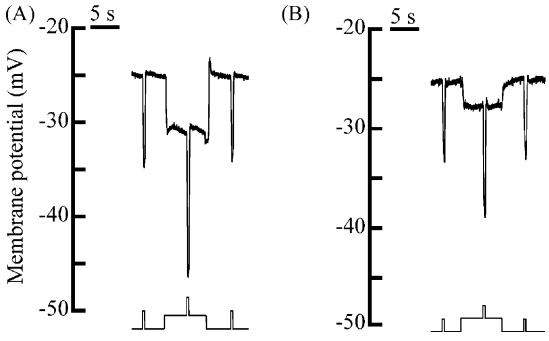

Fig. 1. The interaction of red- and green-cone signals in LHC. (A) LHC's response to green test light (518 nm, $\log I = -2.03$) before, during, and after the presence of a dim red background illumination (701 nm, $\log I = -2.23$). The dim red background hyperpolarized LHC and greatly enhanced the cell's response to the green flash. (B) LHC's response to red light (702 nm, $\log I = -2.04$) before, during, and after the presence of a dim red background light (504 nm, $\log I = -4.5$). Similarly, the dim green background light hyperpolarized LHC and remarkably increased the cell's red response.

the mean (±S.D.) values of hyperpolarization of the cell's membrane potential, induced by red and green background light, being 6.1 ± 3.70 and 2.4 ± 1.60 mV, respectively. Consequently, our results are in agreement with previous reports of mutual color enhancement [11,16].

Since the pharmacological experiments yielded similar results for the protocols of green test flash with red background illumination and red test flash with green background illumination, we will thus focus our pharmacological results on the red background-induced enhancement of LHC's response to green test flash in the subsequent parts of this report. The mechanism should also apply to the green background-induced enhancement of LHC's response to red stimulus.

Previous reports suggested that the negative feedback pathways from horizontal cell to various cone systems might mediate the interaction of different cone signals in LHC [11,22]. So we first examined the role of the GABAergic activity in the mutual color enhancement in LHC. Fig. 2A showed that the application of 4 mM GABA hyperpolarized LHC by about 3 mV and reduced its response amplitude to green flash from 8.5 to 6.7 mV. The enhancement effects of red background on the cell's responsiveness, before and during GABA perfusion, were 142 and 133%, respectively. In a total number of four cells investigated, significant enhancement of LHC's green response induced by the dim red background was found (150 ± 27.4%, $P < 0.05$, $n = 4$), during GABA perfusion, although the degree of enhancement is somewhat attenuated as compared to the observation made in normal Ringer solution (184 ± 34.1%, $n = 4$). On the other hand, administration of 200 μM picrotoxin depolarized LHC by 10 mV and increased its response amplitude from 6.7 to 8.1 mV (Fig. 2B), whereas exerted little effect on the red background-induced enhancement of LHC's green response. The relative response amplitudes were 169 and 156% before and during the application of picrotoxin. In a total number of four cells, significant enhancements of LHC's green response induced by the dim red background were found whether at the absence or presence of 200 μM picrotoxin (143 ± 19.5% versus 137 ± 16.0%, $P < 0.05$). Taken together, it seems unlikely that the GABAergic pathway was responsible for the signal enhancement.

To test whether or not the presynaptic mGluRs, existing at the terminals of cone photoreceptors [9,12], contribute to the mutual color enhancement in LHC, we investigated the effect of MCPG, an antagonist of groups II and III mGluRs [3]. As illustrated in Fig. 3, during normal Ringer solution, LHC's green response was increased, from 7.5 to 9.7 mV, in the presence of a dim red background illumination; the application of 500 μM MCPG depolarized the cell's resting membrane potential by about 5 mV, and reduced its green response amplitude to 5.5 mV. At the same time, the green flash induced a photoresponse of LHC by only 5.4 mV in the presence of red background illumination. Statistical result showed that the mean value of the response enhancement during the drug application was 103 ± 5.2%, which is significantly diminished as compared to that during control 124 ± 5.4% ($P < 0.05$, $n = 4$). Therefore, inhibition of presynaptic mGluRs eliminated the effect of the red background on LHC's green response.

It has been shown that there existed abundant glutamate transporters on the membrane of photoreceptors and Müller cells in the retina [6]. Blockade of glutamate uptake may promote glutamate spillover and hence facilitate the activation of presynaptic mGluRs [18]. We, therefore, investigated the effect of D-aspartate (D-Asp), a competitive blocker of

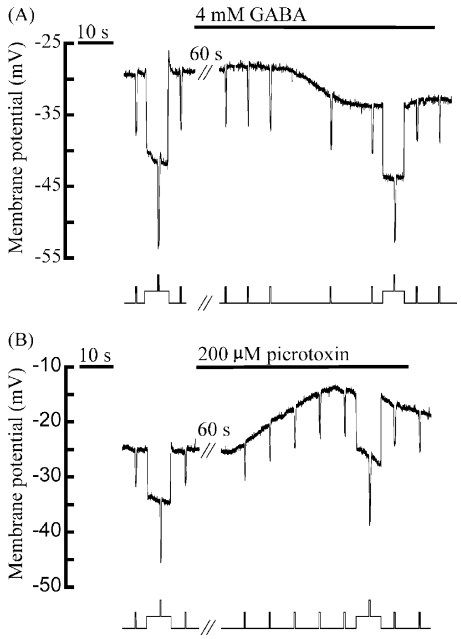

Fig. 2. The role of GABAergic activity on the red background-induced enhancement of LHC's green response. (A) Administration of 4 mM GABA induced a hyperpolarization in LHC and reduced its light response amplitude. However, the red background illumination could still enhance LHC's green response remarkably. (B) Administration of 200 μM picrotoxin depolarized LHC and increased the cell's light response whereas it had little effect on the enhancement of LHC's green response induced by the red background light.

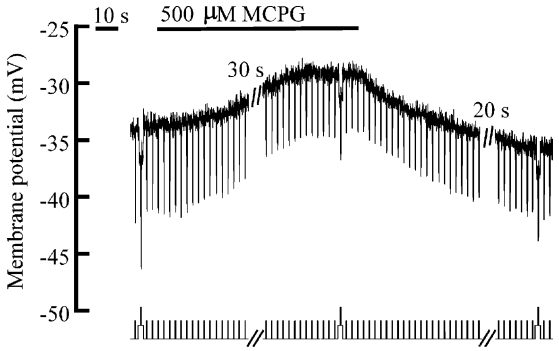

Fig. 3. Inhibition of mGluRs eliminated enhancement effect of red background light on LHC's green response. The perfusion of 500 μM MCPG depolarized the membrane potential of LHC and decreased its light response amplitude. Conspicuously, the red background light no longer elicited enhancement of LHC's green response.

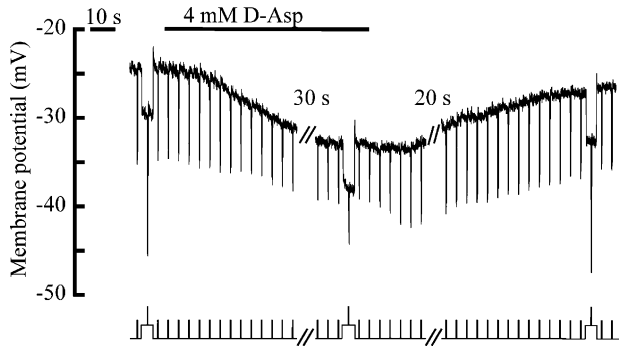

Fig. 4. Blockade of glutamate uptake interrupted the enhancement effect of red background on LHC's green response. In the presence of 4 mM D-Asp, LHC's membrane potential was hyperpolarized and the cell's light response amplitude was decreased. Concurrently, the red background-induced enhancement of LHC's light response to green flash was completely abolished.

glutamate transporter. As presented in Fig. 4, when retina was perfused using normal Ringer solution, LHC's response to the green flash was 10.0 mV in the dark and was increased to 15.7 mV under the dim red background light. The application of 4 mM D-Asp hyperpolarized the cell's resting membrane potential by about 8 mV, and reduced its green response amplitude to 6.7 mV. The green flash, imposing on the dim red background light, now induced LHC's response of only 6.2 mV. The relative response amplitudes were 157 and 92%, respectively, during normal Ringer and D-Asp perfusion. Significant difference was detected between the relative percentages of enhancement in normal Ringer and during the drug application ($148 \pm 15.6\%$ versus $103 \pm 12.0\%$, $P < 0.05$, $n = 6$).

Similar result was found while D-Asp was substituted with THA, another glutamate transporter blocker. The presence of 1 mM THA remarkably hyperpolarized the cell's resting membrane potential and obliterated the response enhancement induced by the dim red background. The color enhancement was remarkable ($160 \pm 12.6\%$) during normal Ringer and almost undetectable ($108 \pm 11.5\%$) during THA application, which was significantly different ($P < 0.05$, $n = 5$).

4. Discussion

The neural interactions of various photoreceptor inputs fed forward onto horizontal cells (HCs) have been investigated over the years, and various hypothetical models have been proposed to pry the underlying mechanism [11,22].

In this study we found that the red background light greatly enhanced LHC green response and vice versa, which was consistent with the previous reports of mutual color enhancement [16]. But potentiation or repression of GABAergic activity exerted little effect on the background-induced enhancement of LHC's light responsiveness, although it significantly modulated the membrane potential and light response amplitude of LHC, which suggests that the GABAergic feedback pathway was not necessarily responsible for the signal interaction. However, our results indicated that inhibition of mGluRs successfully abolished the enhancement effect of red background light on LHC green response and vice versa (data not shown), which implied that activation of mGluRs may serve as a mechanism underlying the mutual color enhancement. In the central nervous system, it has been demonstrated that intersynaptic diffusion of neurotransmitter could mediate the crosstalk of nearby synapses, i.e. transmitters released from one synapse lead to significant activation of receptors at an adjacent synapse and modulate its synaptic transmission [21]. For excitatory glutamatergic synaptic contact, especially, glutamate may spill over from the synaptic cleft and diffuse to activate extrasynaptic receptors on presynaptic and/or postsynaptic membrane [14]. Activation of mGluRs may suppress the glutamate release from the presynaptic terminals, which was thought to mediate the hetero-synaptic repression between distinct synapse [15]. The diffusion of transmitter out of the synaptic cleft is subject to the presynaptic release, transporter uptake, and synaptic geometries [20]. Evidence suggested that the complex geometries of cone pedicle would not limit the clearance of glutamate out of the synaptic cleft [20]. Furthermore, the Müller cells, containing high-affinity glutamate transporters, rarely projected their processes into the outer plexiform layer near the ribbon synapses [17]. Thus, it would be reasonable to assume that tonic glutamate release and low activity of glutamate uptake at the cone terminals during the dark [7,20] may allow glutamate to spread out of the synapse and escape to the neighboring synapses, where they may activate the presynaptic mGluR on the membrane of cone photoreceptors. Immunohistochemical and electrophysiological studies have shown that L-AP4-sensitive group III mGluRs are present at the photoreceptor terminals [12]. Activation of mGluRs could inhibit the glutamate release from cone photoreceptors [9], which therefore make LHC respond to the flash stimulus in a repressed manner. In the case of red background-induced enhancement of LHC's green responsiveness, the dim red background light hyperpolarizes red cone, which reduces the glutamate release and strengthens the activity of glutamate transporter of red cone. Both contribute to the reduction of glutamate spillover from red cone synapse to neighboring green cone and thus diminish the inhibition of glutamate release from the green cone and enhance LHC's photoresponse to the imposing green stimulus. Inhibition of presynaptic mGluRs, with antagonist MCPG, eliminates the action of diffused glutamate on the process of transmitter release from the cone photoreceptors. As a result, the dim red background light no longer enhances LHC's green response (this also applies to the mechanism underlying green background-induced red response enhancement, data not shown).

Blockade of glutamate uptake with D-Asp or THA completely eliminated the mutual color enhancement, which agreed with the suggestion that inhibition of glutamate

transporter facilitated the activation of presynaptic mGluRs [18]. In the vertebrate retina, photoreceptors tonically release glutamate in the dark, the clearance of which depends largely upon the activity of glutamate transporter. While the glutamate transporter is blocked, it causes accumulation of glutamate at the synaptic cleft, which promotes the spillover of glutamate and sustain activation of presynaptic mGluRs. The application of background illumination can no longer exert modulatory effect on the glutamate spillover, so the mutual enhancement effect is interrupted.

One thing unexpected is that in our experiment, the application of D-Asp or THA hyperpolarized the membrane potential of horizontal cells, which was in contrast with previous reports [7,20]. Although inhibition of glutamate uptake and the subsequent accumulation of ambient glutamate would depolarize the postsynaptic horizontal cells, it may also result in other presynaptic and/or postsynaptic consequence. Rapid desensitization of AMPA receptors by high concentration of glutamate could lead to decreased amplitude of the EPSC [8,23]. Additionally, increase of extracellular glutamate could activate the presynaptic mGluRs and depress the glutamate release from cone photoreceptors [4,13]. Taken together, these factors may affect the membrane potential of LHC. However, the exact mechanism remains further investigation.

In summary, glutamate spillover from synaptic cleft may depress the transmitter release through activation of mGluRs, presumably at the perisynaptic region of the cone terminals. This process provides a mechanism for sensing neighboring activity in neural circuits and fine adjustment of the efficacy of synaptic transmission, and for signaling the context of stimulation imposed on. The latter may be responsible for the interaction of red- and green-cone signals in LHC.

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