Desensitization of the GABA_A Receptor Shifts the Dynamic Range of Retinal Horizontal Cells Due to Light and Dark Adaptation

Fusao KAWAI¹, Yoshimi KAMIYAMA, and Shiro USUI

Department of Information and Computer Sciences, Toyohashi University of Technology, Toyohashi, 441 Japan

Abstract: The effects of the GABAergic pathway on the dynamic range of the light response of cone-driven horizontal cells (HCs) in the carp retina were investigated using conventional intracellular recordings and computer simulations. The amplitude of the HC responses to repetitive test flashes was altered by background illumination in control Ringer's solution, but was not changed following the application of picrotoxin or

bicuculline, which suggests that GABA_A receptors mediate the subsequent change in light response. This phenomenon was simulated by introducing the desensitization mechanism of the GABA_A receptor into a cone–HC network model. These results suggest that desensitization of the GABA_A receptor shifts the dynamic range of the HCs due to light and dark adaptation. [*Japanese Journal of Physiology*, 47, 417–429, 1997]

Key words: GABA_A receptor, desensitization, horizontal cell, retina, ionic current model.

Horizontal cells (HCs) are the second-order retinal neurons that receive photoreceptor outputs. In teleost fish retinas, HCs consist of four subtypes; three cone-driven types (L, R/G, Y/RB) and a rod-driven cell [1–3]. The L-type HCs (LHCs) receive sign-preserving synaptic outputs primarily from red-sensitive cones via glutamate, and they feedback to red- and green-sensitive cones via sign-inverting synapses. GABA is the putative chemical transmitter mediating the negative feedback from LHCs onto these cones [4–11]. The LHCs continuously release GABA in the dark, and hyperpolarization of the LHCs reduces the concentration of GABA in the outer plexiform layer [12, 13]. The R/G-type HCs (R/G HCs) receive sign-preserving outputs from green-sensitive cones which consist of responses derived from green-sensitive cone and from the LHCs through the negative feedback synapse. This synaptic connection results in hyperpolarizing responses to short-wavelength stimuli and depolarizing responses to long-wavelength stimuli in R/G HCs. Y/RB-type HCs receive sign-preserving outputs from blue-sensitive cones which consist of responses derived from blue-sensitive cones and from

the R/G HCs through negative feedback synapses. These negative feedback systems are thought to be involved in color and spatial information processing [14–17].

It has long been believed that the dynamic range of the light response of retinal neurons such as photoreceptors, HCs, and bipolar cells is modulated by the biochemical mechanisms in the outer segment of the photoreceptor due to light and dark adaptation [18, 19]. In contrast, synaptic transmission mechanisms such as the GABAergic or dopaminergic pathways modulate the gap junctions between the HCs or the spinules between HCs and cones due to adaptation [20–22]. However, it is still unclear whether these synaptic transmission mechanisms modulate the dynamic range of the light response of retinal neurons due to light and dark adaptation. In the experiments reported here, the effects of the GABAergic and dopaminergic pathways on the dynamic range of the cone-driven HCs in the carp retina were investigated. Different types of GABA receptors are expressed in the terminals of cone (GABA_A [11, 23]), and in HCs (GABA_C [24–26]). In order to analyze the effects of

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¹ Present address: Department of Information Physiology, National Institute for Physiological Sciences, Myodaiji, Okazaki, 444 Japan
Correspondence should be addressed to: Shiro Usui, Department of Information and Computer Sciences, Toyohashi University of Technology, Toyohashi, 441 Japan. Tel: +81-532-44-6764, Fax: +81-532-46-7806, E-mail: usui@bpel.tutics.tut.ac.jp

GABA receptors on the dynamic range, we made intracellular recordings of LHC and R/G HC responses following application of either GABA_A or GABA_C antagonists. The HC responses were also recorded following application of dopamine to study the effects of the dopaminergic pathway on the dynamic range of the HC responses. In addition, we simulated the light responses of the HCs using an ionic current model to analyze how the kinetics of synaptic transmission mechanisms modulate the dynamic range due to light and dark adaptation.

MATERIALS AND METHODS

Preparation. Carp (*Cyprinus carpio*) were kept in an aquarium under natural daylight conditions. Prior to an experiment, a fish was dark-adapted for more than 2 h and decapitated under a dim red light. The eye balls were enucleated and hemisected. A retina was isolated from the pigment epithelium, mounted, receptor side up, on a Millipore filter, and bathed in Ringer's solution.

Photostimulation system. The retina was irradiated from the receptor side by a dual-beam photostimulator. Two independent light beams, whose intensities and wavelengths could be adjusted by neutral-density and interference filters, were provided by a xenon source. Full field illumination was used in order to eliminate the effects of the gap junctions on the light responses of HCs. The unattenuated intensity (0 log unit) of the light on the retinal surface was 1.15×10^{13} (quanta $\cdot$ s⁻¹ $\cdot$ cm⁻²). A test flash (duration, 50 ms; intensity, 0, -0.5, -1, -1.5, or -2 log unit) was applied to the retina every 2 s during and after the termination of background illumination (duration, 30 s; intensity, 0 log unit).

Recording procedure. Intracellular recordings from LHCs and R/G HCs were made using microelectrodes pulled on a Sutter P-87 puller. When filled with 3 M potassium acetate solution, the microelectrodes had a resistance of about 100 M Ω . For recording, the isolated retina was mounted onto a recording chamber (0.8 ml) and continuously perfused (at a rate of 2.0 ml/min) with control Ringer's solution or Ringer's solution containing various agents which are described in the next section. Cells were classified by standard criteria; spectral sensitivity and dependence on test spot diameter [2, 27]. The membrane potential of HCs was continuously monitored on an oscilloscope screen and a chart recorder, and recorded on a VTR tape recorder. The data were digitized after the experiment for analysis. All experiments were performed at room temperature (20–25°C).

Bathing solution. Normal Ringer's solution contained 100 mM NaCl, 28 mM NaHCO₃, 2.6 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, and 5 mM glucose, continuously bubbled with 95% O₂ and 5% CO₂ (pH=7.9). To study the effects of GABA receptors on the light response of HCs, picrotoxin (500 μ M) or bicuculline methiodide (200 μ M) was dissolved in normal Ringer's solution. Dopamine (5 μ M) was dissolved in the Ringer's solution with ascorbic acid (25 μ M) to reduce the oxidation of dopamine for studying the effects of the dopaminergic pathway on HC responses. These solutions were prepared fresh prior to each experiment.

RESULTS

Changes in HC responses following background illumination

LHC and R/G HC responses to test flashes were subsequently changed by background illumination, as shown in Fig. 1. In order to hyperpolarize red-sensitive cones selectively, we used deep red (700 nm) background illumination. Background illumination caused rapid and sustained hyperpolarization of an LHC (Fig. 1A). The peak amplitude of the LHC response to the first green test flash during background illumination was 4 mV, and the amplitude of the response to successive test flashes decreased with time (peak amplitude was 1 mV at the last test flash during background illumination). Similar characteristics were obtained in ten different cells. This phenomenon is consistent with previous observations [28]. The termination of background illumination caused rapid and sustained depolarization, which was accompanied by a subsequent increase in the amplitude of the response to green test flashes. Similar phenomena were observed when red (620 nm) test flashes were applied to the retina. The subsequent change in the amplitude of the response to red test flashes was slightly smaller than the response to green test flashes (data not shown).

In contrast, red background illumination caused gradual hyperpolarization of an R/G HC after rapid depolarization (Fig. 1B). The peak amplitudes of the R/G HC responses to the first and last green test flashes during background illumination were 23 and 19 mV, respectively; i.e., the amplitude of the response to successive green test flashes decreased over time during background illumination ($n=10$). The termination of background illumination increased the amplitude of the response to test flashes over time. The amplitudes of the responses to the first and last test flashes after the termination of background illumination were 12 and 17 mV, respectively.

Fig. 1. L-type and R/G-type horizontal cell responses to successive test flashes during and after the termination of background illumination.

A: L-type horizontal cell (LHC) response. **B:** R/G-type horizontal cell (R/G HC) response. A test flash was applied to the retina every 2 s. Light flash: wavelength, 520 nm; intensity, -1 log unit (1.15×10^{12} (quanta $\cdot$ s $^{-1}$ $\cdot$ cm $^{-2}$)); spot diameter, 4.6 mm; duration, 50 ms. Background illumination: wavelength, 700 nm; intensity, 0 log unit (1.15×10^{13} (quanta $\cdot$ s $^{-1}$ $\cdot$ cm $^{-2}$)); spot diameter, 4.6 mm; duration, 30 s.

Fig. 2. The light responses of the same LHC and R/G HC as shown in Fig. 1 following the application of 500 μM picrotoxin (PTX). A: LHC response. **B:** R/G HC response. The light stimulus conditions were the same as that in Fig. 1.

Effects of GABA receptors on changes in HC responses due to background illumination

In order to study the effects of the GABAergic pathway on these phenomena, we recorded HC responses following the application of GABA receptor antagonists. Figure 2 shows the light responses of the same HCs as shown in Fig. 1 following the application of picrotoxin, an antagonist for both GABA_A and GABA_C receptors. The base line of membrane potential and peak amplitude to test flashes were not changed significantly over time by background illumination ($n=5$). Similar effects were observed following the application of 200 μM bicuculline, a competitive GABA_A receptor antagonist (data not shown, $n=3$).

The depolarizing response of the R/G HC to red background illumination was almost eliminated following the application of picrotoxin (Fig. 2B); consistent with previous observations [7, 8]. The peak amplitude of the R/G HC responses to successive test flashes did not change over time ($n=3$). Similar results were observed in the presence of 200 μM bicu-

culline (data not shown, $n=3$). This observation suggests that GABA_A receptors are involved in the changes in LHC and R/G HC responses during background illumination.

Effects of the dopaminergic pathway on the light responses of HCs

Negishi and co-workers [29] have shown that a GABA_A receptor antagonist affects HCs through dopaminergic neurons in the carp retina. To determine whether changes in HC responses by background illumination might be modulated via dopaminergic cells, we recorded HC responses following the application of 5 μM dopamine (DA). An example is shown in Fig. 3. The base line of membrane potential and peak amplitude to test flashes changed over time, in a similar manner to that seen in Fig. 1 ($n=3$). This result eliminates the possibility that the effect of background illumination is modulated by dopaminergic cells.

Fig. 3. LHC and R/G HC responses to successive test flashes during and after the termination of background illumination following the application of $5 \mu\text{M}$ dopamine (DA). A: LHC response. B: R/G HC response. The light stimulus conditions were the same as that in Fig. 1.

Fig. 4. LHC responses to test flashes of various intensities during and after the termination of background illumination in normal Ringer's solution. A test flash was applied to the retina every 2 s. The light intensities of the test flashes are indicated in the inset. Light flash: wavelength, 520 nm; intensity, 0, -0.5 , -1 , -1.5 , -2 log unit; spot diameter, 4.6 mm; duration, 50 ms. Background illumination: wavelength, 700 nm; intensity, 0 log unit; spot diameter, 4.6 mm; duration, 30 s.

Voltage-intensity relationship of HCs

We found that the response amplitude of the HC changed over time as the result of background illumination via the GABAergic pathway. These observations raised the possibility that the GABA_A receptor may modulate the dynamic range of HCs, which is in-

teresting since it has long been believed that the dynamic range of the retinal neurons is modulated by photochemical adaptation mechanisms within the photoreceptors [18, 19].

To verify this possibility, we studied the effects of GABA_A receptors on the dynamic range of HCs. Fig-

Fig. 5. Voltage-intensity relationship of the same LHC as shown in Fig. 4 during and after the termination of background illumination. A: Voltage-intensity relationship during background illumination. Each symbol shows the re-

sponse amplitude to a test flash at 1 ($\square$), 15 (Δ), and 29 s ($\circ$). **B:** Voltage-intensity relationship after the termination of background illumination. Each symbol shows the response amplitude to a test flash at 31 ($\blacksquare$), 45 ($\blacktriangle$), and 59 s ($\bullet$).

ure 4 shows LHC responses to successive test flashes of various intensities during and after the termination of background illumination. The peak amplitude of the LHC responses to test flashes was decreased during background illumination (Fig. 4B-E). The flash responses after the termination of background illumination increased gradually at low intensities (Fig. 4D and E). However, the HC responses to high-intensity test flashes did not markedly change over time (Fig. 4A). No changes in LHC response were observed during or after the termination of background illumination following the application of picrotoxin or bicuculline. Similar phenomena were also observed for R/G HCs (not shown).

Figure 5 shows the voltage-intensity relationship of the same LHC as shown in Fig. 4. During background illumination, the peak amplitude of the LHC response to the first test flash (at 1 s, $\square$) was 11 mV at -2 log unit, and was increased when the test flash was intensified (18 mV at 0 log unit). The voltage difference between -2 log and 0 log unit at 1 s ($\square$) was approximately 7 mV (Fig. 5A). In contrast, the voltage difference between -2 log and 0 log unit at 15 (Δ) and 29 s ($\circ$) was approximately 15 mV. This value was larger than that at 1 s ($\square$). This result suggests that the dynamic range of LHC was shifted to a higher intensity over time due to light adaptation.

After the termination of background illumination, the peak amplitudes at 45 ($\blacktriangle$) and 59 s ($\bullet$) were larger than that at 31 s ($\blacksquare$) at lower intensities (-2 , -1.5 log). This result suggests that sensitivity to dim test flashes was increased by the termination of background illumination; i.e., the dynamic range of the LHC was shifted to a lower intensity due to dark adaptation. These phenomena were not observed following the application of picrotoxin or bicuculline.

Fig. 6. A GABA-gated Cl^- channel model. "A" and "R" are the GABA molecule and GABA receptor, respectively. Two GABA molecules, "A," bind to the receptor, "R," in a stepwise manner to form " A_2R ," which isomerizes to " A_2R^* ." "D" is the desensitized state. The parameters estimated by Bormann and Clapham [31] were used for the binding rate constants (k_1 and k_{-1}) and gating rate constants (α and β). The rate constants of desensitization, α' and β' , were estimated from the time course of the desensitization of the GABA-induced current [45]. $k_1=123$ ($\text{M}^{-1} \text{s}^{-1}$), $k_{-1}=123$ (s^{-1}), $\alpha=123$ (s^{-1}), $\beta=467$ (s^{-1}), $\alpha'=0.1$ (s^{-1}), $\beta'=2.0$ (s^{-1}).

Similar phenomena were observed for the R/G HCs. Therefore, these results suggest that the GABA_A receptors shift the dynamic range of the HCs due to light and dark adaptation.

A GABA-gated Cl^- channel model

In the perfusion system we used to study the effects of GABA receptor antagonists on HC responses, picrotoxin affected the GABA_C receptor in LHCs as well as the GABA_A receptor in the cone terminal. Therefore, the specific GABA receptor involved in the shift of the dynamic range of the HCs could not be determined. However, since the dynamic range of the HCs did not shift following the application of bicuculline, the GABA_A receptor in the cone terminal may be involved in this phenomenon. To verify this possibility, we simulated the light responses with a cone-

HC network model whose neuron included a GABA-gated Cl^- channel (GABA_A and GABA_C receptor) model.

Figure 6 shows a GABA-gated Cl^- channel model based on single-channel recordings [30]. Two GABA molecules, "A," bind to the receptor, "R," in a step-wise manner to form " A_2R ." The two binding steps are in agreement with the Hill coefficients of ~ 2 (bovine chromaffin cells [31]; turtle cones [10, 11]). Following isomerization of the biliganded receptor " A_2R ," the channel is converted to open-state " A_2R^* ." The inset of Fig. 6 shows the responses of the GABA-gated Cl^- channel model to a sustained increase in GABA concentration. The time course of the open-state " A_2R^* "

shows the desensitization clearly. The mechanism of these kinetics is explained in DISCUSSION.

Light responses of a cone-HC network model

Figure 7 shows the electrical circuit model of a cone-HC network. The above-mentioned GABA-gated Cl^- channel model was incorporated into the cone model proposed by Baylor and colleagues [19]. The GABA-gated Cl^- channel model was also incorporated into the LHC model [32, 33] previously developed using data from voltage-clamp studies of HCs [34–38]. However, since GABA receptors have not been found on the R/G HCs, the GABA-gated Cl^- channel model was not incorporated into the R/G HC

Fig. 7. Electrically equivalent circuits of a cone-HC network model. The mathematical formulations of each ionic current (I_{Na} , I_K , I_{Cl} , I_{glu} , I_A , I_{Ka} , I_{Kv} , I_{Ca} , or I_l) are shown in Tables 1 and 2. I_C is a capacitive current. E_{Na} , E_K , E_{Cl} and E_{Ca} are the equilibrium potentials for the various ionic channels.

Table 1. Mathematical formulations of the ionic currents of the red- and green-sensitive cones.

<p>I_{Na} Sodium Current</p> $I_{Na} = G_{Na} \cdot V_{Na}$ $G_{Na} = \frac{2}{1 + \frac{1}{10}} [\text{unit}]$ $\left(\frac{d}{dt} + 83.3\right) y_5 = 83.3^4 \cdot I(t)$ $\frac{dz_1}{dt} = 83.3 \cdot y_5 - \kappa_{12} \cdot z_1 - \kappa_{21} \cdot z_2$ $\frac{dz_2}{dt} = \kappa_{12} \cdot z_1 - (\kappa_{21} + 25) \cdot z_2 + 0.01 \cdot z_3$ $\frac{dz_3}{dt} = 2.5 \cdot z_2 - 10 \cdot z_3$ $25 \cdot \kappa_{21} = \kappa_{12}$ $\kappa_{12} = 25 + \left[\frac{113}{75 + 1.5 \cdot z_2}\right]$ $V_{Na} = V - E_{Na}$ $E_{Na} = 0 [\text{mV}]$	<p>I_K Potassium current</p> $I_K = \overline{G_K} \cdot V_K$ $\overline{G_K} = 1 [\text{unit}]$ $V_K = V - E_K$ $E_K = -80 [\text{mV}]$ <p>I_{Cl} GABA-gated Chloride Current</p> $I_{Cl} = G_{Cl(GABA)} \cdot V_{Cl}$ $G_{Cl(GABA)} = 19 \cdot A_2 R^*$ $\frac{dR}{dt} = 123 \cdot AR - 2 \cdot 123 \cdot A \cdot R$ $\frac{dAR}{dt} = 2 \cdot 123 \cdot A \cdot R - (123 \cdot A + 123) \cdot AR + 2 \cdot 123 \cdot A_2 R$ $\frac{dA_2 R}{dt} = 123 \cdot A \cdot AR - (2 \cdot 123 + 467 + 2.0) \cdot A_2 R + 167 \cdot A_2 R^* + 0.1 \cdot D$ $\frac{dA_2 R^*}{dt} = 467 \cdot A_2 R - 167 \cdot A_2 R^*$ $\frac{dD}{dt} = 2.0 \cdot A_2 R - 0.1 \cdot D$ $V_{Cl} = V - E_{Cl}$ $E_{Cl} = -67 [\text{mV}]$
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I_{Na} and I_K are based on the cone model proposed by Baylor and colleagues [19]. I_{Na} was changed by the light stimulus. I_{Cl} was formulated based on the GABA-gated Cl^- channel model presented in the text.

Table 2. Mathematical formulations of the ionic currents of the LHC.

I_{Ca}	Calcium Current	I_{Ka}	Anomalous rectifying K current
	$I_{Ca} = -\overline{G}_{Ca} \cdot h_{Ca} \cdot m_{Ca}^4 \cdot E_{Ca}$		$I_{Ka} = \overline{G}_{Ka} \cdot m_{Ka}^4 \cdot V_K$
	$\overline{G}_{Ca} = 250[\text{nS}]$		$\overline{G}_{Ka} = 50 \cdot [1 - 0.5 \cdot Glu(t)][\text{nS}]$
	$\frac{dm_{Ca}}{dt} = \alpha_{mCa} \cdot (1 - m_{Ca}) - \beta_{mCa} \cdot m_{Ca}$		$m_{Ka} = \frac{1}{1 + \exp(V_K/30)}$
	$\alpha_{mCa} = \frac{5 \cdot (70 - V)}{\exp[(70 - V)/28] - 1}$		$V_K = V - E_K$
	$\beta_{mCa} = 2 \cdot \exp(-V/15)$		$E_K = -80[\text{mV}]$
	$\frac{dh_{Ca}}{dt} = -0.25 \cdot h_{Ca} + 0.25 \cdot h_{Ca\infty}$	I_{Kv}	Delayed rectifying K current
	$h_{Ca\infty} = \frac{0.7}{1 + 5 \cdot (E_{Ca} \cdot m_{Ca}^4)^{0.5}}$		$I_{Kv} = \overline{G}_{Kv} \cdot m_{Kv}^4 \cdot V_K$
	$E_{Ca} = \frac{1}{1 + \exp[(V - 20)/8]}$		$\overline{G}_{Kv} = 0.25[\text{nS}]$
I_A	Transient outward K current		$\frac{dm_{Kv}}{dt} = \alpha_{mKv} \cdot (1 - m_{Kv}) - \beta_{mKv} \cdot m_{Kv}$
	$I_A = \overline{G}_A \cdot m_A^3 \cdot h_A \cdot V_K$		$\alpha_{mKv} = \frac{10 \cdot (120 - V_K)}{\exp[(120 - V_K)/20] - 1}$
	$\overline{G}_A = 2[\text{nS}]$		$\beta_{mKv} = 5 \cdot \exp(-\frac{V_K}{20})$
	$m_A = \frac{\alpha_{mA}}{\alpha_{mA} + \beta_{mA}}$		$V_K = V - E_K$
	$\alpha_{mA} = \frac{40 \cdot (140 - V_K)}{\exp[(140 - V_K)/15] - 1}$		$E_K = -80[\text{mV}]$
	$\beta_{mA} = 8 \cdot \exp(-\frac{V_K}{20})$	I_{glu}	Glutamate sensitive current
	$\frac{dh_A}{dt} = \alpha_{hA} \cdot (1 - h_A) - \beta_{hA} \cdot h_A$		$I_{glu} = \overline{I}_{glu} \cdot \frac{\exp((V+3)/125) - 1}{\exp[(3-V)/125] + 1}$
	$\alpha_{hA} = 0.01 \cdot \exp(-\frac{V_K}{20})$		$\overline{I}_{glu} = 600 \cdot Glu(t)[\text{pA}]$
	$\beta_{hA} = \frac{2}{\exp[(30 - V_K)/10] + 1}$	I_l	Leakage current
	$V_K = V - E_K$		$I_l = \overline{G}_l \cdot (V - E_l)$
	$E_K = -80[\text{mV}]$		$\overline{G}_l = 0.5[\text{nS}]$
I_{Cl}	GABA-gated Chloride Current		$E_l = -80[\text{mV}]$
	$I_{Cl} = G_{Cl(GABA)} \cdot V_{Cl}$		
	$G_{Cl(GABA)} = 9.5 \cdot A_2 R^*$		
	$\frac{dR}{dt} = 123 \cdot A \cdot R - 2 \cdot 123 \cdot A \cdot R$		
	$\frac{dAR}{dt} = 2 \cdot 123 \cdot A \cdot R - (123 \cdot A + 123) \cdot AR + 2 \cdot 123 \cdot A_2 R$		
	$\frac{dA_2 R}{dt} = 123 \cdot A \cdot AR - (2 \cdot 123 + 467 + 2.0) \cdot A_2 R + 167 \cdot A_2 R^* + 0.1 \cdot D$		
	$\frac{dA_2 R^*}{dt} = 467 \cdot A_2 R - 167 \cdot A_2 R^*$		
	$\frac{dD}{dt} = 2.0 \cdot A_2 R - 0.1 \cdot D$		
	$V_{Cl} = V - E_{Cl}$		
	$E_{Cl} = -20[\text{mV}]$		

Each current was formulated using Hodgkin-Huxley type equations based on voltage-clamp studies [34–38].

model. Tables 1 and 2 show the mathematical formulations of the cone and LHC models, respectively.

Figure 8 shows the HC model responses corresponding to the conditions of Figs. 1 and 2. To simulate the light stimulus, the Na⁺ conductances of the red- and green-sensitive cone models were changed based on the spectral sensitivity of the cones in the carp retina [39]. Under simulated normal Ringer's solution, background illumination caused rapid and sustained hyperpolarization of the LHC model (Fig. 8A). The peak amplitude of the LHC model responses to successive test flashes during background illumination was reduced over time. Termination of the background illumination caused rapid and sustained depolarization of the LHC model, which was accompanied by a subsequent increase in response amplitude. Fol-

lowing the simulated application of picrotoxin, these kinetics were eliminated (Fig. 8B). These characteristics were also suppressed following the simulated application of bicuculline, which selectively blocks the GABA_A receptor in the cone terminal. These results were consistent with the experimental observations (Figs. 1A, 2A).

The changes in the test flash responses of the R/G HC model were also consistent with the experimental observations (compare Fig. 8C and D with Figs. 1B and 2B). These results suggest that the kinetics of the GABA_A receptor in the cone terminal change the membrane potential and response amplitude of the LHCs and R/G HCs over time during and after the termination of background illumination.

Fig. 8. The light responses of the LHC and R/G HC models corresponding to Figs. 1 and 2. A, B: The light responses of the LHC model in simulated control Ringer's solution (A), and following the simulated application of picrotoxin (B). **C, D:** The light responses of the R/G HC model under simulated control conditions (C), and following the simulated application of picrotoxin (D). Following the simulated application of picrotoxin, GABA-gated Cl^- currents in the cone and HC models were fixed at 0 pA.

Effects of the kinetics of the GABA-gated Cl^- channel model on light and dark adaptation

Using the ionic current model, it is possible to analyze the cone model responses to light stimulation in addition to the HC model responses. Figure 9 shows the light responses of the various components in the cone-HC network model. Simulated red background illumination decreased the Na^+ conductances (G_{Na}) of the red-sensitive cone model ($G_{\text{Na}}^{\text{Red}}$ in Fig. 9E), which caused hyperpolarization (Fig. 9A). In response to light, the GABA-gated Cl^- conductance of the red-sensitive cone model ($G_{\text{Cl(GABA)}}^{\text{Red}}$ in Fig. 9I) rapidly decreased due to a decrease in GABA concentration (Fig. 9L), and then increased gradually due to desensitization. The gradual increase in $G_{\text{Cl(GABA)}}^{\text{Red}}$ during background illumination caused sustained hyperpolarization of the red-sensitive cone model, since the equilibrium potential for the Cl^- (E_{Cl}) of the cones was more negative than the membrane potential of the cones ($E_{\text{Cl}} = -55 \text{ mV}$ [11]; $E_{\text{Cl}} = -67 \text{ mV}$ [23]). The sustained increase in $G_{\text{Cl(GABA)}}^{\text{Red}}$ also progressively reduced the sensitivity of the red-sensitive cone model to test flashes, which decreased the flash responses of the red-sensitive cone model over time. The response amplitudes to the first and last test flashes during background illumination were 5 and 3 mV, respectively. Termination of the simulated background illumination increased $G_{\text{Na}}^{\text{Red}}$, which caused rapid and sustained depolarization of the red-sensitive cone model due to the desensitization of $G_{\text{Cl(GABA)}}^{\text{Red}}$. The flash responses increased over time because of the sustained decrease in the membrane conductance

caused by the desensitization of $G_{\text{Cl(GABA)}}^{\text{Red}}$.

The voltage response of the red-sensitive cone model changed the glutamate concentration (Fig. 9F) that was released onto the LHC model and evoked the light response (Fig. 9B). The kinetics of the LHC model responses were similar to those of the red-sensitive cone model. These results suggest that the light response of the LHC model is more strongly affected by $G_{\text{Cl(GABA)}}^{\text{Red}}$ than by $G_{\text{Cl(GABA)}}^{\text{LHC}}$, since the membrane potential of the LHC model was dominantly modulated by the E_{Cl} of the red-sensitive cone model (-67 mV) rather than the E_{Cl} of the LHC model (-20 mV). This result also suggests that the shift of the LHC dynamic range due to light and dark adaptation is caused mainly by the GABA_A receptor in the cone terminal.

Hyperpolarization of the LHC model decreased the GABA concentration (Fig. 9L), which caused rapid depolarization of the green-sensitive cone model (Fig. 9C) due to the rapid decrease in $G_{\text{Cl(GABA)}}^{\text{Green}}$ (Fig. 9K). The sustained increase in $G_{\text{Cl(GABA)}}^{\text{Green}}$ during background illumination caused gradual hyperpolarization of the green-sensitive cone model after rapid depolarization, and also caused a sustained decrease in flash responses due to desensitization. This light response in the green-sensitive cone model was transmitted to the R/G HC model via glutamate (Fig. 9H). The R/G HC model response (Fig. 9D) was similar to the green-sensitive cone model response.

We also analyzed the light responses of the cone-HC network model following the simulated application of picrotoxin. Under these conditions, $G_{\text{Cl(GABA)}}$

Fig. 9. The light responses of the cone-HC network model corresponding to Fig. 1. **A-D:** The light responses of the red-sensitive cone model (A), LHC model (B), green-sensitive cone model (C), and R/G HC model (D). **E, G:** The changes in sodium conductance of the red-sensitive cone model (E) and green-sensitive cone model (G). **F, H:** The co-

efficient changes of the glutamate-sensitive current of the LHC model (F) and R/G HC model (H). **I, K:** The conductance changes of the GABA-gated Cl⁻ channel of the red-sensitive cone model (I), LHC model (J), and green-sensitive cone model (K). **L:** GABA concentration changes.

Fig. 10. The simulated voltage-intensity relationship of the LHC model corresponding to Fig. 5. **A:** Simulated voltage-intensity relationship during background illumination. Each symbol shows the response amplitude to test flashes

at 1 (□), 15 (Δ), and 29 s (○). **B:** Simulated voltage-intensity relationship after the termination of background illumination. Each symbol shows the response amplitude to a test flash at 31 (■), 45 (▲), and 59 s (●).

was fixed at zero to simulate the effects of picrotoxin on the GABA receptor. Following the simulated application of picrotoxin, the subsequent changes in the membrane potential and flash responses of the HCs were eliminated (Fig. 8B, D). A similar result was obtained by the model simulation following the applica-

tion of bicuculline, which blocks only the GABA_A receptor in the cone terminal (not shown). These results suggest that desensitization of the GABA_A receptor in the cone terminal modulates the subsequent changes in membrane potential and flash responses of the LHCs and R/G HCs due to light and dark adaptation.

Voltage-intensity relationship of the HC model

To study the effect of desensitization of GABA_A receptors on the dynamic range of the HC responses, we simulated the same experiment as shown in Fig. 5. Figure 10 shows the voltage-intensity relationship of the LHC model. The voltage difference between the -2 log and 0 log unit at 15 (Δ) and 29 s ($\circ$) during background illumination was approximately 15 mV, while that at 1 s ($\square$) was about 5 mV (Fig. 10A); i.e., the dynamic range at 15 and 29 s was wider than that at 1 s. This result suggests that the dynamic range of the LHC model is shifted to a higher intensity due to light adaptation.

After the termination of simulated background illumination, the peak amplitudes of $\blacktriangle$ and $\bullet$ were larger than that of $\blacksquare$, indicating that the sensitivity to test flashes was increased over time due to dark adaptation. Following the simulated application of bicuculline or picrotoxin, these characteristics were eliminated. Therefore, these results suggest that desensitization of the GABA_A receptor in the cone terminal shifts the dynamic range of the HC responses due to light and dark adaptation.

DISCUSSION

In the experiments presented here, we studied the effects of the GABAergic pathway on the dynamic range of the cone-driven HCs in the carp retina using conventional intracellular recordings and computer simulations. The dynamic ranges of the LHCs and R/G HCs were shifted over time by background illumination. These phenomena were not observed following the application of bicuculline, a GABA_A receptor antagonist, or picrotoxin, a GABA_A and GABA_C receptor antagonist, suggesting that the GABA_A receptor is involved. In order to verify whether it is the cone terminal or the LHC GABA receptor that is involved in shifting the dynamic ranges of the HCs, we simulated the light responses of the HCs using an ionic current model of the cone-HC network. The simulations suggest that desensitization of the GABA_A receptor in the cone terminal shifts the dynamic range of the HCs due to light and dark adaptation.

Effects of background illumination on HC responses. The peak amplitude of the HC response to successive test flashes during background illumination decreased over time in control Ringer's solution (Fig. 1). In contrast, Yang and Wu [40] have reported that the peak amplitude of HC response to test flashes increased in the tiger salamander retina after

the onset of steady background illumination. HCs in the tiger salamander retina receive inputs from both cones and rods, and the suppression of cone inputs to HCs by the rods might be responsible for this phenomenon [40]. However, the LHCs and R/G HCs in the carp retina receive their input from cones exclusively [1, 2, 17]. Thus, the different synaptic input from photoreceptors may produce different HC responses.

The depolarizing response of the R/G HC to red background illumination was blocked following the application of picrotoxin or bicuculline in this study (Fig. 2B). This observation is consistent with previous observations [7, 8]. The depolarizing response of the R/G HC to red light stimuli is thought to be generated by the GABAergic feedback pathway from LHCs to green-sensitive cones [3, 6–8, 16]. In support of this, the depolarizing response of the R/G HCs was eliminated by blocking the GABAergic feedback pathway by the application of picrotoxin or bicuculline.

GABA receptor subtypes in the retina.

Three classes of GABA receptors (GABA_A, GABA_B, and GABA_C) have been identified [24, 41]. Kaneko and Tachibana [11] showed that muscimol, a GABA_A receptor agonist, evoked a GABA-induced current in isolated turtle cones, while baclofen, a GABA_B receptor agonist, evoked no responses. They also reported that the GABA-induced current in turtle cones was antagonized by picrotoxin or bicuculline. Similar observations have also been reported for tiger salamander cones [23]. These observations suggest that the cones in turtle and tiger salamander retinas express the GABA_A receptor rather than the GABA_B receptor [11, 23]. On the other hand, it was reported that HCs express GABA_C receptors in various species (white perch [24]; catfish [25, 26]). Bicuculline- and baclofen-resistant GABA receptors are called GABA_C receptors [42]. GABA_A receptors desensitize rapidly (<10 s) [11], whereas GABA_C receptors do not desensitize [24–26]. In this study, the changes in HC responses due to test flashes were eliminated not only by picrotoxin but also by bicuculline, suggesting that these phenomena are mediated by GABA_A receptors rather than GABA_C receptors.

Effects of photochemical adaptation on HC responses. According to previous studies on photochemical adaptation [18, 19], background illumination should exert effects on phototransduction not only under control Ringer's conditions but also following the application of GABA receptor antagonists. However, changes in HC response due to photochemical adaptation in the cones were not observed following the application of picrotoxin or bicuculline in these

experiments (Fig. 2A, B). Schnapf and co-workers [43] showed that steady light illumination desensitized the photocurrent of the cones in monkey retina to less than 100 ms. In this study, the test flash was applied to the retina every 2 s. Therefore, since the photochemical adaptation kinetics were much faster than the time interval of the successive test flashes, no changes in HC response due to photochemical adaptation were observed following the application of GABA_A receptor antagonists.

The GABA-gated Cl⁻ channel model. As shown in Fig. 6, the GABA-gated Cl⁻ channel model presented here showed desensitization. The mechanism of this desensitization can be explained as follows. The sustained increase in GABA concentration rapidly increased the A_2R and A_2R^* since the binding (k_1 and k_{-1}) and gating (α and β) rate constants were large. The increase in A_2R increased D slowly due to the small rate constants of desensitization (α' and β'), which decreased A_2R over time. A gradual decrease in A_2R subsequently decreased A_2R^* , causing the desensitization of A_2R^* .

In order to verify whether the cone terminal or the HC GABA receptor is involved in shifting the dynamic range of the HCs, we simulated the light responses of the HCs with an ionic current model of a cone-HC network. The rate constants of the GABA-gated Cl⁻ channel model were inferred from the single-channel kinetics of the GABA-gated Cl⁻ channel in the adrenal chromaffin cells [31]. It is not certain that the binding and gating rate constants of the GABA-gated Cl⁻ channel in the carp retina are equal to those of the adrenal chromaffin cells. However, the rate constants were much faster than the time course of the subsequent changes in the HC response, and thus a small change in rate constants exerted little effect on the response kinetics of the HC model.

Some ligand-gated channels including NMDA channels are voltage-dependent [44]. This raises the possibility that the GABA-gated Cl⁻ conductance might be modulated not only by the GABA concentration but also by membrane potential. However, the I - V relationship of GABA-gated Cl⁻ channels in the cones and HCs was found to be approximately linear [11, 45]. Therefore, we formulated the components of the membrane potential in the GABA-gated Cl⁻ channel model presented here as a linear function (Table 1, 2).

Functional implications of GABA_A receptor kinetics in the outer retina. In the above simulations, we have shown that the response kinetics of the LHC model are similar to those of the red-sensitive cone model. If the light responses of the LHC models

are modulated mainly by $G_{Cl(GABA)}^{LHC}$ rather than $G_{Cl(GABA)}^{Red}$, simulated background illumination should cause sustained depolarization of the LHC model after rapid hyperpolarization since the E_{Cl} of the HCs is approximately -20 mV (catfish HC [26]; cyprinid fish HC [46]; stingray HC [47]). However, in our simulations, background illumination caused sustained hyperpolarization of the LHC model due to the negative voltage of the E_{Cl} of the cones (turtle [11]; tiger salamander [23]); suggesting that the light response of the LHC is more strongly affected by $G_{Cl(GABA)}^{Red}$ than $G_{Cl(GABA)}^{LHC}$. This result is consistent with previous observations that the cones express the GABA_A receptor [11, 23] and HCs the GABA_C receptors [24–26], since the subsequent changes in HC responses by background illumination were suppressed following the application of bicuculline.

It has long been believed that the dynamic range of retinal neurons is controlled by photochemical adaptation. However, recent studies have shown that the dynamic range of HCs is modulated by the kinetics of the GABA_A receptor due to light and dark adaptation. Since the desensitization kinetics of the GABA_A receptor were slower than the desensitization of the photocurrent in the cones [43], GABA_A receptor kinetics might be involved in the slow visual adaptation of HCs.

The bipolar cells also receive synaptic inputs from the cone terminal [48, 49]. Therefore, this observation raises the possibility that the dynamic range of the bipolar cells might also be affected by the desensitization kinetics of the GABA_A receptor in the cone terminal. Further experiments are needed to study the effects of GABA_A receptors kinetics on bipolar cell responses.

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