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OFF response of bullfrog cones is shaped by terminal ionotropic GABA receptors

Jian Liu [a,b], Xiong-Li Yang [a,*]

[a] *Institute of Neurobiology, Institutes of Brain Science, Fudan University, 138 Yixueyuan Road, Shanghai 200031, PR China*
[b] *Shanghai Institutes for Biological Sciences, Graduate School, Chinese Academy of Sciences, 320 Yueyang Road, Shanghai 200031, PR China*

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Abstract

We recently reported an ionotropic GABA receptor expressed at the bullfrog retinal cone terminal that is potentiated by the $GABA_A$ receptor antagonist bicuculline (BIC) and suppressed by the $GABA_C$ receptor antagonist imidazole-4-acetic acid (I4AA) [13]. In this study, by using the patch clamp technique in current clamp mode, we show that activation of this GABA receptor causes voltage changes of cones, which are closely dependent on the membrane potential level in relation to the chloride equilibrium potential of the cells. Furthermore, the OFF overshoot of cone light responses is enhanced or diminished when this receptor is potentiated by BIC or suppressed by I4AA, suggesting the involvement of this GABA receptor in shaping OFF light responses of bullfrog cones.

Keywords: Retina; Chloride channel; Bicuculline; Light response; Membrane potential; Equilibrium potential

1. Introduction

In the outer retina, γ-aminobutyric acid (GABA) released from horizontal cells has been thought to be involved in feedback from horizontal cells to cone photoreceptors (for review see [17]). Even though the GABA-mediated feedback hypothesis is recently challenged by the calcium hypothesis and other hypothesis [2,5–7,20,24], the existence of both $GABA_A$ and $GABA_C$ receptors at vertebrate retinal cone photoreceptor terminals [11,15,16] suggests that GABA must somehow modulate cone signal. We recently reported an ionotropic GABA receptor with novel pharmacology expressed at bullfrog cone terminals [13] that is different in pharmacological characteristics from either conventional $GABA_A$ or $GABA_C$ receptor. Especially, the currents mediated by these GABA receptors are potentiated by the $GABA_A$ receptor antagonist bicuculline, but suppressed by the $GABA_C$ receptor antagonist I4AA. In the present work, we further studied GABA-induced voltage changes of cones in bullfrog retinal slice preparations by whole-cell current clamp recording. Our results show that such voltage changes are closely dependent on the dark membrane potential (V_m) in relation to the chloride equilibrium potential (E_{Cl}) of the cones. Furthermore, the OFF overshoot of cone light responses is enhanced by BIC and suppressed by I4AA, suggesting the involvement of this receptor in shaping OFF light responses of the cones.

2. Materials and methods

2.1. Retinal slice preparations

Adult bullfrogs (*Rana catesbeiana*) were used in the present work. All efforts were made to minimize animal pain and discomfort in accordance with the NIH guidelines for animal experimentation. Retinal slices were prepared following the procedures reported previously [14]. Briefly, the dark-adapted retina was isolated and then cut into 150-μm-thick slices in Ringer's using a manual cutter (ST-20, Narishige, Tokyo, Japan). The slices were then transferred into a recording chamber with the cut side up and they were held mechanically in place by a grid of parallel nylon strings glued onto a U-shape frame of platinum wire. All these procedures were performed under dim red illumination. The glass-bottomed recording chamber with a volume of approximately 1.0 ml was continuously perfused with the oxygen-bubbled extracellular solution, which was fed in and out of the chamber through inlets by a peristaltic pump (Minipulse 3, Gilson Medical Electronics, Viuiers Le Bel, France) at a rate of 3–5 ml/min. All experiments were performed at room temperature (25 °C).

2.2. Solutions and drug application

Ringer's with an osmolality of 250 m Osm/kg H_2O consisted of (in mM) 100 NaCl, 25 $NaHCO_3$, 2.5 KCl, 1.6 $MgCl_2$, 2.0 $CaCl_2$, and 10 glucose, pH

* Corresponding author. Tel.: +86 21 6422 1975; fax: +86 21 6422 1975.
E-mail address: xlyang@fudan.edu.cn (X.-L. Yang).

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adjusted to 7.4 by bubbling with 95% O_2 and 5% CO_2. Ringer's containing GABA was pressure-ejected by nitrogen gas via a Picospritzer II (General Valve, Houston, TX, USA), which was triggered by the Pulse software (PulseFit 8.65, HEKA, Lambrecht/Pfalz, Germany), and focally applied to the cone photoreceptor terminal using a puff pipette with a motor-driven micromanipulator (MP-285, Sutter, Novato, CA, USA). The position and pressure of the puff pipette with a tip size of 3–5 μm in diameter were adjusted to obtain consistent drug-induced effects. When no pressure was applied to the pipette, small amount of the bath medium was sucked into the pipette by capillary attraction, thus preventing the drug solution from leaking out. The actual concentration of GABA around the terminal was surely much lower because of bulk flow, diffusion and potent uptake systems in the retina. Drugs other than GABA were applied by administrating drug-containing Ringer's into the bath medium through another inlet.

The intracellular solution for whole-cell current clamp recording in retinal slice preparations consisted of (in mM) 126 D-gluconic-K, 8.5 NaCl, 5.0 $MgCl_2$, 1.0 $CaCl_2$, 2.5 Mg_2-ATP, 1.1 ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 10 HEPES, with an osmolality of 240 m Osm/kg H_2O. To change E_{Cl}, KCl was substituted by equimolar D-gluconic-K. The pH of the pipette solution was adjusted to 7.2 with Trizma Base.

All chemicals were purchased from Sigma Chemicals (St. Louis, MO, USA), and freshly dissolved in Ringer's when used.

2.3. Whole-cell current clamp recording and light stimulation

The recording chamber containing retinal slices was placed on a fixed stage microscope (Zeiss, Axioskop 2 FS Mot, Jena, Germany), which was equipped with infrared differential interference contrast (DIC) optics. A water-immersion objective with a working distance of 1.4 mm (Zeiss, ×63 W, 0.95 NA) was used. During the experiments, the cells were imaged by an infrared video camera (C2400-79, Hamamatsu, Shizuoka Pref., Japan) and visualized on a TV monitor (Panasonic, Osaka, Japan).

Recording patch electrodes were pulled from borosilicate glass (BF150-86-10, Sutter) with a multi-stage horizontal puller (P-97, Sutter). Electrodes of 8–10 MΩ, when filled with the intracellular solution, were used. The recording pipette was connected to a patch amplifier (EPC9/2, HEKA) and the liquid junction potential was calculated and auto-compensated by the software (Pulse 8.65, HEKA). Current clamp data were acquired at a sample rate of 10 kHz, filtered at 2 kHz, and the displayed traces were further low-pass filtered at 200 Hz.

Light responses of cones were recorded in current clamp mode (or voltage-follower mode) in the dark condition, which was achieved by shutting off background illumination and keeping the preparation in the dark for at least 8 min. The light source was a stimulator-driven (SEN-7203, Nihon Kohden, Tokyo, Japan) super-high-light white LED (Light Emission Diode, Toshiba, Tokyo, Japan), which delivered a full field white test light to the retinal slice preparation through the upright microscope binocular ergotube. The LED light intensity was 5.0 μW/mm^2 at the surface of the retinal slice preparation, as measured with a calibrated photometer (UDT-114A, United Detector Technology, Hawthorne, CA, USA).

3. Results

In order to explore possible physiological roles of GABA-induced currents from cones, we first studied how application of GABA to the terminals of cones may affect the V_m of these cells in the dark. The dark V_m of cones was monitored in current clamp mode (voltage-follower mode) and the chloride concentration in the recording pipette was carefully adjusted to achieve an E_{Cl} of −43 mV in all experiments described in the present work, unless otherwise specified, which has been demonstrated to be within the physiological range in amphibian cones [21]. Our results showed that GABA-caused changes in V_m basically depended on the V_m levels of the cones in relation to E_{Cl}. Three examples are shown in Fig. 1A–C. For the cell with a V_m of −37 mV, GABA of 1 mM caused a hyperpolarization of 3.3 mV (Fig. 1A), whereas GABA application depolarized the cell with a V_m of −50 by 3.0 mV (Fig. 1C). When the V_m of the cell (−46 mV) was very close to the E_{Cl}, no change in V_m was detectable in response to GABA application (Fig. 1B). The cells, which did not show GABA-caused changes of V_m in the dark, responded to 1 mM GABA with a small depolarization of 2–3 mV when they were hyperpolarized by a light flash (data not shown). The statistical results obtained from the cones that could be categorized into these three groups are shown in Table 1. When E_{Cl} was experimentally shifted to +5.1 mV by adjusting the chloride concentration in the recording pipette, the cone with a V_m of −44 mV responded to application of 1 mM GABA with a larger depolarization (see the inset in Fig. 1C). Under this condition, 1 mM GABA-caused an averaged depolarization of 9.0 ± 0.8 mV in six cones with V_m ranging between −36 and −45 mV.

Totally, 34 cones with V_m more negative than −30 mV were examined and the change of V_m caused by 1 mM GABA is plotted as a function of V_m in Fig. 1D. Puff of 1 mM GABA-caused a depolarization in cells with V_m of more negative than −49.00 mV (n = 6, 17.6% of the cells tested), but a hyperpolarization in those with V_m more positive than −43.00 mV (n = 9, 26.5%). In cells with V_m ranging from −43.50 to −49.00 mV (n = 19, 55.9%), changes in V_m were less than 1 mV. With some exceptions, the size of the V_m change was positively correlated with the size of the deviation of V_m from E_{Cl}, which could be described by linear regression ($y = -19.60 - 0.44x$) with a correlation coefficient (γ) of −0.92.

Since cones are hyperpolarized by light and the V_m of cones is thus more deviated from E_{Cl} during light illumination, a large increase of GABA released from horizontal cells could be expected at the cessation of a light flash. We therefore hypothesized that GABA might contribute to shaping response component appearing at the offset of a light flash. To test this hypothesis, we examined the effects of imidazole-4-acetic acid (I4AA) and bicuculline (BIC) on OFF responses of cones. Fig. 2A shows the response of a cone to a light flash of 500 ms in Ringer's, which exhibited an initial ON transient and then sagged to a plateau. When the light was switched off, an OFF overshoot (arrow) could be distinguished. With continuous perfusion of 1 mM I4AA, which has been demonstrated to suppress the GABA

Table 1
Dependence of GABA-induced voltage changes of bullfrog cones on dark V_m

Membrane potential change	V_m (mV), mean ± S.E.M.	GABA-induced voltage change (mV), mean ± S.E.M.	n
Depolarization	−50.33 ± 0.42	3.50 ± 0.36	6
No change	−44.28 ± 0.46	–	19
Hyperpolarization	−38.80 ± 1.26	2.68 ± 0.45	9