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Depolarizing effect of GABA in rod bipolar cells of the mouse retina

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Abstract

Gamma-amino butyric acid (GABA) has been characterized as inhibitory neurotransmitter through chloride mediated channels in the adult nervous system. However, using gramicidin perforated patch-clamp recordings from rod bipolar cells dissociated from retinas of adult mice, we find that GABA is capable of inducing cell depolarization. Currents mediated by $GABA_A$ and $GABA_C$ receptors were further isolated by the use of GABA receptor specific blockers. In rod bipolar cells dissociated from the mouse retina, activation of $GABA_A$ receptors located at the cell dendrites induces ionic currents which show a reversal potential of −33 mV. However, local activation of $GABA_C$ receptors located at the axon terminal induces ionic currents with a reversal potential of −60 mV. According to Nernst equation, the dendrites of rod bipolar cells of the mouse retina would have a high intracellular chloride concentration ($[Cl^-]_i$) and there must be an intracellular gradient in $[Cl^-]_i$, being the $[Cl^-]_i$ more elevated in the dendrites than in the axon terminal. The depolarizing effect of GABA at the dendrites of rod bipolar cells may contribute to the lateral interaction in the mammalian retina, thereby enhancing visual discrimination of stimuli input.

Keywords: Retina; Bipolar cell; GABA receptors; Chloride transporters

1. Introduction

It has been shown in the hippocampal and retinal neurons that the polarity of the GABA induced responses reverses from depolarization in neonate to hyperpolarization in adult (Ben-Ari, 2002; Zhang, Sterling, & Vardi, 2003), thus suggesting that the intracellular chloride concentration ($[Cl^-]_i$) decreases during development. Excitatory effect of GABA mediated chloride conductance has also been shown recently in different mammalian adult brain regions, as in cerebellar network (Chavas & Marty, 2003), substantia nigra (Gulacsi et al., 2003), hippocampus (Woodin, Ganguly, & Poo, 2003) and cortex (Gulledge & Stuart, 2003).

Chloride concentration is regulated primarily by two transporters, the Na–K–Cl cotransporter (NKCC) and the K–Cl cotransporter (KCC2). NKCC maintains $[Cl^-]_i$ high, while KCC2 keeps the $[Cl^-]_i$ low (see for review Russell, 2000). Probably, the switch of the chloride transporter from NKCC to the KCC2 is responsible for the developmental change of $[Cl^-]_i$ (Lu, Karadsheh, & Delpire, 1999; Plotkin, Snyder, Hebert, & Delpire, 1997; Rivera et al., 1999).

In the distal retina of adult mammals, horizontal cells (HCs) and ON type bipolar cells (BCs) express the NKCC (Vardi, Zhang, Payne, & Sterling, 2000) on their dendrites, at the first synapse of the visual system. Thus, $[Cl^-]_i$ is predicted to be high in those cell types, and the chloride equilibrium potential (E_{Cl}) to be more positive than the resting membrane potential.

At the first synapse of the visual pathway, photoreceptors release glutamate on their postsynaptic neurons, HCs and BCs. Light-induced hyperpolarization in

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photoreceptors decreases their glutamate release, while dark-induced depolarization of photoreceptors increases their glutamate release. Metabotropic glutamate receptors (mGluR6) are expressed at the dendrites of ON type BCs, both cone bipolar cells (CBCs) and rod bipolar cells (RBCs). Ionotropic AMPA/kainate glutamate receptors are expressed at the dendrites of Off type CBCs (see for review Vardi, Morigiwa, Wang, Shi, & Sterling, 1998). Horizontal cells are able to release GABA upon cell depolarization (Schwartz, 1982; Vardi, Kaufman, & Sterling, 1994) and GABA receptors have been localized at the BCs dendrites (Fletcher, Koulen, & Wässle, 1998; Greferath, Muller, Wässle, Shivers, & Seeburg, 1993, 1994; Koulen, Brandstatter, Enz, Bormann, & Wässle, 1998; Vardi & Sterling, 1994) and HCs dendrites (Greferath et al., 1993, Greferath, Grünert, Müller, & Wässle, 1994; Pourcho & Owczarzak, 1989; Vardi, Masarachia, & Sterling, 1992). It is actually accepted that in the distal retina, the direct glutamatergic input from photoreceptors to BCs generates the center response, while the GABAergic input from HCs may contribute to the generation of the lateral response. GABA released from HCs at the triad, must depolarize presynaptic HCs and ON BCs, but hyperpolarize Off BCs. These effects have been proposed to be mediated by the GABA receptors localized at the dendrites of HCs and BCs (Vardi et al., 2000).

According to this hypothesis it is necessary that GABA must induce cell depolarization in retinal ON type BCs. However, there is currently confusing data about the $[Cl^-]_i$ in BCs (Billups & Attwell, 2002; Satoh, Kaneda, & Kaneko, 2001; Vardi et al., 2000). The present study shows evidence of a high E_{Cl} at the dendrites of RBCs enzimatically dissociated from the mouse retina. This high E_{Cl} justifies the depolarizing effect of GABA at this level.

2. Materials and methods

2.1. Cell-isolation procedure

Bipolar cells were prepared from mouse (NMRI strain) retinas, following procedures described previously (De la Villa, Kurahashi, & Kaneko, 1995; Vaquero & De la Villa, 1999). Animals were handled according to the European Union statement for the use of laboratory animals. Mice were sacrificed by cervical dislocation before enucleating procedure. Then, the eye was hemisected, the anterior segments and the vitreous body removed, and the retina detached from the pigment epithelium. Afterwards, the retina was incubated for 30 min in a standard mammalian saline (control solution) containing (in mM): 135 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES and 10 glucose (pH 7.4), containing 40 U/ml papain (Worthington, Freehold, NJ, USA) and 0.1 mg/ml L-cysteine (Sigma Chemical Co., St Louis, MO, USA) at 37 °C. The retinal pieces were rinsed with the standard mammalian saline containing 0.1 mg/ml bovine serum albumin (BSA) (Sigma Chemical Co., St Louis, MO, USA) and triturated using a glass pipette. One to 2 drops of the cell suspension were dispersed in a plastic culture dish containing ca. 2 ml BSA + standard solution. The bottom of the plastic dish was substituted by a cover-glass coated with 1 mg/ml concanavalin A (Sigma Chemical Co., St Louis, MO, USA). Cells were stored at 4 °C and experiments were performed at room temperature within 1–4 h after dissociation.

2.2. Identification of cellular types

Dissociation procedure produced a mixture of cells. Bipolar cells dissociated from the mouse retina were unequivocally identified from other retinal cell types by their own characteristic morphology. Rod bipolar cells constitute ca. 50% of BCs of the mouse retina and they can be easily identified after dissociation by their typical morphology. It has been shown that RBCs of the mammalian retina show protein kinase C-like immunoreactivity (PKC-IR) (Greferath, Grünert, & Wässle, 1990). In a series of experiments, PKC-IR was performed on isolated BCs just after finishing the recording (for methods, see De la Villa et al., 1995) and it was confirmed that they corresponded to the RBCs subtype.

2.3. Whole-cell patch-clamp recording

Cells were viewed at 400× magnification by using an inverted microscope with phase-contrast optics (Nikon, TMD, Garden City, NY, USA). Cells were continuously perfused (0.5 ml/min) at room temperature (21–25 °C), with an *extracellular solution* containing in mM: 135 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, 10 glucose, and 0.1 mg/ml bovine serum albumin (pH 7.4). A stainless steel ring was placed into the dish to minimize the dead space of the recording chamber (total volume 150 μl). Ionic currents and voltages were recorded under the whole-cell configuration of the patch-clamp technique (Hamill, Marty, Neher, Sakmann, & Sigworth, 1981) using an Axopatch 200A amplifier (Axon instruments, Foster City, CA, USA). Patch pipettes were made from Pyrex glass tubing (1.2 mm o.d.) pulled in two steps on a vertical pipette puller (Narishige mod. P88, Narishige, Tokyo, Japan). After heat polishing, the inner diameter of the pipette was about 0.5–1 μm.

Pipettes were then filled with *intracellular solution*; two main intracellular solutions were used. A *high chloride* solution, containing (in mM): 10 NaCl, 110 KCl, 5 EGTA, 0.5 $CaCl_2$, 1 $MgCl_2$, 1 GTP, 0.1 cGMP, 1 ATP, 0.01 cAMP and 10 glucose (pH 7.2), and a *low chloride*