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In vivo electroretinographic studies of the role of $GABA_C$ receptors in retinal signal processing

Jing Wang [a], Deb Kumar Mojumder [a, b], Jun Yan [c, d], An Xie [e, f], Robert F. Standaert [c, g], Haohua Qian [e, h], David R. Pepperberg [e], Laura J. Frishman [a, *]

[a] College of Optometry, University of Houston, Houston, TX 77204, USA
[b] Department of Neurology, Texas Tech University Health Sciences Center, Lubbock, TX 79430, USA
[c] Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA
[d] Chengdu Kanghong Pharmaceutical Co., Ltd., Sichuan, People's Republic of China
[e] Lions of Illinois Eye Research Institute, Department of Ophthalmology and Visual Sciences, Illinois Eye and Ear Infirmary, University of Illinois at Chicago, Chicago, IL 60612, USA
[f] Lifespan Cardiovascular Institute and the Warren Alpert School of Medicine of Brown University, Providence, RI 02906, USA
[g] Department of Biochemistry and Molecular & Cellular Biology, University of Tennessee, Knoxville, TN 37996, USA
[h] National Eye Institute, National Institutes of Health, Bethesda, MD 20892, USA

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ABSTRACT

All three classes of receptors for the inhibitory neurotransmitter GABA (GABAR) are expressed in the retina. This study investigated roles of GABAR, especially $GABA_CR$ (GABA(A)-ρ), in retinal signaling *in vivo* by studying effects on the mouse electroretinogram (ERG) of genetic deletion of $GABA_CR$ versus pharmacological blockade using receptor antagonists. Brief full-field flash ERGs were recorded from anesthetized $GABA_CR^{-/-}$ mice, and WT C57BL/6 (B6) mice, before and after intravitreal injection of $GABA_CR$ antagonists, TPMPA, 3-APMPA, or the more recently developed 2-AEMP; $GABA_AR$ antagonist, SR95531; $GABA_BR$ antagonist, CGP, and agonist, baclofen. Intravitreal injections of TPMPA and SR95531 were also made in Brown Norway rats. The effect of 2-AEMP on GABA-induced current was tested directly in isolated rat rod bipolar cells, and 2-AEMP was found to preferentially block $GABA_CR$ in those cells. Maximum amplitudes of dark (DA) and light-adapted (LA) ERG b-waves were reduced in $GABA_CR^{-/-}$ mice, compared to B6 mice, by 30–60%; a-waves were unaltered and oscillatory potential amplitudes were increased. In B6 mice, after injection of TPMPA (also in rats), 3-APMPA or 2-AEMP, ERGs became similar to ERGs of $GABA_CR^{-/-}$ mice. Blockade of $GABA_ARs$ and $GABA_BRs$, or agonism of $GABA_BRs$ did not alter B6 DA b-wave amplitude. The negative scotopic threshold response (nSTR) was slightly less sensitive in $GABA_CR^{-/-}$ than in B6 mice, and unaltered by 2-AEMP. However, amplitudes of nSTR and photopic negative response (PhNR), both of which originate from inner retina, were enhanced by TPMPA and 3-APMPA, each of which has $GABA_B$ agonist properties, and further increased by baclofen. The finding that genetic deletion of $GABA_CR$, the $GABA_CR$ antagonist 2-AEMP, and other antagonists all reduced ERG b-wave amplitude, supports a role for $GABA_CR$ in determining the maximum response amplitude of bipolar cells contributing to the b-wave. $GABA_CR$ antagonists differed in their effects on nSTR and PhNR; antagonists with $GABA_B$ agonist properties enhanced light-driven responses whereas 2-AEMP did not.

1. Introduction

GABA (γ-aminobutyric acid) is a major inhibitory neurotransmitter in the central nervous system, including in the retina (Lukasiewicz et al., 2004). Three classes of retinal GABA receptors have been described: ionotropic $GABA_A$ and $GABA_C$ (also known as GABA(A)-ρ) receptors that are linked to Cl^- ion channels, and metabotropic $GABA_B$ receptors that are coupled to G-proteins and work through second messenger systems to modulate K^+ or Ca^{2+} channels (Enz and Cutting, 1998).

In mammals, $GABA_A$ receptors ($GABA_ARs$) are expressed throughout the retina including on bipolar cell dendrites in the

* Corresponding author. College of Optometry, University of Houston, 4901 Calhoun, 505 J. Davis Armistead, Houston, TX 77204-2020, USA.

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outer plexiform layer (OPL) and terminals of bipolar cells, processes of amacrine and ganglion cells in the inner plexiform layer (IPL). $GABA_C$ receptors ($GABA_CR$) are more localized to bipolar cells than $GABA_AR$, mainly to axon terminals, but also have been observed in dendrites of bipolar cells in mouse retina (Haverkamp and Wässle, 2000; McCall et al., 2002). The presence of $GABA_CR$ in photoreceptors and horizontal cells is unresolved in mice. $GABA_CR$-gated current has been reported in horizontal cells in white perch (Qian and Dowling, 1993, 1994), but $GABA_CR$ antibody immunoreactivity has not been detected in photoreceptors in mammalian retina (Enz et al., 1996; Ogurusu et al., 1997) or in horizontal cells (Koulen et al., 1998a). $GABA_B$ receptor immunoreactivity has been observed presynaptically in amacrine and retinal ganglion cells, and in the processes of horizontal cells in mouse and rat retina (Koulen et al., 1998b; Zhang et al., 1998). Retinal Müller cells have $GABA_AR$s, but not $GABA_CR$ or $GABA_BR$, and they have GABA transporters that remove GABA from the extracellular space (Newman and Reichenbach, 1996).

GABA is released from amacrine cells in the retina and also has been reported to be released from horizontal cells (Deniz et al., 2011). GABA-mediated feedback and feed-forward inhibition are critical to normal processing of visual signals in the inner retina. Across species, the retinal GABA-induced current mediated by $GABA_AR$s is a fast transient response that quickly desensitizes, while the $GABA_C$ component is slow, sustained, and desensitizes more slowly (Lukasiewicz and Shields, 1998).

$GABA_CR$ also mediates a spontaneous tonic current, which is regulated by GAT-1 GABA transporters (Jones and Palmer 2009; (Ichinose and Lukasiewicz, 2002).

The electroretinogram (ERG) is a mass potential representing the summed activity of all retinal cells. In the dark-adapted (DA) ERG, responses to weak stimuli called the positive and negative scotopic threshold response (pSTR and nSTR) are related to activation of inner retinal neurons, i.e. the amacrine cells and/or ganglion cells, and mediated by Müller glia currents (Frishman and Steinberg, 1989; Saszik et al., 2002; Sieving et al., 1986). The initial negative a-wave of the DA-ERG is mainly associated with photoreceptor activity, but includes postreceptoral contributions (Hood and Birch, 1990; Robson et al., 2003). The positive going b-wave originates primarily from the rod bipolar cells in scotopic ERG (Robson and Frishman, 1995, 1998; Robson et al., 2004). Small waves superimposed on the leading edge of b-wave are oscillatory potentials (OPs) which reflect high-frequency activity of inner retinal circuits (Wachtmeister, 1998). In the light-adapted (LA) ERG, the a-wave originates mainly from cone photoreceptors and Off pathway neurons (Bush and Sieving, 1994; Robson et al., 2003). The b-wave originates from the activity of On- and Off-bipolar cells and is shaped by horizontal cell feedback onto cones (Sieving et al., 1994).

The ERG can be used to study the functional role of GABA receptors in the retina *in vivo*. Kapousta-Bruneau (2000) reported that a $GABA_C$ receptor antagonist, 3-APA (3-aminopropylphosphonic acid, 500 μM) (Vien et al., 2002) reduced b-wave maximum amplitude, and enhanced the negative scotopic threshold response (nSTR) and oscillatory potentials (OPs) of the dark-adapted ERG recorded from rat retina. Dong and Hare (2002) also found that a $GABA_CR$ antagonist TPMPA (1,2,5,6-tetrahydropyridine-4-yl-methylphosphinic acid) (Ragozzino et al., 1996) reduced b-wave amplitude in rabbit retina. However, McCall et al. (2002) reported that $GABA_CR^{-/-}$ mice lacking expression of both ρ1 and ρ2 subunits of $GABA_CR$ in the retina did not show reduced b-wave amplitudes, but did have enhanced OPs in the DA ERG, compared to those of wild type mice. More recently, Herrmann et al., 2011 observed reduced b-wave amplitudes in mice lacking functional $GABA_cR$. They suggested that $GABA_CR$ participates in modulating rod-driven bipolar cell responses by a mechanism involving tonic GABA-induced Cl^- current, and that this current sets the resting membrane potential to a more hyperpolarized level than would occur in the absence of active $GABA_CR$, thereby allowing a greater range over which the cells can depolarize. Because assessment of the b-wave is commonly used in clinical diagnostic procedures, a clear understanding of its origins should benefit clinical applications as well as basic research.

$GABA_C$ receptor function in retinal signal processing is not fully understood, and new receptor effectors could be useful for future studies. Chowdhury et al. (2007) reported the synthesis of 2-aminoethyl methylphosphonate (2-AEMP, chemical structure in Fig. 1 along with the structure of GABA and of other known $GABA_CR$ antagonists) and found this GABA analog to be a competitive antagonist to homomeric ρ1 $GABA_C$ receptors expressed in Xenopus oocytes. Further work showed competitive antagonism of 2-AEMP in neuroblastoma cells transfected with human GABA ρ1 subunit (Xie et al., 2011). To date, however, this relatively new antagonist has not been tested *in vivo*.

One aim of the present study was to investigate the role of traditionally inhibitory GABARs in retinal visual signal processing *in vivo* by studying their effects on ERGs, with a focus on $GABA_CR$ in shaping responses originating from bipolar cells in both rod and cone pathways. Another aim was to test the more recently synthesized $GABA_CR$ antagonist 2-AEMP by comparing its effect with the effect of $GABA_CR^{-/-}$, and of other known $GABA_CR$ antagonists on the mouse ERG. Preliminary results of this study have been reported in abstract form (Wang et al., *Invest Ophthalmol Vis Sci* 2009: E-Abstract 2179, Wang et al., *Invest Ophthalmol Vis Sci* 2011: E-Abstract 1603, Xie et al., *Soc Neurosci Abstr* 34:608.10).

2. Methods

2.1. Animals

Subjects were adult C57BL6 mice, 2–6 months old (Simonsen Labs, USA; Jackson Labs, USA, n = 76), to be referred as C57BL6/J (B6) mice, $GABA_CR^{-/-}$ mice, 2–3 months old (n = 12; from Dr. Maureen McCall, back crossed with B6 mice for over nine generations), and adult Brown Norway (BN) rats (n = 5, Charles River Laboratories, Inc.), 7 weeks to 6 months of age. Rats used for the isolated bipolar cell experiments at University of Illinois at Chicago

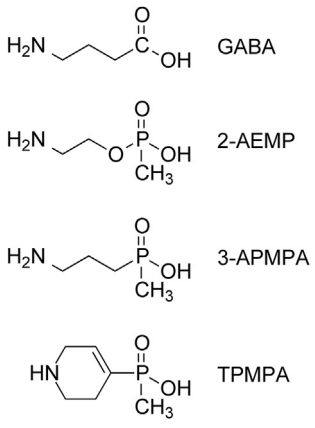

Fig. 1. Chemical formulae for GABA, 2-AEMP, TPMPA and 3-APMPA. The figure shows the structural similarities and differences between the inhibitory neurotransmitter, GABA and the $GABA_CR$ antagonists used in this study that varied in specificity for $GABA_CR$ (see Discussion). GABA, ã-aminobutyric acid; 2-AEMP, 2-aminoethyl methylphosphonate; phosphonic analogs: 3-APMPA (3-aminopropyl-(methyl) phosphinic acid) and TPMPA (1,2,5,6-tetrahydropyridine-4-yl-methylphosphinic acid).