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Voltage-gated sodium channels contribute to the b-wave of the rodent electroretinogram by mediating input to rod bipolar cell $GABA_C$ receptors

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ABSTRACT

Voltage-gated sodium (Na_v) channels are known to augment cone bipolar cell light responses, increasing the electroretinogram (ERG) b-wave in response to stimulus strengths above the cone threshold. However previous *in vivo* studies on a number of animal models have not found a role for Na_v channels in augmenting the b-wave in scotopic conditions below the cone threshold. We recorded ERGs from mice and rats using a series of TTX concentrations and tested retinal output to ensure complete Na_v channel block. We found that TTX concentrations sufficient to completely suppress retinal output caused large (~40%) decrease in the scotopic electroretinogram (ERG) response to high stimulus strengths (1.0 log cd s/m^2). In addition the b-wave was reduced by ~20% even at stimulus strengths that should predominately excite the rod pathway (−2.2 log cd s/m^2). Modulating stimulus strength and background luminance showed that Na_v channel contribution to the b-wave is strongest in mesopic conditions with low strength stimuli. Blocking $GABA_C$ receptors indicted that Na_v channels predominately contribute to the b-wave by supporting $GABA_C$ input to rod bipolar cells in addition to directly amplifying the light response of cone ON bipolar cells. We also determined that saturating levels of TTX reduced the rat b-wave below cone threshold. Na_v channels increase the ERG b-wave in both rod and cone bipolar cell-dominated circuits. In circuits involving rod bipolar cells the effect is mediated indirectly via GABAergic inhibitory cells, while Na_v channels directly located on cone bipolar cells amplify light responses in the cone pathways.

1. Introduction

Voltage-gated sodium (Na_v) channels are found in most of the cell types in the retina. *In vitro* electrophysiological studies have recorded Na_v currents in retinal ganglion cells (Lipton and Tauck, 1987; Skaliora et al., 1993; Tsai et al., 2011; Weick and Demb, 2011), in some amacrine cell subtypes including AII, (Tian et al., 2010; Wu et al., 2011), A17 (Hartveit, 1999), dopaminergic (Feigenspan et al., 1998; Gustincich et al., 1997), and starburst (Cohen, 2001), and more recently in cone bipolar cells (Ichinose et al., 2005; Ichinose and Lukasiewicz, 2007; Ma et al., 2005; Pan and Hu, 2000; Saszik and DeVries, 2012; Zenisek et al., 2001).

Na_v channels in ON cone bipolar cells have recently been demonstrated to contribute to the amplitude of the positive component of the ERG generated predominately by the ON bipolar cells (Robson and Frishman, 1998; Robson et al., 2004; Xu and Karwoski, 1994) referred to as the b-wave (Mojumder et al., 2007, 2008). This result has been repeated in a number of species including rat (Bui and Fortune, 2004, 2006; Li et al., 2005; Mojumder et al., 2007, 2008), mouse (Miura et al., 2009), and frog (Popova and Kupenova, 2010), and in most cases Na_v channels on cone ON bipolar cells increase the dark-adapted ERG b-wave at stimulus strengths sufficiently high to stimulate cones (but see Bui and Fortune, 2004). Interestingly in spite of *in vitro* electrophysiological, *in situ* hybridization, and immunohistochemical results

Abbreviations: CNQX, 6-Cyano-7-nitroquinoxaline-2,3-dion; ERG, electroretinogram; GABA, gamma-aminobutyric acid; Na_v, voltage-gated sodium channel; NMDA, *N*-Methyl-d-aspartate; PDA, *cis*-piperidine dicarboxylic acid; RBC, rod bipolar cell; TPMPA, (1,2,5,6-Tetrahydropyridin-4-yl) methylphosphinic acid; TTX, tetrodotoxin; VEP, visual-evoked potential.

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showing Na_v to be present in a majority of the cell types in the retina (Côté et al., 2005; Fjell et al., 1997; Mojumder et al., 2007; Kaneko and Watanabe, 2007; O'Brien et al., 2008) including amacrine cells that synapse with rod bipolar cells (RBCs) (Chávez et al., 2006, 2010) previous results have not shown that Na_v channels contribute to the rod-generated portion of the ERG.

Na_v channels, probably in retinal ganglion cells, have been shown to contribute to ERGs in response to weak stimuli (Bui and Fortune, 2006; Mojumder et al., 2007, 2008; Popova and Kupenova, 2010). However, in most studies blocking Na_v channels has little effect on the scotopic ERG b-wave at stimulus strengths above the scotopic threshold response but below the range of sensitivity of the dark-adapted cone bipolar cells (Bui and Fortune, 2004; Li et al., 2005; Mojumder et al., 2007, 2008; Popova and Kupenova, 2010; Viswanathan et al., 2004) consistent with a lack of evidence of Na_v channels in rod bipolar cells (RBC) (Pan and Hu, 2000). In rabbits however after a short dark adaptation TTX increases the b-wave, an effect attributed to third order inhibitory neurons (Dong and Hare, 2003) the findings of Awatramani (2001) in salamander. A recent *in vivo* study in rats found that TTX strongly reduced the amplitude of the b-wave in the range where rods should be contributing the b-wave when the retina was partially light adapted by a dim background (Mojumder et al., 2008). This effect was suggested to act via the secondary rod pathway which passes rod signals to the ON cone pathway via gap junctions between rods and cones (Abd-El-Barr et al., 2009), or via an Na_v channel-dependent interneuron that contributes to the b-wave only under mesopic conditions (Mojumder et al., 2008).

Recently it has been shown that lateral inhibition in the inner retina is mediated primarily by $GABA_c$ receptors on RBCs (mouse: Eggers and Lukasiewicz, 2006) and is strongly dependent on Na_v channels (rat: Chávez et al., 2006, 2010). Similarly lateral inhibition of bipolar cells is Na_v channel-dependent in many species (Cook and McReynolds, 1998; Shields and Lukasiewicz, 2003; Vigh, 2011; Volgyi et al., 2002). In addition, $GABA_c$ receptors on RBCs have recently been shown to mediate chloride currents hyperpolarizing the cell membrane and increasing the drive for cations to enter the cell during a light response (Herrmann et al., 2011). Blocking $GABA_c$ receptors substantially reduces the amplitude of the scotopic b-wave (Dong and Hare, 2002; Herrmann et al., 2011; Kapousta Bruneau 2000; Mojumder et al., 2008; Möller and Eysteinsson, 2003) suggesting that Na_v channels on amacrine cells in the inner retina could, in theory, increase the scotopic b-wave generated by rods. However inhibitory cells in both the inner and outer retina release GABA onto RBCs and the relative contribution of amacrine vs. horizontal cells to the b-wave is unknown.

Here we investigated the contribution of Na_v channels to the rodent ERG b-wave and found that in both mice and rats Na_v channels contribute strongly to the scotopic b-wave at stimulus strengths too low to excite cone bipolar cells. At saturating TTX concentrations the scotopic b-wave was reduced by approximately 40%, indicating that Na_v channels substantially amplify both rod and cone pathways. We also found an increased contribution of Na_v channels in response to dim but not bright stimulus strengths in mesopic conditions. The effects of TTX on RBCs were found to act predominately on $GABA_c$-mediated input.

Isolating the photoreceptor-to-bipolar cell circuit by blocking ionotropic glutamate receptors showed a direct contribution of Na_v channels to ON bipolar cells isolated to stimulus strengths that stimulate cones in addition to rods. The combined indirect contribution of Na_v channels on $GABA_c$-mediated input and direct contribution of Na_v channels on cone bipolar cells explain the majority of Na_v contributions to the ERG b-wave across diverse lighting conditions.

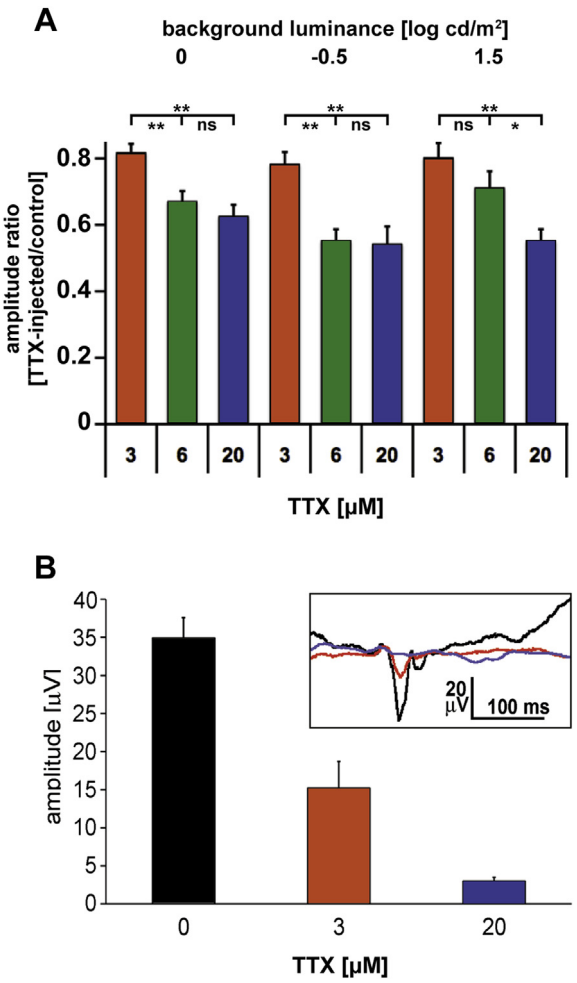

Fig. 1. Comparison of the effects of varying concentrations (3, 6, and 20 μM) of TTX on the amplitude of the b-wave in response to a high-strength stimulus. A) Normalized b-wave responses to high strength stimuli (1.0 log cd s/m^2) over scotopic, mesopic (−0.5 log cd/m^2), and photopic (1.5 log cd/m^2) backgrounds. TTX-treated eyes were compared to un-injected control eyes using paired Student's *t*-test. All TTX concentrations reduced the b-wave significantly compared to un-injected controls. The result of an ANOVA post-hoc analysis is indicated by significance levels * = $p \leq 0.01$. B) Amplitude of cortical visual evoked potentials at 0, 3, and 20 μM TTX. Inset depicts example traces. Error bars are standard error.

2. Materials and methods

2.1. Ethics approval

All animal procedures were completed in accordance to animal care guidelines established by the Canadian Council on Animal Care and in accordance with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research. Mice and rats were housed under a 12-h light/dark cycle with free access to food and water.

2.2. Electroretinography

C57Bl-6 mice between 8 and 16 weeks of age (Charles River Laboratories, St. Constant, QC, Canada) were dark-adapted for at least 8 h before being anesthetized under dim red light by intraperitoneal injection of Avertin (2,2,2 Tribromoethanol, Sigma Aldrich, St-Louis, MO) dissolved in amylene hydrate (tertiary amyl alcohol, 275 mg/kg, Sigma Aldrich). The pupils were dilated with the mydriatic agent cyclopenolate HCl 0.5% (Alcon, Fort-Worth, TX)