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Recovery of rod-mediated a-wave during light-adaptation in mGluR6-deficient mice

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Abstract

The purpose of this study was to compare the a-waves of mGluR6-deficient mice (KO) to that of wild-type mice (WT), and to determine whether the light-adapted electroretinogram of the KO mice originate exclusively from cones. Dark-adapted a-waves were recorded under the same conditions from both types of mice. With a 96-cd/m^2 background, the a-wave from both types of mice showed a rapid recovery over a 50-min period. The analysis of the a-waves in KO mice indicated that the recovery was determined mainly by the rod component. The light-adapted b-wave of WT mice showed no corresponding recovery. We conclude that rod contribution must be considered in the analyses of the light-adapted a-waves of KO mice.

Keywords: Mouse; mGluR6; Electroretinogram; a-Wave; Light-adaptation

1. Introduction

Altering the genome of mice is relatively easy, and such transgenic mice have served as valuable models of human retinal disease (Review: Peachey & Ball, 2003). The electroretinogram (ERG) can be used to assess the function of the outer retinal neurons, and the similarity in the origin of the different components of the ERG in humans and mice allows mice to be used in these studies. In addition, the techniques used for recording full-field ERGs from mice and humans have been established, and the feasibility of using ERGs as a screening tool for retinal dysfunction in mice has been demonstrated (Dalke et al., 2004).

Mouse models with specific alterations of their genome have also been used to study basic retinal functions, especially when they are related to the phototransduction pathway (Review, Lem & Makino, 1996; Peachey & Ball, 2003). In some transgenic mice, the physiology of the cone photoreceptors has been altered (Seeliger et al., 2001; Yang et al., 1999). This is important because, as opposed to rod photoreceptors, in vitro electrophysiological recordings from individual cones are limited in mammals other than primates (Nunn, Schnapf, & Baylor, 1984; Schnapf, Kraft, & Baylor, 1987). This is probably true because there are fewer cones, they are more widespread, and their shape and size are not conducive to electrical recordings. Thus, in vivo ERG analyses have been used to investigate the properties of the cone photoreceptors in mice.

It has been established that the a-wave of the ERG originates from the activity of the photoreceptor cells (Penn & Hagins, 1969), and the b-wave from the ON-type bipolar

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cells (Green & Kapousta-Bruneau, 1999; Gurevich & Slaughter, 1993; Kofuji et al., 2000; Stockton & Slaughter, 1989). The case may not be as straightforward, because the total ERG response, a-wave and b-wave, is a sum of two components; PII generated in inner nuclear layer and PIII generated by photoreceptors (Hood & Birch, 1992). In the primate ERG, the a-wave originates mainly from photoreceptor activity, mixed with small postreceptoral signals (Jamison, Bush, Lei, & Sieving, 2001; Robson, Saszik, Ahmed, & Frishman, 2003; Sieving, Murayama, & Naarendorp, 1994). In the photopic ERGs of rats, the contribution of second order neurons to the negative a-wave was reported to be much smaller (Xu, Ball, Alexander, & Peachey, 2003). Some of these ERGs were recorded after suppressing the b-wave with intravitreal injections of 2-amino-4-phosphonobutyric acid (APB or L-AP4).

Similar to rats under light-adapted conditions, the cone-mediated a-waves of mice are very small because they are masked by the large b-waves (Ekesten, Gouras, & Moschos, 1999; Peachey, Goto, Al-Ubaidi, & Naash, 1993). The use of intravitreal APB to isolate photoreceptor activity has been limited in mice probably because of the size of their eyes. Therefore, the pure cone-driven photoresponse in mice are mostly analyzed from the initial portion of the a-waves before the intrusion of the b-wave from bipolar activity (Lyubarsky, Chen, Simon, & Pugh, 2000, 2001; Pennesi, Howes, Baehr, & Wu, 2003). On this point, mouse strains lacking b-wave could serve as a helpful model, and mGluR6-deficient mouse (Masu et al., 1995), *nob* (no b-wave) mouse (Candille, Pardue, McCall, Peachey, & Gregg, 1999; Krishna, Alexander, & Peachey, 2002), and Gαo deficient mouse (Dhingra et al., 2000) have been studied.

The metabotropic glutamate receptor, mGluR6, is a subtype of the glutamate receptor that is sensitive to APB (Nakajima et al., 1993). mGluR6 regulates the entry of ions into the depolarizing ON-type bipolar cells responding to the excitatory amino acid released from photoreceptor cells of both rods and cones (Masu et al., 1995; Ueda, Iwakabe, Masu, Suzuki, & Nakanishi, 1997). A mGluR6-deficient mouse line has been created by conventional homologous recombination methods, and the ERGs of these mice have a negative shape with the absence of a b-wave, as might be expected. Unfortunately, the physiological properties of mGluR6-deficient mice have not been studied in detail. These mGluR6-deficient mice can serve as substitutes for APB-injected mice, and are thus valuable for studying the physiology of the photoreceptors. The mGluR6-deficiency would also remove the APB-sensitive feedback to the a-wave from second order neurons (Jamison et al., 2001; Robson et al., 2003). However, some caution is necessary when interpreting the results because of possible compensatory responses to the genetic deficiency.

The purpose of this study was twofold: first, to verify the similarity of the a-wave of wild-type mouse (WT) to that of mGluR6-deficient mouse (KO); and second, to determine whether the mouse light-adapted ERGs originate exclusively from the cone system. This latter is important because any contamination by rods would have to be taken into account in the interpretation of cone function in mGluR6-deficient animals.

2. Materials and methods

All experimental procedures were performed according to the Guidelines for Animal Experiments of Kyoto University, and the principles embodied in the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Experimental animals, kindly supplied by Dr. Shigetada Nakanishi of Kyoto University, were created on a 129/SvJ mouse strain (Masu et al., 1995) and maintained on a C57BL/6J background. The mice were 2- to 10-months-old and weighed 25–35 g. For the ERG recordings, the mice were anesthetized by an intraperitoneal injection of urethane (0.67 g/kg), xylazine (1.7 mg/kg), and ketamine (13.3 mg/kg). The pupils were dilated with 0.5% phenylephrine hydrochloride and 0.5% tropicamide. The anesthetized animals were placed on a heating pad, and a temperature probe was placed between the mouse body and the heating pad and maintained at around 35 °C.

A contact lens electrode, the inner rim of which was lined with gold wire (Kyoto Contact Lens, Kyoto, Japan), was placed on the anesthetized (0.4% oxybuprocaine hydrochloride) cornea covered with a layer of hydroxyl ethyl cellulose. A tungsten reference electrode was inserted subcutaneously on the forehead, and a ground electrode was attached to the tail. ERGs were recorded after more than 12 h of dark-adaptation.

Two 500 W Xenon lamps (LX-500 and LX-500F, Sanso, Tokyo, Japan) were used for the stimuli and steady background light. A pulse generator (SEN-7203, Nihon Koden, Tokyo, Japan) was used to control the duration and sequence of a guillotine-type shutter. Two short-arc power flash lamp units were used for the paired flash stimuli (SA-200F, Nissin Electronic, Tokyo, Japan). A detailed spectral distribution output of the Xenon light sources was not determined.

Fiber optic light bundles were used to carry the light stimuli into a Faraday cage and into a full-field stimulus dome (6.5 cm diameter, Sanso). Although no UV filter was used in the optical system, UV wavelengths shorter than 380 nm were not transmitted from the stimulus light through the glass fiber optics. The unattenuated luminance of the stimulus on the surface of full-field dome was 7000 cd/m^2 (ND 0) for the stimulus and 9600 cd/m^2 (bg-ND 0) for the background, calibrated in photopic units. In our Xenon system, photopic cd/m^2 almost equaled to scotopic cd/m^2, as calibrated by the manufacturer. Neutral density (ND) filters (Toshiba, Tokyo, Japan) were used to reduce the stimulus and background intensities. The stimulus protocols will be presented in detail in the Section 3.

To study the cone responses, four tandem 500 ms pulse stimuli were presented on a background illumination to suppress the rod responses. For the paired flash studies, the first