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Focal cone ERGs of rhodopsin Pro347Leu transgenic rabbits

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ABSTRACT

A rhodopsin P347L transgenic (Tg) rabbit, a model of retinitis pigmentosa, has been generated in our laboratory. The purpose of this study was to determine the properties of focal areas of the retina in this rabbit model during the course of retinal degeneration. To accomplish this, we recorded focal ERGs from wild-type (WT) and Tg rabbits at ages 3, 6, and 12 months. A 15° stimulus spot was used to elicit the focal ERGs from the center of the visual streak and from four surrounding areas. We found that the amplitudes of the focal cone ERG b-waves and oscillatory potentials (OPs) of the Tg rabbits in the five areas decreased progressively with increasing age and became almost non-recordable at 12 months. There were no significant regional differences in the b-waves of Tg rabbits recorded from the 5 areas. The amplitudes of the OPs were better preserved than the b-waves and the OPs/b-wave ratio was higher than that in WT rabbits at every recording area. The summed OPs amplitudes, which most likely originate from the amacrine and/or ganglion cells, recorded from the area superior to the optic disc was significantly larger than that from other areas at 3- and 6-months-old. This indicated that the inner retinal neurons were not altered equally after photoreceptor degeneration in this rabbit model.

1. Introduction

Retinitis pigmentosa (RP) is a group of inherited retinal diseases caused by mutations of genes related to the retina. These mutations result in degeneration of the rod photoreceptors followed by a gradual loss of cones. Photoreceptor degeneration is followed by the death of the inner retinal cells (Heckenlively, 1988; Weleber, Gregory-Evance, et al., 2006). The retinal degeneration usually begins in the periphery, and the function and morphology of cone-rich central area are relatively well preserved until the late stages of the disease process.

Different subjective and objective examinations have been used to assess the macular function in patients with RP. Among the objective methods, focal ERGs (Ikenoya et al., 2007; Sugita et al., 2008) and multifocal ERGs (Chan & Brown, 1998; Hood et al., 1998; Seeliger et al., 1998; Vajaranant et al., 2002) have been used. Our laboratory has developed a technique for recording focal cone ERGs while monitoring the location of the stimulus on the fundus with an infrared camera during the recordings (Kondo et al., 2008; Miyake, 1990; Miyake et al., 1989; Shiroyama & Miyake, 1990). In our system, focal ERGs can be recorded even in patients without good fixation, and the a- and b-waves and oscillatory potentials (OPs) elicited by focal stimuli resemble full-field cone ERGs.

To study the pathophysiology of RP, we have generated a rhodopsin P347L transgenic (Tg) rabbit using bacterial transgenes (Kondo et al., 2009). We found that the rod function of Tg rabbits was reduced at an early age whereas the cone function was relatively well preserved. We also noted that the oscillatory potentials (OPs), which are believed to originate mainly from amacrine and ganglion cells (Dong, Agey, & Hare, 2004; Wachtmeister, 1998), were larger than those of wild type rabbits (Sakai et al., 2009). Although the exact mechanism(s) underlying these secondary changes in the postreceptoral neurons has not been fully determined, our results indicated that the activities of the inner retinal neurons were altered in Tg rabbits (Marc et al., 2007).

The visual streak of the rabbit retina is a horizontal band lying inferior to the optic nerve head where the densities of rods and cones are higher than elsewhere in the retina (Famiglietti & Sharpe, 1995; Juliusson et al., 1994) Our histopathological study of Tg rabbits showed that the retinal degeneration developed earlier in the visual streak than in other areas (Kondo et al., 2009).

The conclusions made from these earlier ERG studies were mainly based on the results obtained from full-field ERGs, and we do not know whether there are local functional changes in the retina in this rabbit model. Thus, the purpose of this study was to determine the characteristics of local areas of the retina of Tg rabbits by recording focal ERGs. To accomplish this, we

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recorded and analyzed the changes in the b-waves and the OPs from 5 regions of the retina of Tg rabbits at 3, 6, and 12 months.

2. Materials and methods

2.1. Animals

The experiments were performed on 8 Tg and 9 wild type (WT) pigmented rabbits whose ages ranged from 3 to 12 months. After recording the ERGs, 1 Tg rabbit was euthanized at 3 months, and 3 WT and 3 Tg rabbits were euthanized at 6 months. All protocols were approved by the Institutional Review Board of Nagoya University Graduate School of Medicine and adhered to the EU Directive 2010/63/EU for animal experiments.

The techniques used for generating the Tg rabbits has been described in detail (Kondo et al., 2009). We used pigmented rabbits, a cross of Dutch pigmented rabbits and New Zealand albino rabbits because the stray light effects are lower in pigmented eyes. The animals were anesthetized with an intramuscular injection of 25 mg/kg ketamine and 2 mg/kg xylazine to record the ERGs.

2.2. Stimuli for focal ERGs

The system for recording focal ERGs consisted of a modified infrared fundus camera and an electronic pulse generator that controlled the light-emitting diodes (LEDs) used for the stimulus and background illumination (Kowa, Nagoya, Japan). An infrared television fundus camera was modified so that the stimuli were presented in Maxwellian view. The images from this fundus camera were fed to a television monitor with a 45° view of the posterior pole of the eye. The position of the stimulus spot on the fundus was monitored on the television screen and could be moved by the examiner with a joystick. A white LED was used for the stimulus and background illumination that covered a retinal area of 45°. The size of the stimulus spot was 15°, and the luminance of the background was fixed at 3 phot cd/m^2 for photopic conditions. The luminance of the stimulus spot was 30 phot cd/m^2, and the stimulus duration was 100 ms for photopic conditions. The luminance of the stimulus spot was 3 phot cd/m^2 and the stimulus duration was 10 ms for scotopic conditions. The stimulus repetition rate was fixed at 2 Hz. The luminance of the stimulus and background illumination was measured at the corneal surface and then converted to the value at the retinal surface. These luminances were measured with a photometer (Model IL 1700; International Light, Newburyport, MA).

2.3. Focal ERG recordings

The cornea was anesthetized with topical 1% tetracaine, and the pupils were dilated with topical 0.5% tropicamide and 0.5% phenylephrine HCl. The ERGs were recorded with a Burian-Allen bipolar contact lens electrode (Hansen Ophthalmic Development Laboratories, Iowa City, IA), and the ground electrode was attached to the ipsilateral ear. The responses were amplified, and the band pass filters were set at 0.5 to 1000 Hz. The ERGs were digitized at 5 kHz, and 500 responses were averaged (MEB-9100 Neuropack; Nihon Kohden, Tokyo, Japan).

To confirm that the stimulus and recording conditions elicited focal ERGs, we investigated the effect of stray light on the responses. We made an approximately 15° laser scar on the visual streak to two other WT pigmented rabbits (Fig. 1A), and we recorded the focal ERGs elicited by stimulating the visual streak and the photocoagulated area.

A fundus photograph of a 3-months-old WT rabbit is shown in Fig. 2, and the gray ellipse shown below the optic disc outlines the

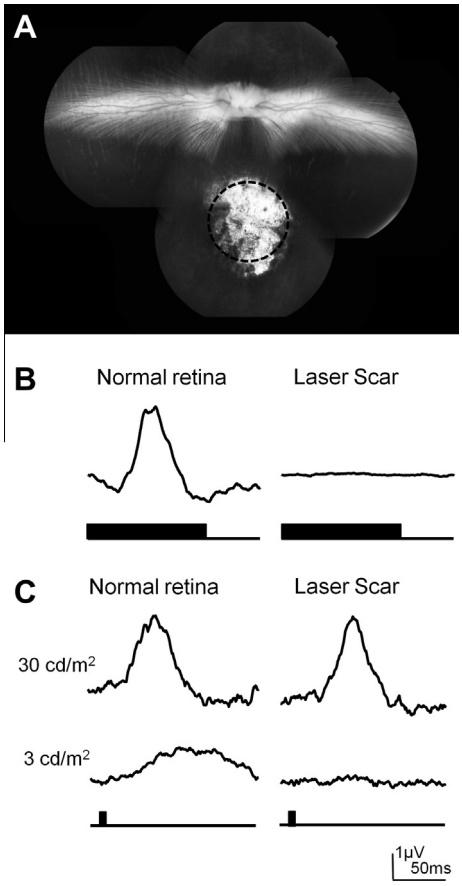

Fig. 1. Experiments to determine whether the focal ERGs arise from only the stimulated area. (A) Fundus photograph showing laser scar in the visual streak of a wild rabbit. Dotted circular indicates the area of stimulation to elicit focal ERGs. (B) Focal cone ERGs from normal retina (visual streak) and laser scar ERGs were recorded with a 3 cd/m^2 steady background. The luminance of the stimulus spot was 30 phot cd/m^2, and the stimulus duration was 100 ms. The focal ERGs were almost non-recordable from the area photocoagulated. (C) Focal rod ERGs from normal retina (visual streak) and laser scar. ERGs were recorded without background light. The ERGs recorded with stimulus intensity of 30 cd/m^2 with 10 ms duration are shown in the upper row. ERGs were recorded even from the area photocoagulated due to stray light. The ERGs recorded with stimulus intensity of 3 cd/m^2 with 10 ms duration are shown in the lower row. The focal ERGs were almost non-recordable from the area photocoagulated.

visual streak (Famiglietti & Sharpe, 1995; Juliusson et al., 1994). To compare the local retinal function of Tg and WT rabbits, we recorded ERGs from 5 areas of the retina as shown in Fig. 2. The ERGs were elicited by a 15° stimulus spot placed superior to the disc (A), nasal to the visual streak (B), center of the visual streak (C), temporal to the visual streak (D), and inferior to the visual streak (E).

2.4. Measurement of b-wave and OPs

The amplitudes of the b-waves and OPs were measured by a masked researcher. The amplitude of the b-wave was measured from the bottom of the negative a-wave to the top of the positive wave (Fig. 2, lower). To determine the appropriate band pass filters for isolating the OPs of WT and Tg rabbits, we first analyzed the ERGs by Fast Fourier Transform (FFT) as reported (Sakai et al., 2009). Based on the results, we chose band pass settings of 50–300 Hz for isolating the focal OPs (Fig. 2, lower). We measured the amplitude of each OP wavelet from the trough to the peak of the filtered waveforms, and one masked researcher checked the original waveform and decided on the position of the trough and peak of each OP. When a clear oscillation could not be found, we