Rod and Cone Components in the Dog Electoretinogram during and after Dark Adaptation

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ABSTRACT. In anesthetized beagle dogs, electoretinograms (ERGs) were recorded with full-field stimuli during, and after, dark adaptation. With blue stimuli, a cornea-positive b-wave appeared at 13 min in the dark, and increased in amplitude and peak latency with dark adaptation, reaching near-plateau level approximately 61 min after bleaching. This b-wave was considered to be derived from rods (scotopic b-wave) based on its spectral sensitivity function. After about 31 min, oscillatory potentials and a small cornea-negative wave preceding the b-wave appeared. With red stimuli, a small cornea-positive b-wave with a short peak latency appeared immediately after the start of dark adaptation. The amplitude and peak latency of this wave remained substantially unchanged for 16 min. It then gradually became obscure after the appearance, and increase in amplitude, of scotopic components. After 2 hr dark adaptation, ERGs elicited by blue and red stimuli showed essentially the same waveforms, and in contrast to the situation in humans and monkeys, the cone component was not easily distinguished in the waveform elicited by red stimulus. It was confirmed that the ERG elicited by red stimulus, under blue-green background light sufficient to eliminate rod components, included a cone-derived photopic b-wave. The ERGs elicited by red stimuli were not different from those elicited by blue stimuli, because the photopic b-wave had a very small amplitude relative to the scotopic b-wave and a peak latency very similar to that of the scotopic cornea-negative small wave.—KEY WORDS: canine (beagle), cone, dark adaptation, electoretinogram, rod.

The electoretinogram (ERG) is a bioelectrical potential recorded when the retina is stimulated by light. It represents the composite activity of different types of retinal cells, extending from the retinal pigment epithelium to at least the inner nuclear layer. The ERG has been studied in humans and other animal species for more than a century and has proved to be a good indicator for objective assessment of the retinal function [6, 10]. In recent human ophthalmology, the ERG has been used not only as a general examination of the functional integrity of the retina, but also as a valuable method for the detailed assessment of the retinal function in clinical and basic research studies [7–9].

The ERG has also been used as an examination in veterinary ophthalmology for various purposes such as the evaluation of the preoperative retinal function before cataract extraction, the diagnosis of retinal defects, and the classification of photoreceptor abnormality [1–3]. Like humans and monkeys [19], dogs have two basic types of retinal photoreceptors, rods and cones, and the ERG represents various combinations of rod- and cone-derived responses which are dependent upon conditions such as intensity, color and duration of stimulus light as well as adaptation conditions [10]. In order to successfully interpret ERG responses, it is thought to be vital to evaluate the rod and cone contributions to the recorded ERG.

In the present study, we recorded ERGs in anesthetized beagle dogs with full-field stimuli under several recording conditions, and examined the characteristics of the rod and cone components (mainly b-wave) in the recorded ERG during and after dark adaptation.

MATERIALS AND METHODS

Eight healthy purebred beagle dogs were used in this study. After pre-treatment with atropine sulfate (0.05 mg/kg s.c.), the animals were sedated with intravenous ketamine hydrochloride (5 mg/kg), and an endotracheal intubation was performed immediately after an intravenous injection of 40 μg/kg vecuronium bromide (Musculax Intravenous, Sankyo, Tokyo). During the experiment, the animals were paralyzed by a continuous infusion of vecuronium bromide in physiological saline (approximately 400 μg/kg/hr), and anesthesia was maintained by artificial ventilation with a mixture gas of 25% oxygen and 75% nitrous oxide. The end-tidal CO\(_2\) level, rectal body temperature, electrocardiogram and non-invasive arterial blood pressure were monitored continuously. Mydriasis was achieved with topical 0.5% tropicamide and 0.5% phenylephrine hydrochloride (Mydrin-P, Santen, Osaka) drops.

The recordings were made with a Pt contact lens electrode on the cornea. Skin electrodes were attached to the center of the forehead and inner pinna for use as reference and ground electrodes respectively. The signals were amplified (42 dB, 0.3–300 Hz at 3 dB) using a bioelectrical preamplifier, and the responses were then displayed on a CRT and stored on magnetic tape by a PCM recorder (PC204, Sony, Tokyo). After the experiment, the analogue responses were converted to digital by a signal processor (7T23S, NEC San-ei, Tokyo), and recorded on paper by a digital plotter (7550, Hewlett Packard, U.S.A.) via a personal computer (HP382, Hewlett Packard, U.S.A.).

A two channel optical system with a 150 W xenon arc
lamp light source (XL-151F, Wacom, Tokyo) was used as an optical stimulator. The test and background light were beamed onto a white translucent hemisphere (halved table-tennis ball) in front of the animal's eye, thus creating a full-field stimulus. The surface luminance of the inner hemisphere was measured by a luminometer (LS-110, Minolta, Tokyo), and the maximum surface luminance of the test and background lights, zero log unit intensity, were about 90.0 and 13.5 cd/m² respectively. The color and the intensity of the light was controlled by inserting Kodak Wratten color and neutral density filters (Kodak, U.S.A.) in each light path, and 8 msec stimuli were given by an electromagnetic shutter. Light adaptation (bleaching) was obtained by an intense white light (15,200 cd/m²) led by optical fiber from a 150 W tungsten light source.

The spectral sensitivity functions were determined by calculating the spectral intensity necessary to reach the criterion voltage for each interference filter used.

RESULTS

Figure 1 shows ERGs elicited by blue stimuli (Wratten No. 47B and ND 1.0) during dark adaptation after bleaching for 10 min. Stimuli were given from 1 to 91 min at 3 min intervals. Almost no responses were recorded up to 10 min. At 13 min, a cornea-positive b-wave was recorded, which then gradually increased in amplitude and in peak latency along with dark adaptation. After about 31 min, oscillatory potentials superimposing the ascending b-wave and a small cornea-negative potential preceding the b-wave were produced. The amplitude of the b-wave reached near-plateau level at about 61 min after bleaching. The mean amplitude and peak latency of the b-wave at 91 min were 287.5 μV and 81.6 msec respectively. The spectral sensitivity functions of this b-wave after 2 hr dark adaptation were obtained in three dogs (Fig. 2). The spectral sensitivity of the b-wave almost corresponded with that of the human rod system measured psychophysically (C.I.E. V’ λ [20]), and its peak wavelength was about 500–510 nm, a value thought to be well consistent with the absorption spectrum of the canine rod visual pigment [13]. On the basis of the results, the b-wave appearing at about 13 min was considered to be a rod-derived scotopic b-wave.

Figure 3 shows ERGs elicited by red stimuli (Wratten No. 26) during dark adaptation. The bleaching conditions and stimulation interval were the same as in the experiment.

Fig. 1. ERGs recorded by blue stimuli (Wratten No. 47B and ND 1.0) during dark adaptation after bleaching. Recordings were made every 3 min from 1 to 91 min after bleaching. From 61 min, responses are presented every 6 min. The bottom trace shows the time course of the stimulus.

Fig. 2. Spectral sensitivity functions of cornea-positive b-wave in Fig. 1 after 2 hr dark adaptation. The dashed curve represents the human scotopic luminous efficiency function (C.I.E. V’ λ [20]). The response criterion voltage was 100 μV. Different symbols represent measurements carried out on different dogs. Each set of symbols was vertically shifted for the fitting to the C.I.E. scotopic luminosity curve by eye. Measurements were made on three dogs.
described above. In contrast to the recordings with blue stimuli, a small cornea-positive b-wave with a short peak latency appeared immediately after the start of dark adaptation (1 min). The amplitude and peak latency of this wave remained almost unchanged from 4 to 16 min in the dark. The mean amplitude and peak latency at 10 min were 13.1 µV and 32.9 msec respectively. Due to the appearance and increase in amplitude of scotopic components, this small b-wave became obscure after about 19 min. After dark adaptation, the ERG waveform by red stimulus was almost the same as that elicited by blue stimulus. In humans and monkeys, it is possible to distinguish the photopic b-wave in ERG waveforms elicited by scotopically balanced red stimuli [10, 12], however, this was not the case in the dog ERG in the present study. To determine whether ERGs elicited by red stimuli included the cone component after dark adaptation, we recorded ERGs by red and blue stimuli in the presence of blue-green background light (Wratten No. 45) sufficient to eliminate the rod-derived responses (Fig. 4). In the absence of background light, ERG elicited by red stimulus and that elicited by blue stimulus showed closely similar waveforms, and a photopic b-wave was not distinguishable in the ERG elicited by red stimulus. As the intensity of background light increased, the scotopic b-wave gradually decreased in amplitude. No response to blue stimulus was recorded in the presence of most intense background light (right column, 0 log units). In contrast, a small cornea-positive b-wave was elicited by red stimulus in the presence of the same background light (left column, 0 log units). This small b-wave resembled those recorded early in dark adaptation by red stimuli and was thought to be a cone-derived photopic b-wave. Figure 5 is a superimposed illustration of the responses in Fig. 3, elicited by red stimuli during dark adaptation. This figure clearly shows that the

Fig. 3. ERGs recorded by red stimuli (Wratten No. 26) during dark adaptation from 1 to 91 min. From 61 min, responses are shown every 6 min. The bottom trace shows the time course of the stimulus.

Fig. 4. ERG waveforms recorded by scotopically balanced blue (right column) and red (left column) stimuli. Top row was recorded in the dark adapted state. Responses recorded in the presence of increasing intensity of blue-green background light (Wratten No. 45) are shown successively in the 2nd to 7th traces. The numbers on the left indicate the log relative intensity of the background light. The bottom trace shows the time course of the stimulus.

Fig. 5. Superimposition of the ERGs in Fig. 3. From 22 min, waveforms are shown every 6 min to facilitate observation of the relationship between the photopic b-wave and scotopic components. The bottom trace shows the time course of the stimulus.
amplitude of the photopic b-wave was very small relative to the scotopic b-wave and that its peak latency was almost the same as that of the small cornea-negative wave preceding the scotopic b-wave.

DISCUSSION

Since Piper's first report in 1911 [15], many investigators have studied the ERG of dogs, and they have proved that the ERG can be used as a tool for the objective assessment of the retinal function [1–3, 11, 16]. Particularly, in some inherited retinal degenerations such as progressive retinal atrophy in some breeds, the ERG is the only diagnostic procedure for early detection of affected dogs before the ophthalmoscopical abnormality becomes apparent [4, 5, 14]. For the exact diagnosis and classification of the various types of retinal degeneration, independent evaluation of rod and cone function is thought to be required. Gouras suggested that the starting point of any clinical ERG laboratory should be to separate the rod from cone components in the ERG [10].

In humans and monkeys, both the rod and cone components can be identified in the ERG recorded after dark adaptation [10, 12]. In the present study in dogs however, ERG waveforms elicited by scotopically balanced red and blue stimuli after dark adaptation showed closely similar waveforms. Although the red stimuli used were confirmed to elicit the photopic b-wave in recordings in the presence of background light sufficient to eliminate rod responses, the cone component could not be clearly distinguished in the waveform recorded under the dark adapted state by red stimulus. This phenomena has also been observed by other investigators [4, 14, 17, 18] but the reason for this species difference has not been investigated in detail, although Shaeppi and Liverani suggested that it may be due to the low sensitivity of the cone system in dogs [17]. The ratio of rods to cones in dogs is not so much different from that of humans and monkeys [19], however, it was reported that the light sensitivity of the cone system in dogs is only 1/30 of that in humans, whereas the rod system has the same sensitivity to white light flashes [18]. In the present study, it was confirmed that the amplitude of the photopic b-wave was much smaller than that of primates, whereas that of the scotopic b-wave was comparable. This small amplitude of the photopic b-wave seems to be one of the major factors making the photopic component undetectable in the ERG elicited by red stimulus, as suggested by Shaeppi and Liverani [17, 18]. In addition, the relationship between the peak latency of the photopic b-wave and those of scotopic components, particularly the small cornea-negative wave, is thought to be another major factor. In the recordings elicited by red stimuli during dark adaptation, a cone-derived photopic b-wave was recorded early in dark adaptation, but it gradually became obscured by the appearance and growth of the scotopic components. Furthermore, the photopic b-wave can not be detected after the appearance of the small cornea-negative wave preceding the scotopic b-wave, because its peak latency is closely similar to that of the small cornea-negative wave (Fig. 5).

We have shown in the present study that unlike in humans and monkeys, it is difficult to analyze the cone components of the canine ERG record elicited by scotopically balanced red stimuli after dark adaptation. We therefore conclude that it will be necessary to record the cone responses predominantly, by using intense background light sufficient to eliminate the scotopic components, or by using rapid flickering stimuli to which the rod responses fuse, for the detailed examination of the cone component in the canine ERG.

REFERENCES


