Doc Ophthalmol (2009) 119:79–88
DOI 10.1007/s10633-009-9173-x

ORIGINAL RESEARCH ARTICLE

Comparison of the effects of three different combinations of general anesthetics on the electroretinogram of dogs

Man Bok Jeong · Kristina Narfström · Shin Ae Park · Je Min Chae · Kang Moon Seo

Received: 15 October 2008 / Accepted: 28 March 2009 / Published online: 14 April 2009

Abstract The objective of this study is to compare the effects of three different anesthetic combinations on the electroretinogram in the same animals under similar laboratory conditions. Thiopental–isoflurane (TI), medetomidine–ketamine (MK), and xylazine–ketamine (XK) were used on each of 12 healthy miniature schnauzer dogs (MS) with a period of at least 3 weeks in between subsequent anesthesia protocols, using the Dog Standard Protocol. The scotopic ERGs consisted of scotopic low stimulus strength (S) responses designated S1, S2, S3, S4, and S5, at 1, 5, 10, 15, and 20 min after dark adaptation, respectively, and scotopic standard stimulus strength (S-ST) responses. The photopic ERGs consisted of a photopic single flash (P) response and 31 Hz flicker (P-FL) responses. For S-ST (2.5 cd s/m^2), the amplitude of the a-wave using TI was significantly lower than that using MK (adjusted $P = 0.05$) and XK (adjusted $P = 0.03$), and the implicit time of the a-wave was significantly shorter than that using MK (adjusted $P = 0.04$). For P (2.5 cd s/m^2), the amplitude of the b-wave using XK was significantly higher than that using MK (adjusted $P = 0.01$). The implicit times of the b-wave using TI was significantly longer and shorter than that of MK for S1, S2 and P-FL and for S4 and S-ST, respectively, and than that of XK for S2 and P-FL and for S5 and S-ST, respectively. The results of the present study showed that TI affected both the amplitude and the implicit time of the a-wave for S-ST and the implicit time of the b-wave relatively more so than was the case when using XK or MK. Therefore, it appears that either XK or MK could be advantageous to use rather than TI for clinical studies.

Keywords Dog standard protocol · Electroretinography · Medetomidine–ketamine · Thiopental–isoflurane · Xylazine–ketamine

Abbreviations

cd	Candela
ECVO	European College of Veterinary Ophthalmologists

M. B. Jeong · S. A. Park · J. M. Chae · K. M. Seo (✉)
Department of Veterinary Surgery and Ophthalmology, College of Veterinary Medicine and BK21 Program for Veterinary Science, Seoul National University, San 56-1, Sillim 9-dong, Gwanak-gu, Seoul 151-742, Republic of Korea
e-mail: kmseo@snu.ac.kr

M. B. Jeong · K. Narfström
Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO 65211, USA

K. Narfström
Department of Ophthalmology, Mason Eye Institute, University of Missouri, Columbia, MO 65211, USA

J. M. Chae
Department of Ophthalmology, School of Medicine, Johns Hopkins University, 600 N. Wolfe st. 255 Woods Bd., Baltimore, MD 21287, USA

ERG	Electroretinography
IM	Intramuscular injection
TI	Thiopental–isoflurane
MK	Medetomidine–ketamine
MoM	Multiple of the medians
MS	Miniature schnauzer dogs
P	Photopic single flash
P-FL	31 Hz flicker
S	Scotopic low stimulus strength
SC	Subcutaneous injection
IV	Intravenous injection
S-ST	Scotopic standard stimulus strength
XK	Xylazine–ketamine

Introduction

Full field flash electroretinography (ERG) records the summed transient electrical responses obtained from the entire retina elicited by a light stimulus, allowing an objective evaluation of retinal function in humans and animals [1]. The technique has been broadly used for the evaluation and diagnosis of hereditary retinal degenerative disease processes, sudden acquired retinal degeneration, and optic neuritis in animals [2, 3]. It is also used for the evaluation of new drugs or devices in toxicity studies and in the appraisement of preoperative retinal function for cataract surgery in animals, including dogs and cats [4–6].

In order to obtain reliable ERG recordings, it is important to appropriately immobilize the animals tested [7]. Movements such as a blink, an eyelid twitch or movement of the globe by conscious animals, may interfere with the delivery of light to the eye and cause noise in the recordings [4]. Movement of the animal tested may change the waveform, which can result in a misdiagnosis of a possible retinal disease. Therefore, it is imperative to restrain the animals tested using general anesthesia throughout the ERG procedure, especially, when evaluating hereditary retinal degenerations.

However, many of the commonly used anesthetics have prominent effects on retinal function, shown for humans and animals [8–11]. Some anesthetics affect the ERG in a dose-dependent manner. Even the route of administration and species differences may affect the ERG [12–14]. The effect of the anesthetics, particularly anesthetic combinations, may disguise slight changes in amplitude and/or the implicit time of the a- and b-waves of the ERG when studying retinal electrical responses to low light stimulus strength, and particularly in the early stages of an inherited retinal degeneration like progressive retinal atrophy. It is therefore extremely important to study the effect of anesthetics on the ERG. It is also crucial to think about the species variation, and to elucidate the anesthetic method suitable for each type of animal [15]. The anesthetic agent and dosage that interferes the least with phototransduction and neuronal function can then be recommended. It is also important that the personnel involved with the anesthetic protocol are comfortable with the specific method used.

Many studies have been performed and reported in order to elucidate the effects of various anesthetics on the retina of dogs, using different ERG protocols. The anesthetics that have been used are medetomidine–ketamine (MK), xylazine–ketamine (XK), isoflurane, halothane, sevoflurane, and propofol [9, 10, 14, 16–18]. It is therefore reasonable for investigating the effects of different anesthetic combinations on the ERG to directly compare the ERG results obtained using a standardized ERG and anesthetic protocol in the same animal. Further, to our knowledge, there have been no reports comparing thiopental–isoflurane with other anesthetics in dogs.

The purpose of the present study was therefore to compare three different anesthetic combinations frequently used clinically in dogs: thiopental–isoflurane, medetomidine–ketamine, and xylazine–ketamine, using the dog ERG protocol recommended by ECVO [19] in the same MS under similar laboratory conditions.

Materials and methods

Animals

Twelve healthy MS (12 eyes, 8 males, 4 females) were used for the present study. The age range of animals was from 1 to 4 years (mean ± SD; 2.1 ± 0.9) and their weight was 5.5 to 11.2 kg (mean ± SD; 8.1 ± 1.8). Ophthalmic examinations, including slit lamp biomicroscopy (SL-202P, Shih-nippon commerce, Tokyo, Japan) and indirect ophthalmoscopy (Vantage, Keeler instruments Inc,

Broomall, PA, USA), showed no abnormalities in the tested eyes. The experiments adhered to the strict guidelines of the "Guide for the Care and Use of Laboratory Animals" of the Institute of Laboratory Animal Resources of Seoul National University, Korea.

Preparation of animals for ERG

All animals were fasted for at least 12 h before performing the ERG recordings. Maximal pupillary dilation was obtained by applying one drop of 1% tropicamide (Mydriacyl, Alcon Inc, Puurs, Belgium) to the test eye, every 30 min from at least 1 h prior to beginning the ERG session. Animals were brought to the examination room and anesthesia was induced under ambient light. Each of three different anesthetic combinations was used on each of the 12 dogs with a period of at least 3 weeks in between subsequent anesthesia protocols.

Anesthetic combinations

Thiopental/isoflurane (TI)

The dogs were premedicated with atropine sulfate 0.04 mg/kg, SC (Je-Il atropine sulfate, Je-Il pharmacy, Daegu, Korea). They were kept in a cage in a silent area under ambient light for ~10 min. Induction of anesthesia was performed by administering thiopental sodium 15 mg/kg, IV (Thionil, Daihan Pharm Co Ltd, Ansan-si, Korea) in the cephalic vein, after which the dogs were endotracheally intubated. Anesthesia was maintained via inhalation of clinically indicated concentrations of 2.5% isoflurane (Terrell, Mindra Inc, Bethlehem, PA, USA) delivered in 100% oxygen with flow rates of 1 l/min. The anesthesia was monitored by a veterinarian. For most of the dogs, the concentration of isoflurane was kept constant during the ERG procedure. The exhaled CO_2 was removed by an absorbent in the canister.

Medetomidine/ketamine (MK)

Each of the 12 experimental animals was given medetomidine 60 μg/kg, IM (Domitor, Pfizer animal health Korea Ltd, Seoul, Korea). The dog was kept in a cage in a silent area under ambient light for ~10 min. After that, ketamine 5 mg/kg, IM (Yuhan Ketamine, Yuhan Corporation, Gunpo-si, Korea) was administered to provide for general anesthesia.

Xylazine/ketamine (XK)

The dogs were premedicated with atropine sulfate (0.04 mg/kg, SC). They were kept in a cage in a silent area under ambient light for ~10 min. Tranquilization was induced by administration of xylazine 2.2 mg/kg, IM (Rompun, Bayer Korea, Ansan-si, Korea). After 10 min, general anesthesia was achieved by the injection of ketamine (11 mg/kg, IM).

ERG procedures

Electroretinograms were recorded from the left eye of each dog using the ERG equipment (RETIport, Roland Consult, Brandenburg, Germany). A contact lens electrode (Kooijman/Damhof ERG lens, Medical Workshop BV, Groningen, Netherland) with a built-in white LED 4 W as an active electrode was placed on the cornea after application of 0.3% hydroxypropyl methylcellulose (Artear, Unimed pharm, Seoul, Korea). Platinum subdermal needle electrodes (Model F-E2, Astro-Med Inc, West Warwick, RI, USA) were used for the reference and ground electrodes, and were placed approximately 2 cm caudal to the lateral canthus, and over the external occipital protuberance, respectively. Topical 0.5% proparacaine hydrochloride (Alcaine, Alcon, Puurs, Belgium) was utilized to anesthetize the cornea. The electrodes were connected to a preamplifier, and signals were amplified with a band pass filter between 1 and 300 Hz. Lights in the examining room were turned off ~10 min after induction of anesthesia by thiopental and after injection of medetomidine or xylazine for initiation of dark adaptation.

The LED stimulating unit of the ERG system used in the study delivered white flashes of light 1.0 cm from the cornea. Signal averaging of ERG responses was not performed in the present study. Each ERG session consisted of scotopic and photopic ERGs. Two different stimuli were used for scotopic ERGs: scotopic low stimulus strength (S) responses for rods and scotopic standard stimulus strength (S-ST) responses for mixed rod and cone responses. The S responses were elicited using a 0.025 cd s/m^2 single flash at 1, 5, 10, 15, and 20 min after dark adaptation

(designated S1, S2, S3, S4, and S5, respectively). After the 20 min recording, the light stimulus strength was increased to 2.5 cd s/m^2 and the S-ST responses were recorded. The photopic ERGs for evaluation of the cone system consisted of two different responses: after 10 min of light adaptation (background luminance: 25 cd/m^2), a photopic single flash (P) response for evaluation of cones, and 31 Hz flicker (P-FL) responses for evaluation of the fast components of the cone pathways were recorded with a 2.5 cd s/m^2 flash or flashes using the same 25 cd/m^2 background luminance. The duration of the stimulus flash was 0.005 ms for the S response and 0.5 ms for the S-ST, P, and P-FL responses, respectively. For all procedures, the dogs were positioned on sternal recumbency throughout the recordings and the head positioned using a pack of towels. Conjunctival stay sutures were used to position the eyes and to avoid rotation of the globe.

The amplitudes and implicit times of the a- and b-waves were measured for S-ST and P responses. The a-wave amplitude was measured from the baseline to the trough of the a-wave, and the b-wave amplitude was measured from the trough of the a-wave to the following positive peak. Implicit times of a- and b-waves were measured from the onset of light stimulus to the a-waves trough and b-waves peak, respectively. For the S responses and P-FL responses, only b-wave amplitudes and peak implicit times were obtained and analyzed. Amplitudes of the P-FL responses were measured from the baseline to the positive peak and implicit times from the light onset to the positive peak.

Statistical analysis

The repeated measures analysis of variance (Anova) was used since the same dog was measured under different conditions: three combinations of anesthetics and different levels of light stimulus strength. Least square means (LSM) and a Wald test were used to test differences among the three different combinations of anesthetics, at a given level of light stimulus strength. In view of these multiple tests, the False discovery rate (FDR) was used to control the rate of false discoveries. Thus, for these multiple tests, FDR adjusted P values less than or equal to 0.05 are reported under results. The analysis was done in the mixed procedure using statistical computer software (SAS, version 9.1, SAS Institute Inc., Cary, NC, USA).

For data description purpose only, the multiple of the medians (MoM) and 90% reference range were used [20]. The MoM expresses the data points of the amplitude and implicit time of the a- and b-wave as a proportion of the median value for the three different combinations of anesthetics. The MoM was used to establish normative values for each type of anesthetics used in the MS breed of dogs, in the age group 1–4 years. The lower limit and the upper limit of the 90% reference range are defined as the 5th percentile and the 95th percentile of the MoM distribution, respectively.

Results

Waveform of ERG components

Figure 1 displays examples of typical ERG waveforms obtained from one of the MS using the three different combinations of anesthesia. As shown in the figure, the various combinations of anesthetics used in the study generated rather similar waveforms for the different test sessions. For all anesthetic combinations, the S responses had a prominent b-wave that increased in amplitude and in implicit time during the 20 min of dark adaptation. The a-wave was not recordable using this low light stimulus strength. In the S5 response obtained after 20 min of dark adaptation, 67% of the dogs anesthetized with XK had longer implicit time for the scotopic b-wave than those of TI. Similarly, 58% of the dogs on XK had longer implicit time for the scotopic b-wave than those of MK. The S-ST responses had prominent a- and b-waves, and oscillatory potentials were observed during the ascending phase of the b-wave using all anesthetic protocols. The P response had a smaller but faster b-wave than that of the S response. The P-FL responses consisted of b-waves only.

Parameters of ERG components

Figure 2 demonstrates LSM changes in amplitudes and implicit times of a- and b-waves obtained using the three different anesthetic protocols in the same animals for each ERG test session. For the S-ST responses, the amplitude of the a-wave using TI was

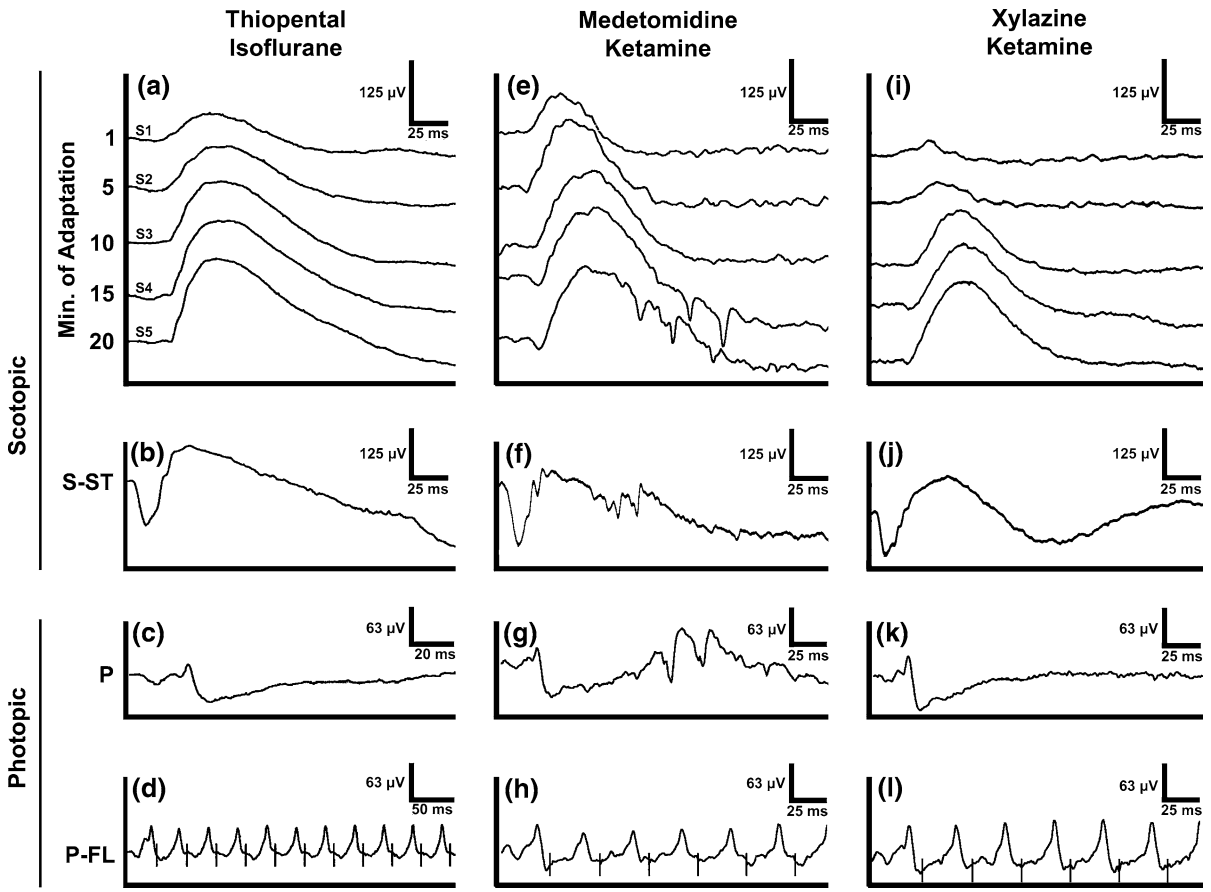

Fig. 1 Actual (original) ERG waveforms recorded during use of three different combinations of anesthesia in the same miniature schnauzer dogs. Scotopic low stimulus strength responses (**a**, **e**, and **i**) are obtained using 0.025 cd s/m^2 of light stimulation at 1, 5, 10, 15, and 20 min, respectively, after dark adaptation designated S1, S2, S3, S4, and S5 (from top to bottom). Scotopic standard stimulus strength responses (**b**, **f**, and **j**) are depicted as S-ST responses and obtained at 2.5 cd s/m^2 of light stimulation. Photopic single flash responses (**c**, **g**, and **k**) and photopic 31 Hz flicker response (**d**, **h**, and **l**) are depicted as P and P-FL, respectively, both at 2.5 cd s/m^2 after 10 min of light adaptation using 25 cd/m^2 of background light. The light stimulus coincides with the *y*-axis of each recording. Note that the time scale varies between some of the photopic recordings

significantly lower than those using MK and XK (Fig. 2a; adjusted $P = 0.05$ and adjusted $P = 0.03$, respectively), while the implicit time of the a-wave was significantly shorter than when using MK (Fig. 2b, adjusted $P = 0.04$). For the P responses, there was no significant difference in the amplitude and implicit time of the a-wave among the three different anesthetic protocols (Fig. 2a, b). The b-wave amplitudes of all responses, except the P responses, were not significantly different for the three different anesthetic protocols (Fig. 2c). For the P responses, the amplitude of the b-wave using XK was significantly higher than when using MK (adjusted $P = 0.01$). For the S1 and S2 responses, the implicit times of the b-waves using TI was significantly longer than when using MK (adjusted $P = 0.01$ and adjusted $P = 0.007$, respectively) and XK for the S2 responses (adjusted $P = 0.03$) (Fig. 2d). For the S4 responses, the implicit times of the b-waves using TI was significantly shorter than when using MK (Fig. 2d, adjusted $P = 0.01$). For the S5 responses, the implicit time of the b-wave using TI was significantly shorter than when using XK (Fig. 2d, adjusted $P = 0.01$). For the S-ST responses, the implicit time of the b-wave using TI was significantly shorter than when using MK and XK (Fig. 2d, adjusted $P = 0.03$ and adjusted $P = 0.002$, respectively). For the P responses, the implicit time

of the b-wave using TI was significantly longer than when using XK (Fig. 2d, adjusted $P = 0.004$). For the P-FL responses, the implicit time of b-wave using TI was significantly longer than when using MK and XK (Fig. 2d, adjusted $P < 0.0001$ and adjusted $P = 0.004$, respectively).

Graphical illustration of the reference ranges

Figure 3 illustrates a graphical representation of the ERG results using the MoM and 90% reference range.

Discussion

The purpose of the present study was to compare the effects of three different combinations of anesthetics on the ERG in the same dog using the same ERG protocol under similar laboratory conditions. To our knowledge, this is the first study that compares TI with MK and XK in dogs, all of which have been widely used in the veterinary practice. Stable and reliable ERG waveforms were obtained through all three different anesthetic methods using the ECVO recommended long protocol for dogs. The most important finding in the present study was that TI markedly affected the amplitude and implicit time of the a-wave for the S-ST response and the implicit time of the b-wave in comparison to MK and/or XK (Fig. 2). Further, on the basis of the results found in the present study, it was observed that XK or MK resulted in significantly higher a-wave amplitudes when using S-ST stimuli compared to responses obtained when using TI (Fig. 2a).

The stable and reliable ERG waveforms that were obtained in the present study for all three different combinations of anesthetics means that the different anesthetic protocols could be equally employed for ERG recordings in dogs for clinical purposes (Fig. 1).

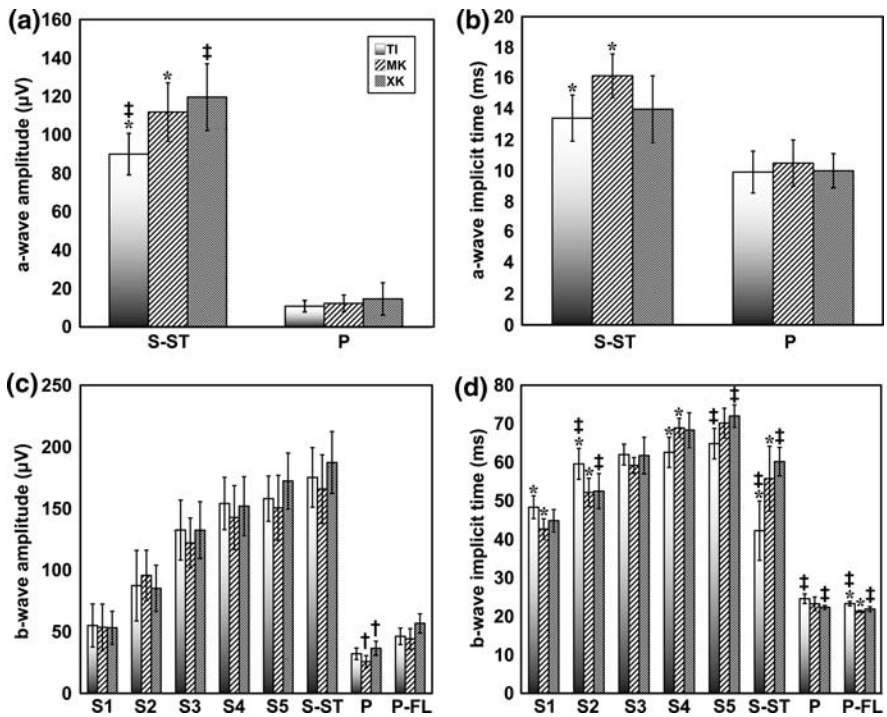

Fig. 2 Comparison of least square means and 95% confidence interval (LSM ± 1.96 SE) of amplitudes (**a** and **c**) and implicit times (**b** and **d**) on the vertical axis of a- and b-waves recorded by TI, MK, and XK. *, †, and ‡ indicate a significant difference between TI and MK, MK and XK, and XK and TI, respectively. On the horizontal axis, S1 to S5 depicts scotopic low stimulus strength responses obtained using 0.025 cd s/m^2 of light stimulation at 1, 5, 10, 15, and 20 min, respectively, after dark adaptation designated S1, S2, S3, S4, and S5. S-ST depicts scotopic standard stimulus strength responses obtained at 2.5 cd s/m^2. A photopic single flash response and 31 Hz flicker responses are depicted as P and P-FL, respectively, both at 2.5 cd s/m^2 after 10 min of light adaptation using 25 cd/m^2 of background light

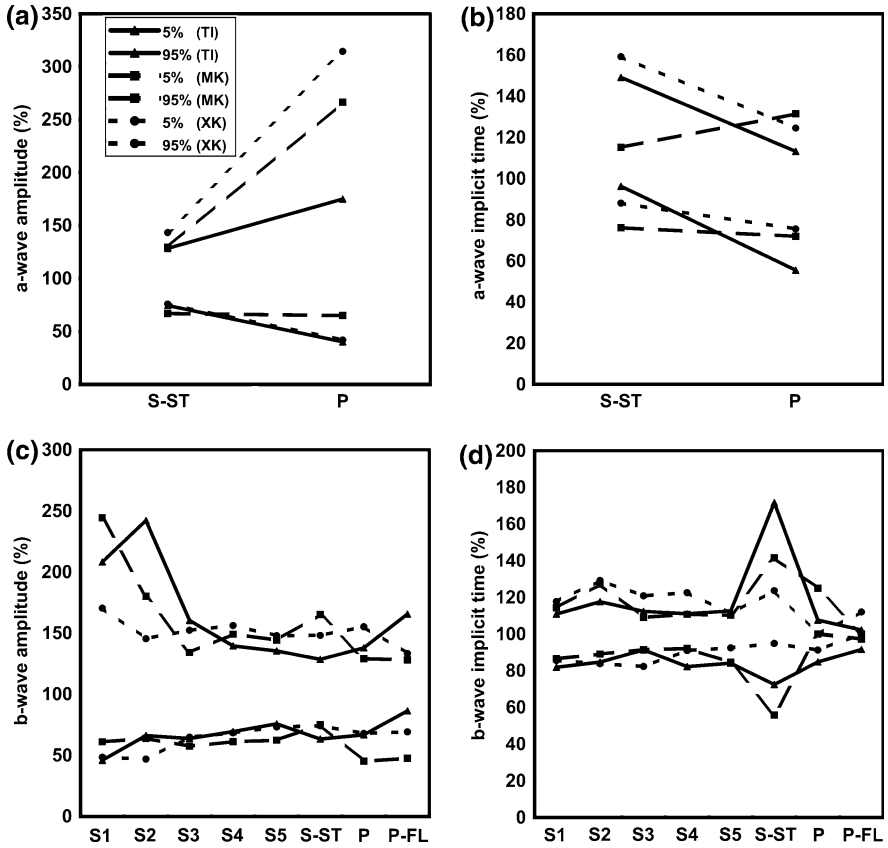

Fig. 3 Graphical illustration of differences in ERG results between TI, MK, and XK using the MoM and 90% reference range. The lower limit and the upper limit of the 90% reference range are defined as the 5th percentile and the 95th percentiles of the MoM distribution, respectively. Amplitude and implicit time values on the vertical axis for the a- and b-waves (MoM distribution), respectively, are shown as a proportion of the median value obtained by the three different combinations of anesthesia when 100% represents the median of these values. On the horizontal axis, S1–S5 depicts scotopic low stimulus strength responses obtained using 0.025 cd s/m^2 of light stimulation at 1, 5, 10, 15, and 20 min, respectively, after dark adaptation designated S1, S2, S3, S4, and S5. S-ST depicts scotopic standard stimulus strength responses obtained at 2.5 cd s/m^2. A photopic single flash response and 31 Hz flicker responses are depicted as P and P-FL, respectively, both at 2.5 cd s/m^2 after 10 min of light adaptation using 25 cd/m^2 of background light

The waveforms obtained were comparable to those of previous reports using each of the three anesthetic protocols [9, 10, 17, 21, 22]. A previous study regarding the effects of isoflurane on the ERG of dogs using the human ERG protocol [17], recommended by the International Society for Clinical Electrophysiology of Vision [23], with similarities to the protocol used in the present study, showed that the resultant waveform elicited by each ERG stimulus was comparable to those obtained in the present study. Some of the waveforms illustrated in Fig. 1 when using MK showed artifacts, ~80–120 ms after the flash stimuli. These artifacts were due to slight muscle twitching and did not affect the peaks of the a- and b-waves (Fig. 1e–g).

In the present study, the amplitude of the a-wave using TI was significantly lower than those using MK and XK; while the implicit time of the a-wave was significantly shorter than when using MK for the S-ST responses (Fig. 2a, b). Considering the origin of the a-wave and contribution to the b-wave of bipolar and Müller cells, it appears that there is very little interference with the hyperpolarization of the photoreceptors themselves after light stimulation when using the MK and XK protocols. This finding suggests that TI influences outer retinal function in dogs. In an earlier study, inner retinal function was shown to be influenced by isoflurane in pigs [24, 25]. Volatile anesthetics, such as isoflurane, enflurane, and halothane, bind directly to ligand-gated ion

channels in the central nervous system and the retina [26], altering kinetics of certain neurotransmitters, including glutamate and γ-aminobutyric acid (GABA), major excitatory and inhibitory neurotransmitters, respectively. Previous studies reported that glutamate release from synaptosomes and the metabolic breakdown of GABA were inhibited by volatile anesthetics in the rat [27–29]. Isoflurane, especially, may bind to $GABA_A$ receptors as other volatile anesthetics do, mediating inhibitory neurotransmission. Similar to thiopental–isoflurane, the combination of thiopentone, halothane, and nitrous oxide anesthesia showed a highly significant decrease in the amplitude of a-wave only when compared to the combination of xylazine and ketamine [10].

Ketamine is a nonbarbiturate, rapid, and short acting anesthetic agent. In contrast to the volatile anesthetics, ketamine appears to induce anesthesia by inhibition of *N*-methyl-D-aspartate receptors, which is a subtype of the glutamate receptors. In order to neutralize ketamine's properties, such as found with dissociative anesthetics, which may cause side effects such as tremor and hallucinations, ketamine is commonly used in combination with xylazine or medetomidine. These have alpha-adrenergic agonist actions leading to analgesia, sedation, and muscular relaxation in dogs. Therefore, there are many previous ERG studies using the combination of ketamine and xylazine or medetomidine in various species [9, 21, 22]. A previous study, which used a combination of medetomidine and ketamine, the same light stimulus strength (2.5 cd s/m^2), and a similar dark adaptation time as the present study, reported the amplitude of the b-wave to be similar to that of the S-ST responses obtained in the present study (Fig. 2c) [22].

The reason for not using atropine before MK is that currently the use of atropine preoperatively continues to be controversial [30, 31]. Conventionally, anticholinergics are usually administered preoperatively to prevent severe bradycardia as a result of surgical manipulation (vagal reflexes). This is also performed before administration of other anesthetics such as α_2-agonists and opioids. Even though medetomidine is similar to xylazine as an α_2-agonist, it was documented that α_2-adrenoreceptor is $\sim$10 times more potent than the latter [32, 33]. As a result, the pharmacological effects of medetomidine can be stronger than that of xylazine on the ERG. The reason is that anticholinergic administration increases the vagal tone transiently, which may increase the incidence of bradyarrhythmias induced by administration of an α_2-agonist. Especially, medetomidine administration is associated with profound increase in arterial blood pressure, systemic and pulmonary vascular resistance, and myocardial workload. Alibhai et al. [34] reported that even though atropine administration could prevent bradycardia caused by medetomidine, its use could result in prolonged and severe hypertension associated with tarchycardia. Ko et al. [35] have also demonstrated that pre-emptive administration of atropine in dogs sedated with medetomidine induces hypertension. Thus, there is a trend not to use atropine as premedication with medetomidine in the veterinary practice.

An earlier study, however, showed that the combination of ketamine and xylazine may increase blood glucose levels and hereby augment the amplitude of the b-wave in the mouse [36]. There are several reports that changes in blood glucose levels may influence retinal sensitivity when evaluated using ERGs in cats and humans [37–39]. The α_2-adrenoceptor agonists, xylazine and medetomidine, inhibit insulin secretion through action on α_2-adrenoceptor in the pancreas β-cells and hereby increase blood glucose level in various species including dogs [40–44]. Further studies would therefore be necessary to investigate the effects of medetomidine and xylazine on the ERG parameters by changing the blood glucose levels in dogs. In contrast with the α_2-adrenoceptor agonists, there was no significant increase in blood glucose levels from baseline to 30 min after isoflurane anesthetics in dogs [45]. This may be another reason why there is a significant difference in the amplitude and implicit time of the a-wave for the S-ST responses among the anesthesia protocols used in the present study (Fig. 2a, b).

With increasing time in the dark, the increase in amplitudes and implicit times of the b-waves for the scotopic responses reflect the process of retinal dark adaptation [46]. In the present study, the change in b-wave amplitude using TI during dark adaptation was comparable with those obtained using the other two anesthetic protocols (Fig. 2c); however, the increment in b-wave implicit time was much lower than that for both MK and XK (Fig. 2d). In the mouse, isoflurane decreased retinal sensitivity more than ketamine/xylazine/acepromazine on the basis of the

dark-adapted b-wave parameter measured [7]. The earlier study suggested that isoflurane, an inhalation anesthetic, influences the permeability of membranes and changes the neurotransmitter release in the photoreceptor and bipolar cells. Somewhat more surprising was the remarkable reduction in the increase of the b-wave implicit time using TI after the first 10 min of dark adaptation (Fig. 2d). Although the present study cannot provide a detailed explanation for these differences, it is likely to be attributed to changes in the dark-adaptative properties of the rods and cones. The faster dark adaptation of the cones contribute to the first 10 min of the dark adaptation time, and achieve their maximum light sensitivity after about 10–12 min, while the slower dark adaptation process of the rods reach a much lower final light threshold, after about 40 min in darkness [47]. The results enable us to assume that the rods may be influenced by isoflurane, which is in agreement with the effects of halothane [10]. Another reason for these differences could be due to the wearing off of thiopental. A previous study showed that there was a significantly longer implicit time for the b-wave when using thiopentone (thiopental) than when using other anesthetics [10].

In Fig. 3, normative ERG values for three different combinations of anesthetics on the ERG of the same dog were established using the MoM and 90% reference range. In this way, the flow of events during scotopic and photopic ERG recordings was illustrated with the three different anesthetic protocols and with limits of normality. In a clinical situation, such an illustration allows for the ERG results obtained from an individual to be plotted into a diagram and the results directly compared to those from a group of normal age-matched dogs, using the recommended dog protocol and a specific anesthetic protocol.

In conclusion, the present study showed that the three different anesthetic protocols all lead to sufficient immobilization of dogs for performing ERGs with stable recordings. It was shown that TI affected the amplitude and implicit time of the a-wave and the implicit time of the b-wave relatively more compared to MK and XK. It could therefore be recommended that XK or MK be used for clinical ERGs rather than TI, due to the higher a-wave amplitudes obtained when using the former, since the a-wave is an important parameter to evaluate in generalized photoreceptor disease.

Acknowledgments Supported by the College of Veterinary Medicine and BK21 Program for Veterinary Science, Seoul National University, Republic of Korea. The authors thank Dr. Youngju Pak for statistical assistance and Howard Wilson for assistance with illustrations and graphics.

References

1. Ofri R (2002) Clinical electrophysiology in veterinary ophthalmology—the past, present and future. Doc Ophthalmol 104:5–16. doi:10.1023/A:1014463514302
2. Vaegan NK (2008) Amax to scotopic Imax diagnoses feline hereditary rod cone degeneration more efficiently than any other combination of long protocol electroretinogram parameters. Doc Ophthalmol 117:1–12. doi:10.1007/s10633-007-9096-3
3. Ropstad EO, Bjerkas E, Narfström K (2007) Electroretinographic findings in the standard wire haired dachshund with inherited early onset cone-rod dystrophy. Doc Ophthalmol 114:27–36. doi:10.1007/s10633-006-9035-8
4. Acland GM (1988) Diagnosis and differentiation of retinal diseases in small animals by electroretinography. Semin Vet Med Surg (Small Anim) 3:15–27
5. Maehara S, Itoh N, Wakaiki S et al (2007) The effects of cataract stage, lens-induced uveitis and cataract removal on ERG in dogs with cataract. Vet Ophthalmol 10:308–312. doi:10.1111/j.1463-5224.2007.00559.x
6. Messias A, Gekeler F, Wegener A et al (2008) Retinal safety of a new fluoroquinolone, pradofloxacin, in cats: assessment with electroretinography. Doc Ophthalmol 116:177–191. doi:10.1007/s10633-007-9081-x
7. Woodward WR, Choi D, Grose J et al (2007) Isoflurane is an effective alternative to ketamine/xylazine/acepromazine as an anesthetic agent for the mouse electroretinogram. Doc Ophthalmol 115:187–201. doi:10.1007/s10633-007-9079-4
8. Tremblay F, Parkinson JE (2003) Alteration of electroretinographic recordings when performed under sedation or halogenate anesthesia in a pediatric population. Doc Ophthalmol 107:271–279. doi:10.1023/B:DOOP.0000005336.08147.fc
9. Kommonen B (1988) The DC-recorded dog electroretinogram in ketamine–medetomidine anaesthesia. Acta Vet Scand 29:35–41
10. Kommonen B, Karhunen U, Raitta C (1988) Effects of thiopentone halothane-nitrous oxide anaesthesia compared to ketamine–xylazine anaesthesia on the DC recorded dog electroretinogram. Acta Vet Scand 29:23–33
11. Chaudhary V, Hansen R, Lindgren H et al (2003) Effects of telazol and nembutal on retinal responses. Doc Ophthalmol 107:45–51. doi:10.1023/A:1024444113700
12. Knave B, Persson HE (1974) The effect of barbiturate on retinal functions. I. Effects on the conventional electroretinogram of the sheep eye. Acta Physiol Scand 91:53–60. doi:10.1111/j.1748-1716.1974.tb05656.x
13. Knave B, Persson HE, Nilsson SE (1974) The effect of barbiturate on retinal functions. II. Effects on the C-wave of the electroretinogram and the standing potential of the

sheep eye. Acta Physiol Scand 91:180–186. doi:10.1111/j.1748-1716.1974.tb05674.x
14. Kommonen B, Hyvatti E, Dawson WW (2007) Propofol modulates inner retina function in beagles. Vet Ophthalmol 10:76–80. doi:10.1111/j.1463-5224.2007.00512.x
15. Narfström K (2006) Electroretinographic testing in larger animals. In: Heckenlively JR, Arden GB (eds) Principles and practice of clinical electrophysiology of vision, 2nd edn. The MIT press, Cambridge, pp 923–933
16. Acland GM, Forte S, Aguirre G (1981) Halothane effect on the canine electroretinogram. 12th Annual Meeting of the American College Veterinary Ophthalmologists, pp 66–83
17. Jones RD, Brenneke CJ, Hoss HE et al (1994) An electroretinogram protocol for toxicological screening in the canine model. Toxicol Lett 70:223–234. doi:10.1016/0378-4274(94)90166-X
18. Yanase J, Ogawa H (1997) Effects of halothane and sevoflurane on the electroretinogram of dogs. Am J Vet Res 58:904–909
19. Narfström K, Ekesten B, Rosolen SG et al (2002) Guidelines for clinical electroretinography in the dog. Doc Ophthalmol 105:83–92. doi:10.1023/A:1020524305726
20. Palomaki GE, Neveux LM (2001) Using multiples of the median to normalize serum protein measurements. Clin Chem Lab Med 39:1137–1145. doi:10.1515/CCLM.2001.180
21. Kommonen B, Raitta C (1987) Electroretinography in Labrador retrievers given ketamine–xylazine anesthesia. Am J Vet Res 48:1325–1331
22. Rosolen SG, Rigaudiere F, Lachapelle P (2002) A practical method to obtain reproducible binocular electroretinograms in dogs. Doc Ophthalmol 105:93–103. doi:10.1023/A:1020539217538
23. Marmor MF, Holder GE, Seeliger MW et al (2004) Standard for clinical electroretinography (2004 update). Doc Ophthalmol 108:107–114. doi:10.1023/B:DOOP.0000036793.44912.45
24. Lalonde MR, Chauhan BC, Tremblay F (2006) Retinal ganglion cell activity from the multifocal electroretinogram in pig: optic nerve section, anaesthesia and intravitreal tetrodotoxin. J Physiol 570:325–338
25. Yang XL (2004) Characterization of receptors for glutamate and GABA in retinal neurons. Prog Neurobiol 73:127–150. doi:10.1016/j.pneurobio.2004.04.002
26. Krasowski MD, Harrison NL (1999) General anaesthetic actions on ligand-gated ion channels. Cell Mol Life Sci 55:1278–1303. doi:10.1007/s000180050371
27. Cheng SC, Brunner EA (1981) Effects of anesthetic agents on synaptosomal GABA disposal. Anesthesiology 55:34–40
28. Cheng SC, Brunner EA (1981) Inhibition of GABA metabolism in rat brain slices by halothane. Anesthesiology 55:26–33
29. Schlame M, Hemmings HC Jr (1995) Inhibition by volatile anesthetics of endogenous glutamate release from synaptosomes by a presynaptic mechanism. Anesthesiology 82:1406–1416. doi:10.1097/00000542-199506000-00012
30. Vainio O, Palmu L (1989) Cardiovascular and respiratory effects of medetomidine in dogs and influence of anticholinergics. Acta Vet Scand 30:401–408
31. Short CE (1991) Effects of anticholinergic treatment on the cardiac and respiratory systems in dogs sedated with medetomidine. Vet Rec 129:310–313
32. Scheinin H, Virtanen R, MacDonald E et al (1989) Medetomidine—a novel alpha 2-adrenoceptor agonist: a review of its pharmacodynamic effects. Prog Neuropsychopharmacol Biol Psychiatry 13:635–651. doi:10.1016/0278-5846(89)90051-1
33. Virtanen R (1986) Pharmacology of detomidine and other alpha 2-adrenoceptor agonists in the brain. Acta Vet Scand Suppl 82:35–46
34. Alibhai HI, Clarke KW, Lee YH et al (1996) Cardiopulmonary effects of combinations of medetomidine hydrochloride and atropine sulphate in dogs. Vet Rec 138:11–13
35. Ko JC, Fox SM, Mandsager RE (2001) Effects of preemptive atropine administration on incidence of medetomidine-induced bradycardia in dogs. J Am Vet Med Assoc 218:52–58. doi:10.2460/javma.2001.218.52
36. Brown ET, Umino Y, Loi T et al (2005) Anesthesia can cause sustained hyperglycemia in C57/BL6 J mice. Vis Neurosci 22:615–618. doi:10.1017/S0952523805225105
37. Sannita WG, Balestra V, Dibon G et al (1993) Spontaneous variations of flash-electroretinogram and retinal oscillatory potential in healthy volunteers are correlated to serum glucose. Clin Vis Sci 8:147–158
38. Barlow RB, Khan M, Farell B (2003) Metabolic modulation of human visual sensitivity (E-abstract). Invest Ophthalmol Vis Sci 44:2708. doi:10.1167/iovs.02-1054
39. McRipley MA, Ahmed J, Chen EP et al (1997) Effects of adaptation level and hypoglycemia on function of the cat retina during hypoxemia. Vis Neurosci 14:339–350
40. Saha JK, Xia J, Grondin JM et al (2005) Acute hyperglycemia induced by ketamine/xylazine anesthesia in rats: mechanisms and implications for preclinical models. Exp Biol Med (Maywood) 230:777–784
41. Ambrisko TD, Hikasa Y (2002) Neurohormonal and metabolic effects of medetomidine compared with xylazine in beagle dogs. Can J Vet Res 66:42–49
42. Hillaire-Buys D, Gross R, Blayac JP et al (1985) Effects of alpha-adrenoceptor agonists and antagonists on insulin secreting cells and pancreatic blood vessels: comparative study. Eur J Pharmacol 117:253–257. doi:10.1016/0014-2999(85)90610-7
43. Brockman RP (1981) Effect of xylazine on plasma glucose, glucagon and insulin concentrations in sheep. Res Vet Sci 30:383–384
44. Hsu WH, Hummel SK (1981) Xylazine-induced hyperglycemia in cattle: a possible involvement of alpha 2-adrenergic receptors regulating insulin release. Endocrinology 109:825–829
45. Benson GJ, Grubb TL, Neff-Davis C et al (2000) Perioperative stress response in the dog: effect of pre-emptive administration of medetomidine. Vet Surg 29:85–91. doi:10.1111/j.1532-950X.2000.00085.x
46. Yanase J, Ogawa H, Ohtsuka H (1995) Rod and cone components in the dog electroretinogram during and after dark adaptation. J Vet Med Sci 57:877–881
47. Lam BL (ed) (2005) Clinical recording techniques. In: Electrophysiology of vision: clinical testing and applications. Taylor & Francis Group, Boca Raton, pp 1–64