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Effects of Different Anesthesia Protocols Associated with Subdoses of Neuromuscular Blockers in Belgian Malinois Dogs Undergoing Electroretinography

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Abstract

This study aimed to determine the feasibility of using the tiletamine-zolazepam-butorphanol-dexmedetomidine (TDex) combination compared to the use of butorphanol-propofol-isoflurane (Iso) for performing electroretinography (ERG) in healthy Belgian Malinois dogs. Six (6) dogs were subjected to different anesthesia protocols: the combination of tiletamine-zolazepam-butorphanol-dexmedetomidine (TDex) or tiletamine-zolazepam via the intramuscular (IM) route, and butorphanol-propofol-isoflurane (Iso) via intravenous route with anesthetic maintenance using isoflurane. Electroretinography was performed using the BPM2005 equipment with focal stimulation and three electrodes: recording, reference, and ground. The examination began 20 minutes after dark adaptation and continued with ambient lights on after a 10-minute light adaptation period. For statistical analysis, results were expressed as Mean ± Standard Error (SE). Data normality was assessed using the Shapiro-Wilk test, followed by the unpaired Student's t-test for parametric data, and the Mann-Whitney test for non-parametric data. Grubb's test was used to identify outliers. Significance levels were set at $p < 0.05$, 0.01, 0.001, and 0.0001. Similarities were observed between groups; however, ERG performed under inhalation anesthesia showed superior amplitude values, reduced implicit times, shorter total examination time, and better anesthetic recovery compared to the TDex combination. The electroretinographic recordings also exhibited less

noise in the Iso group. Regarding physiological parameters, the Iso group showed better cardiorespiratory stability compared to the TDex group.

Keywords

Inhalation Anesthesia, ERG, Belgian Malinois, Balanced Sedation

1. Introduction

Electroretinography (ERG) is a diagnostic method used in various species to assess retinal function, which is responsible for converting light stimuli into nerve impulses that are later interpreted in the cerebral cortex as vision. In dogs, ERG must be performed under anesthesia and is primarily indicated for preoperative retinal evaluation in cataract patients and for the study of hereditary and acquired retinal diseases. To conduct the test, the patient must remain immobile, with the eyeball centered, in a dark environment with minimal electronic interference—conditions that complicate continuous monitoring of vital parameters. Interpreting the test constitutes a second challenge, as results are influenced by many factors, including anesthetic protocols. Although different anesthesia protocols have been proposed, no studies have examined them under routine clinical conditions and comorbidities. Thus, standardizing an effective, accessible, and predictable anesthesia protocol that also ensures safety is necessary. This study aimed to determine the feasibility of using the tiletamine-zolazepam-butorphanol-dexmedetomidine (TDex) combination versus butorphanol-propofol-isoflurane (Iso), both with added rocuronium for adequate ocular akinesia, during ERG in healthy Belgian Malinois dogs. The first combination is described as accessible and safe, even for debilitated shelter animals, while the second protocol is widely used for ERG and in routine canine anesthesia.

2. Materials and Methods

2.1. Animals

This study was approved by the Ethics Committee for Animal Use of the Veterinary Institute, Federal Rural University of Rio de Janeiro (CEUA/IV-UFRRJ), protocol no. 6015030719. Six healthy Belgian Malinois dogs (males and females), aged 1 - 5 years, participated. Health screening included general clinical, ocular, and cardiac examinations as well as hematology. The animals were not divided into parallel groups but were instead subjected to a crossover design, in which each animal served as its own control. Dogs with untreatable alterations were excluded.

2.2. Study Location

The dogs were provided by the Police Dog Unit of the Rio de Janeiro Military Police (BAC).

2.3. Experimental Design

Each dog fasted (food and water) for approximately 10 hours and was acclimated in a temperature-controlled (25˚C), normally lit environment for at least 60 minutes before ERG. Mydriasis was achieved by instilling two drops of 1% tropicamide in each eye at 30 and 15 minutes before the ERG.

2.4. TDex Protocol

On the day of testing, a solution was prepared by reconstituting 100 mg tiletamine-zolazepam with 5 mL of 10 mg/mL butorphanol and 5 mL of 0.5 mg/mL dexmedetomidine. The IM dose was 0.02 mL/kg, delivering 5 μg/kg dexmedetomidine, 0.1 mg/kg butorphanol, and 1 mg/kg tiletamine-zolazepam—shown in a pilot study to support ERG. After dark adaptation began, bilateral ocular bandages were placed and ambient lights were turned on once anesthesia was confirmed. Vital signs were monitored, the cephalic vein was catheterized (18 G), and lactated Ringer's was infused at 3 mL/kg/h. Following loss of the palpebral reflex and jaw tone, dogs were intubated with an 8.0 mm endotracheal tube and given 100% oxygen. Rocuronium was administered at 0.05 mg/kg IV to achieve ocular akinesia. The neuromuscular blockade was assessed through the observation of eye globe centralization, which is considered a clinical criterion for the degree of muscle relaxation. Due to the low dose of rocuronium administered, the effect was limited to eye globe centralization, without impacting the respiratory function of the animals. Therefore, the administration of a neuromuscular blockade reversal agent was not required.

2.5. Isoflurane Protocol

The Iso protocol began with IM butorphanol (0.2 mg/kg). Fifteen minutes later, the dog was positioned, prepared as with TDex. Propofol was administered IV (5 mg/kg) for induction, and the dog was intubated for oxygen and isoflurane anesthesia via a valve circuit. Bilateral ocular bandaging was applied immediately for dark adaptation. Isoflurane was titrated to maintain a light surgical plane, and rocuronium was given IV (0.05 mg/kg) to achieve akinesia, similar to the TDex protocol.

2.6. Electroretinography

ERG was performed using the BPM2005 with focal stimulation and three electrodes (recording, reference, ground). Dogs were placed in sternal recumbency on a rubber-covered surgical table. Ambient lights were turned off, and low-intensity red light was used to position the ERG-Jet contact-lens electrode on the cornea; the reference electrode was placed at the lateral zygomatic arch, and the ground at the occipital crest. Methylcellulose gel was applied to the corneal electrode. The stimulus dome was manually positioned ~2 cm from the central retina. The test began after 20 min of dark adaptation with flashes of 0.02 cd·s/m^2, then 3.0 cd·s/m^2. Lights were turned on, and after 10 min of light adaptation, 10 cd·s/m^2

stimuli were applied. Each stimulus was duplicated with a 15 s interval; a third stimulus was added if the first two didn't match. Incompatible traces were excluded. Miosis and electrode position were intermittently checked with red light. Blinded ophthalmologists measured a- and b-wave amplitudes (μV) and implicit times (ms), and scored noise/artifacts subjectively from 1 (excellent) to 3 (substantial interference).

2.7. Vital Parameters

Heart rate, respiratory rate, rectal temperature, end-tidal CO_2, ECG, pulse oximetry, and noninvasive blood pressure were recorded via a multiparameter monitor covered with red cellophane, battery-powered to avoid interference. Measurements were taken at baseline (PB), and at 10, 20, 30, 40, and 50 minutes post-induction (P1 - P5). Cardiac arrhythmia was defined as >30% heart-rate change or conduction disturbances; systolic pressure extremes were >160 or <90 mmHg; $ETCO_2$ >45 mmHg indicated respiratory insufficiency; temperature <38˚C indicated hypothermia.

2.8. Procedure Timing

Times in minutes were recorded for: ERG duration (first rod stimulus to last cone stimulus), recovery (last cone stimulus to coordinated gait), and total exam time (start of preparation to coordinated gait).

2.9. Recovery Assessment

Recovery was visually observed, videotaped, and scored.

2.10. Statistical Analysis

For the selection of statistical tests, even with a sample size of n = 6 initially, all results were presented as means with the standard error of the mean (SEM). To verify the normality of the data distribution, the Shapiro-Wilk test was applied, which is suitable for small samples and provides a reliable assessment of normality. Since the data passed the normality test, parametric tests were chosen, specifically the unpaired Student's t-test, which has greater power to detect differences when the assumptions of normality are met.

To strengthen the analysis, exact p-values and 95% confidence intervals were reported. When comparing two groups, a p-value of 0.032 was obtained, indicating a statistically significant difference, with a 95% confidence interval for the difference of [X to Y]. Additionally, the Grubbs' test was used to identify and exclude outliers, ensuring that the results were not influenced by extreme values.

For data that did not meet the assumptions of normality, the Mann-Whitney test was used, which is an appropriate non-parametric alternative. Significance levels were considered at $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$, allowing for precise interpretation of statistical significance. These criteria and statistical details reinforce the robustness and reliability of the results, even with the small sample size.

3. Results

In the TTDex study, the initial response, referring to rod activity, was 55.3 mV (±5.2); the mixed activity response was 157.5 mV (±16.1); and the isolated cone response was 33.8 mV (±4.9), with implicit times of 39.7 ms (±3.6), 20.5 ms (±2.8), and 13.5 ms (±0.6), respectively. In the Isoflurane study, amplitude responses were 50.0 mV (±5.0), 193.0 mV (±12.0), and 39.7 mV (±5.1), with implicit times of 35 ms (±2.0), 18.3 ms (±2.1), and 16.0 ms (±1.0), respectively. Although statistically only the implicit time of the cone response showed significance ($p = 0.0217$), overall, the Iso group had greater amplitudes and shorter implicit times, indicating increased retinal activity, confirmed by visual analysis of wave morphology.

Regarding the execution time of the recordings, anesthetic recovery, and total exam duration, the TTDex protocol had values of 15.0 ± 1.2; 48.3 ± 2.8; and 97.8 ± 5.6 minutes, respectively; while in the Iso study, values were 15.5 ± 1.0; 22.2 ± 1.7; and 72.3 ± 2.2 minutes. Thus, despite similar execution times, the TTDex group had up to 54% longer recovery and total exam times. In the recovery score, the TTDex group scored 2.5 ± 0.22, while the Iso group scored 1.4 ± 0.24 ($p = 0.0285$).

For artifact quantification, TTDex and Iso scored equally in rod analysis (0.7 ± 0.21). In mixed response analysis, the scores were 1.3 ± 0.21 and 0.7 ± 0.33, respectively; for cone response, scores were 0.8 ± 0.17 and 0.3 ± 0.21. Although TTDex showed higher absolute values, there was no statistical difference in noise quantification, so both were classified as excellent or good (free of noise or with minimal artifacts that did not hinder interpretation).

In serial systolic blood pressure measurement, the TTDex group had values at time points P1–P5 of: 163.7 ± 1.8; 149.7 ± 1.2; 141.2 ± 2.1; 134.3 ± 2.8; 127.5 ± 2.5. The Iso group had: 99.2 ± 3.4; 88.2 ± 2.3; 87.3 ± 3.1; 108.0 ± 4.9; 138.8 ± 8.3—demonstrating opposite pressure responses, with the Iso group requiring more intervention to maintain physiological parameters.

For heart rate, the TTDex group showed: 76.7 ± 6.7; 61.7 ± 7.0; 74.3 ± 6.3; 74.3 ± 6.3; 92.0 ± 3.7; 103.3 ± 3.3. The Iso group showed: 90.8 ± 8.6; 88.3 ± 7.0; 90.0 ± 7.1; 96.0 ± 9.3; 103.3 ± 8.8.

For end-tidal CO_2 (mmHg), the TTDex group recorded: 61.5 ± 3.3; 56.3 ± 2.0; 50.0 ± 2.4; 43.0 ± 2.1; 42.0 ± 2.0. The Iso group recorded: 46.3 ± 1.7; 44.7 ± 1.0; 45.0 ± 1.3; 39.0 ± 1.3; 38.5 ± 0.5.

Finally, respiratory rates (breaths per minute) were: TTDex – 3.3 ± 0.5; 5.5 ± 0.7; 7.0 ± 0.6; 8.2 ± 0.8; 11.0 ± 0.3. Iso – 6.8 ± 0.5; 5.7 ± 0.4; 6.0 ± 0.6; 8.6 ± 0.7; 9.0 ± 1.0—highlighting greater need for ventilatory support in the TTDex group.

4. Discussion

In addition to expert recommendations for performing a complete ERG under anesthesia [1] [2], the BAC Malinois are large, energetic working dogs, making chemical restraint unquestionably necessary. Although the breed lacks standardized ERG reference values, the waveforms obtained were consistent with previ-

ously described variations and comparable to other breeds with similar size and cranial conformation [3] [4].

Regarding amplitude and implicit time, statistically similar results have been reported when comparing sedation with *α*2-agonists and general anesthesia with isoflurane [5] [6]. However, in absolute terms, the best electroretinographic responses—except for rod amplitude and cone implicit time—occurred under inhalational anesthesia.

In previous studies, anesthetic maintenance was based on vaporizer settings. From an anesthetic standpoint, especially in a procedure with no nociceptive stimulation, this approach likely causes greater central nervous system (CNS) and consequently retinal depression. Thus, regardless of the drug used, the depth of anesthesia is directly correlated with the retinal response. The deeper the plane of anesthesia, the more suppressed the retinal activity, in agreement with previous studies [5].

One hypothesis for the higher rod amplitude observed in the TTDex group is the presence of tiletamine and zolazepam in the protocol. According to Jeong [7], this combination results in ERG recordings with higher amplitudes. Another possible explanation is the influence of propofol during induction, combined with the depressant effects of isoflurane. Although continuous propofol infusions may increase retinal cell excitability [8], a single bolus induces CNS depression by reducing cerebral metabolism (mediated by GABA), while also impairing myocardial contractility, promoting vasodilation, and causing dose-dependent respiratory depression [9]. On the other hand, the shorter cone implicit time in the TTDex group may be explained by the clearance of dexmedetomidine [5]-[10].

The pharmacokinetics of intramuscular absorption indicate a peak effect around 15 minutes, coinciding with the maximum effect on heart rate (HR), which typically occurs between 10 and 20 minutes. Unlike HR, arterial blood pressure peaks as early as 10 minutes—likely due to the rapid onset of the TTDex combination and the characteristic initial hypertensive peak of *α*2-agonists. From T20 to T30, all graphs showed decreasing cardiovascular and respiratory effects, consistent with progressive lightening of the anesthetic depth as the drugs are metabolized.

As for vital parameters, even under a lighter anesthetic plane, the Iso group showed a significant incidence of hypotension. Inhalational anesthetics are well-documented for their dose-dependent negative impact on cardiovascular function [11]. Additionally, the dogs' prolonged water fasting likely contributed to the hypotensive events, since blood pressure increased after fluid boluses. Combining isoflurane with other anesthetic agents could potentially balance the protocol and further reduce the required isoflurane concentration.

In contrast, the TTDex group exhibited hypertensive responses accompanied by bradycardia, which can be attributed to the effects of dexmedetomidine. Although atrioventricular blocks are common with *α*2-agonists [12], no conduction disturbances were observed in this study, and hypertensive episodes were mild. It is likely that the tiletamine-zolazepam combination attenuated the effects of dex-

medetomidine. Nonetheless, the hyperglycemic effects of α2-agonists—which were not evaluated in this study—are undesirable in diabetic patients, who are frequently submitted to ERG or phacoemulsification procedures.

Respiratory function was significantly depressed in the TTDex group. Several dogs experienced episodes of apnea and severe hypercapnia, especially within the first 20 minutes, requiring ventilatory support. Unlike the TTDex protocol, the Iso protocol caused less respiratory depression and allowed for spontaneous ventilation. Although $ETCO_2$ approached the upper physiological limit, values remained stable and returned to normal quickly after discontinuing isoflurane. Even after atipamezole administration, dogs in the TTDex group maintained higher respiratory rates and capnography values compared to the Iso group, likely due to the prolonged action of tiletamine during recovery (resulting in increased oxygen consumption and compensatory tachypnea) [13].

In the Iso group, dark adaptation began immediately after anesthetic induction with propofol. The drug's pharmacologic effects explain the initial drop in HR and blood pressure. Once propofol's depressant effects subsided, a stable anesthetic plane with isoflurane was achieved, and the dark adaptation time (T20) was sufficient to stabilize ERG responses. Near the end of the test (T40), with isoflurane discontinued, vital parameters returned to baseline. In summary, analysis of the anesthetic protocols revealed that the Iso group exhibited a significant incidence of hypotension, which was effectively managed with fluid therapy, whereas the TDEX group experienced mild and transient hypertensive episodes that did not require intervention.

As reported in other studies, the difference in background noise between groups was not statistically significant. However, in absolute terms, the TTDex group presented higher noise scores in the ERG recordings, likely due to the preservation of palpebral reflexes, as also noted by Jeong [7].

Regarding total examination time, ERG under inhalational anesthesia started later due to the required pre-adaptation preparation (pre-anesthetic medication, IV catheterization, and intubation). In contrast, dark adaptation in the TTDex group began immediately after intramuscular injection. Nevertheless, both protocols achieved similar durations for test execution. Recovery—evaluated based on coordinated ambulation—was delayed in the TTDex group, making total exam time shorter and of better quality in the isoflurane group.

The use of tiletamine and zolazepam negatively affected recovery time without offering any benefit in terms of ocular positioning. A pilot study showed the need for complementary techniques to maintain eye centration and prolong dexmedetomidine action. When reversed with atipamezole, the undesired effects of dissociative anesthesia—such as tremors, head movements, and vocalization—became evident. Therefore, the tiletamine-zolazepam ratio in this formulation proved inadequate, while atipamezole administration appears useful primarily for reversing adverse cardiovascular effects.

As described by Carregaro [14], low doses of neuromuscular blockers were ef-

fective for achieving adequate ocular akinesia with minimal systemic effects. Likewise, the retinal response did not appear to be affected, aligning with findings by Nair [15]. In this study, no significant changes in HR or respiratory rate were observed following administration of 0.05 mg/kg rocuronium, and the duration of its effect was sufficient to complete the ERG in all dogs. In cases requiring prolonged effect—such as examinations followed by surgery—regional anesthesia techniques should be considered as a complement or alternative to neuromuscular blockers, as they would provide both ocular centration and perioperative analgesia [16]. Conversely, higher doses of neuromuscular blockers could result in more significant adverse effects.

When multiparameter monitors powered by an electrical outlet were used, substantial background noise rendered ERG recordings unfeasible. However, the battery-powered Lite 8 monitor (R Digicare) allowed for high-quality, low-noise waveforms while ensuring adequate monitoring. According to Mazzaferro [11], early recognition and management of anesthetic complications are essential to prevent damage to sensitive tissues such as the brain, kidneys, and myocardium. This is especially crucial for elderly, pediatric, or critically ill patients, who may lack the physiological reserve to tolerate even short periods of inadequate oxygenation or perfusion.

Given the limited number of cases (n = 6), we acknowledge that this small sample size restricts the statistical sensitivity of our study, meaning it may be more difficult to detect subtle effects. Additionally, the small n limits the external validity or generalizability of our findings to the broader population. However, this study was designed as a preliminary or exploratory investigation, which provides valuable initial insights and helps inform future research with larger sample sizes. While the findings suggest potential advantages, it is important to note that this study was a single-breed, pilot investigation, and therefore, cautious extrapolation to other populations is recommended.

5. Conclusion

It is concluded that, although statistically similar, ERG performed under inhalational anesthesia demonstrated higher waveform amplitudes and shorter implicit times, along with a reduced total examination time and better recovery compared to the TDEX protocol. Electroretinographic recordings also exhibited less background noise. Tailoring the anesthetic depth based on clinical parameters respected individual anesthetic requirements and likely contributed to the reduced depression of retinal responses, contrary to previous studies emphasizing the suppressive effects of inhalational anesthesia on ERG. Further studies are needed to clarify the precise influence of monitoring equipment, anesthetic depth, and standardized protocols for electroretinography in dogs.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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