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SHORT COMMUNICATION

Comparison of two protocols for field immobilization of white-eared opossums (*Didelphis albiventris*)

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Abstract

The aim of this study was to investigate the efficacy of two protocols for field immobilization of white-eared opossums (*Didelphis albiventris*) and compare their effects on immobilization, cardiopulmonary variables, and recovery times. Twenty one opossums were randomly divided into two groups; G1 received ketamine (15 mg kg^{-1})-dexmedetomidine (0.15 mg kg^{-1}) intramuscularly (IM) and G2 received the ketamine-dexmedetomidine combination and isoflurane once induction was achieved. Oxygen was delivered by face mask (1.5 L $minute^{-1}$). Thirty minutes after induction, isoflurane was discontinued (G2) and both groups were administered atipamezole (1.5 mg kg^{-1}) IM. Respiratory (f_R) and heart rate (HR), oxyhemoglobin saturation (SpO_2), and rectal temperature (T) were recorded every 5 min. Induction time, time to first movement (RT_1), and time to achieve standing (RT_2) were recorded. ANOVA and non-parametric tests were used. Level of immobilization was assessed by observation of movements and evaluation of muscle relaxation. The mean induction time was 4.71 min. RT_1 and RT_2 were significantly longer in G2. No significant differences were found in SpO_2 or fR. HR did not vary significantly along time, but was higher in G2. Rectal temperature did not show differences between treatments, but decreased significantly with time in G2. Four of nine animals in G1 showed movements, while no animals in G2 did and muscle relaxation was determined to be better in this latter group. Both protocols were adequate for short-term field immobilization, with minimal alterations of HR and T and relatively short recovery times. Isoflurane provided better immobilization with statistically significant prolongation of recovery times.

Keywords Ketamine · Dexmedetomidine · Isoflurane · Field immobilization · Opossum

Introduction

Wild animals captured as part of biological, epidemiological, or taxonomic studies may require field chemical immobilization even for noninvasive or minimally invasive procedures, as physical restraint could be highly stressful and result in injuries (Chinnadurai et al. 2016).

Ideally, field immobilization protocols should provide rapid induction and recovery to enable release of the animals into their habitats within a short time. Some protocols for field chemical restraint of wild marsupials have been published (Viggers and Lindenmayer 1995, Stoskopf et al. 1999; Kocer and Powell 2009; Tarragona et al. 2014). Few field studies have assessed the chemical restraint of wild opossums (*Didelphidae*) (Stoskopf et al. 1999, Kocer and Powell 2009, Tarragona et al. 2014) and only one describes the use of ketamine and diazepam in wild white-eared opossums (*Didelphis albiventris*), but did not include information about the duration of the procedures, the recovery times, or the impact on cardiopulmonary variables (Tarragona et al. 2014).

Based on previous experiences of field researchers, an intramuscular ketamine-dexmedetomidine combination used empirically in white-eared opossums provided short-term immobilization with short recovery times, but movements have been observed in a high proportion of the animals. Thus, it was hypothesized that the addition of isoflurane would provide better

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immobilization without significant changes in the measured variables and recovery time, becoming a good choice when complete immobility is required, if appropriate equipment for field anesthesia is available. The aim of this study was to compare two protocols containing ketamine-dexmedetomidine or ketamine-dexmedetomidine-isoflurane in wild white-eared opossums regarding immobilization, cardiopulmonary variables, and recovery times. Both protocols included reversal with atipamezole.

Materials and methods

Field work was conducted in Misiones, Argentina, during two 2-week surveys in autumn (April and May 2014) as part of a study on the prevalence of *Trypanosoma cruzi*, the flagellate that causes Chagas disease, in wild populations of small mammals (Argibay et al. 2016). The study area was Campo San Juan Reserve, a protected area (27° 22′ S 55° 34′ W). Animals had to be anesthetized for 30 min to perform sexing, tag marking, venipuncture, and xenodiagnosis (Orozco et al. 2013).

White-eared opossums (*D. albiventris*) were live-captured with Tomahawk traps (Orozco et al. 2013) and transported in their cages to a field laboratory. For personnel safety, animals were visually examined through the bars of the cage before anesthesia. Animals that appeared in poor health (e.g., emaciated, injured) or nursing females were excluded from this study.

Twenty-one opossums (10 females, 11 males) weighing 892 ± 269 g [mean ± standard deviation (SD)] were included in the study. They were randomly divided into 2 groups; G1 ($n = 9$) received ketamine-dexmedetomidine intramuscularly (IM) and G2 ($n = 12$) received the same combination with added isoflurane for maintenance of the anesthetic plane. Both protocols included reversal with atipamezole. All animals were administered ketamine (15 mg kg^{-1}; 5%; Ketonal 50, Richmond, Argentina) and dexmedetomidine (0.15 mg kg^{-1}; Dexdomitor; Orion Pharma, Finland) IM through the bars of the trap into the semimembranosus or semitendinosus muscle. Time for induction was defined as the time from ketamine-dexmedetomidine administration to loss of righting reflex, evaluated by slightly rotating the cage. This action was repeated every 15 s until loss of righting reflex was confirmed. Once immobilized, animals were removed from the trap and placed on a thermal cushioned surface set at 36 °C (Amrra, Argentina). The environmental temperature ranged between 24 and 28 °C. Oxygen (G1) or oxygen and isoflurane (G2) were delivered by face mask through a Jackson-Rees system (1.5 L $minute^{-1}$). Isoflurane (Isoflurane, Piramal Healthcare, UK) was administered through a previously calibrated vaporizer (IsoTec, Datex-Ohmeda GE Healthcare, UK) for 30 min, starting after loss of righting reflex, with the dial set at 3% for the first 5 min, then at 1.5% for the remaining 25 min. Thirty minutes after induction, isoflurane administration was discontinued in G2 and both groups were administered atipamezole (1.5 mg kg^{-1}; Antisedan; Orion Pharma) IM.

Respiratory rate (f_R) was monitored by observation of thoracic excursions. Heart rate (HR), oxyhemoglobin saturation (SpO_2), and rectal temperature (T) were recorded from a multiparameter monitor (Contec CMS6000B-Vet, China). Three adhesive foam electrocardiogram (ECG) electrodes were placed on both thoracic limbs and on the left pelvic limb. The pulse oximeter was placed on the left metacarpus. Oscillometric blood pressure measurement (Contec CMS6000B-Vet, China) was performed using a neonatal size 2 cuff placed around the tail. Systolic (SAP), diastolic (DAP), and mean (MAP) arterial pressures were recorded. All variables were measured as soon as the animal was positioned on the table and every 5 min for the duration of the procedure.

Spontaneous and in response to manipulation movements of head or limbs were recorded during the 30 min procedure in order to assess immobilization. Muscle relaxation was subjectively evaluated, always by the same researcher every time the other variables were measured, by assessing the ease and degree of jaw opening and flexion/extension of the pelvic limbs.

Recovery times (RT), defined as the time to first movement (RT_1) and the time to achieve standing (RT_2) after atipamezole administration, were recorded. Oxygen delivery was maintained in both groups until RT_1. For safety reasons, animals were then placed in a recovery cage and observed for recovery assessment and RT_2. Wild white-eared opossums' behavior when threatened is to become apparently paralyzed and unresponsive to external stimuli (death feigning), an adaptive behavior that could be used as a defense mechanism (Kimble 1997). Consequently, the exact complete recovery time was difficult to assess and, therefore, all animals were released at the capture site a minimum of 7 h after RT_2.

The study was approved by the Institutional Animal Care and Use Committee, Veterinary Sciences School, University of Buenos Aires (protocol 2012/36). Capture and transit permits were obtained from the local government (Regulation no. 120/2012, General Direction of Ecology, MEyRNR, Misiones, Argentina).

Statistical analysis

Statistical analysis was performed using a SPSS 22.0 software. The Shapiro-Wilk test was used to assess normality of the data. According to their distribution, data (f_R, T and RT) were analyzed by non-parametric tests (the Mann-Whitney U test to compare between treatments or the Friedman and Wilcoxon tests to analyze time evolution of the response). Mixed model was used to analyze HR and SpO_2. Each HR or SpO_2 data was introduced as the dependent variable, whereas treatment and time of sampling were entered as fixed factors. Individual identity was included as a random factor. A statistical significance level of $p < 0.05$ was used. Data are presented as mean ± SD.

Data availability All datasets generated and analyzed during the current study are available from corresponding author on reasonable request.

Results

The induction time was 4.71 ± 2.33 min. RT_1 and RT_2 were significantly longer ($U = 4.50$, $p = 0.001$, and $U = 7.50$, $p = 0.001$, respectively) in G2 ($RT_1 = 7.75 \pm 4.07$, $RT_2 = 13.36 \pm 6.79$ min) than in G1 ($RT_1 = 2.15 \pm 0.64$, $RT_2 = 5.78 \pm 1.92$ min).

HR were significantly higher in G2 than in G1 (F mixed model = 6.849; $p = 0.02$) (Fig. 1). Residual variance homoscedasticity and normality rates ($W = 0.988$; $p = 0.391$) have been observed. f_R did not vary with time ($\chi^2 = 6.655$, $p = 0.247$; $\chi^2 = 6.513$, $p = 0.259$) or between treatments ($U > 12.5$, $p > 0.136$). Rectal temperature did not show differences when comparing between treatments ($U > 13.00$, $p > 0.153$), but, when analyzing time evolution of the response within each treatment, it decreased significantly with time in G2 ($\chi^2 = 16.557$, $p = 0.005$) (Fig. 1). SpO_2 values were ≥ 96% in all animals during the procedure and no significant differences were found within each group with time (F mixed model = 0.928, $p = 0.469$) or between groups (F mixed model = 1.761. $p = 0.206$). No statistical analyses of arterial pressure were performed as few data could be obtained.

Movements (both spontaneous and in response to handling) were observed during the procedure in four of nine animals in G1. All movements involved the limbs and occurred at 20–30 min. No animals in G2 moved. Muscle relaxation was subjectively determined as better in animals in G2 all along the procedure.

Overall, no complications were observed and all animals recovered from anesthesia.

Discussion

The administration of ketamine-dexmedetomidine alone or with isoflurane provided adequate immobilization for 30 min in wild white-eared opossums.

Little is known about chemical immobilization in field conditions of wild opossums. The median time to recumbency observed in American opossums (*Didelphis virginiana*) administered with a combination of ketamine (10 mg kg^{-1}), medetomidine (0.1 mg kg^{-1}), and butorphanol (0.2 mg kg^{-1}) was 6 min (Stoskopf et al. 1999) similar to that found in this study (4.71 ± 2.33 min, mean ± SD). However, in American opossums administered with a tiletamine-zolazepam combination, the median time to recumbency was slightly shorter, at 3 and 3.5 min (15 and 30 mg kg^{-1}, respectively) (Stoskopf et al. 1999), and when immobilization was performed in the same species with isoflurane in an induction chamber, the mean induction time was higher (13 min) (Kocer and Powell 2009). Tarragona et al. (2014) evaluated the chemical immobilization of wild white-eared opossums with a combination of ketamine and diazepam IM, but neither time to recumbency, nor recovery time and cardiovascular, or respiratory parameters were mentioned.

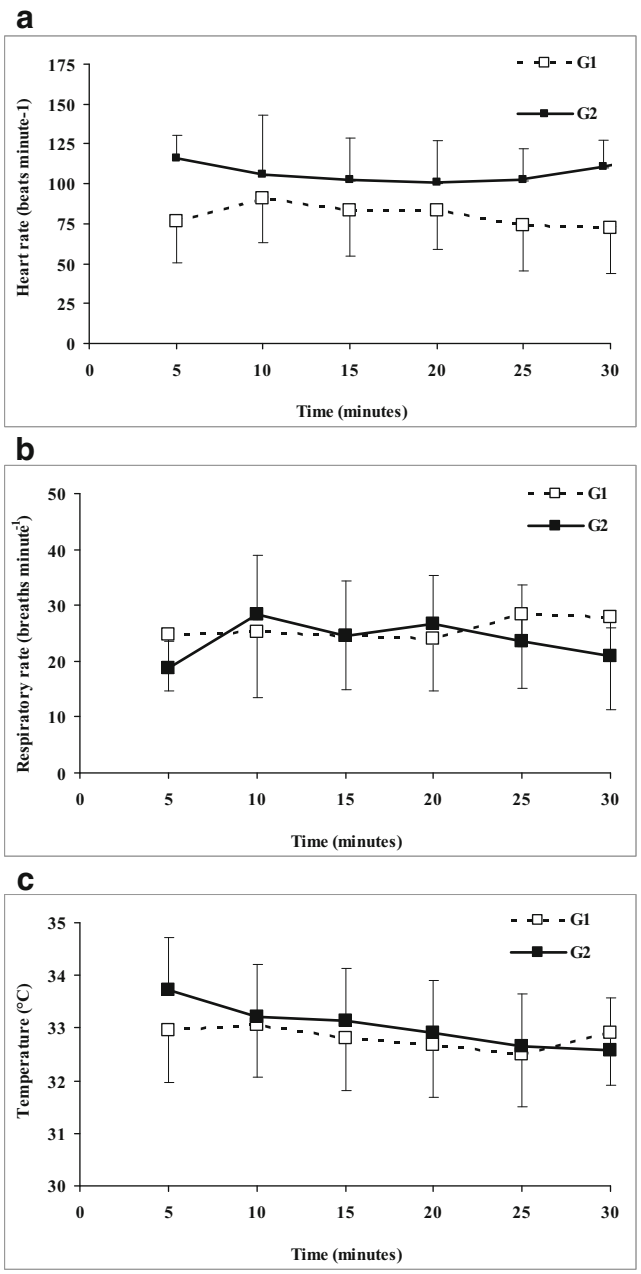

Fig. 1 Mean ± SD heart rate (**a**), respiratory rate (**b**), and body temperature (**c**) recorded during field immobilization of white-eared opossums with a ketamine-dexmedetomidine combination (G1, $n = 9$) or a ketamine-dexmedetomidine-isoflurane combination (G2, $n = 12$)

Minimal changes of the measured parameters were observed.

The lack of difference in SpO_2 was expected as both groups were subject to high FiO_2.

HR was higher in G2 than in G1, which could be due to a compensatory response to a decrease in arterial blood pressure caused by isoflurane. Unfortunately, this phenomenon could not be confirmed as very few measures of SAP, DAP, and MAP could be obtained with the oscillometric method, and, therefore, the statistical analysis was not performed. This could have probably happened due to the small size of the animals and the equipment, which is not designed for the species. Also, alpha-2 agonist associated vasoconstriction could have contributed to the difficulty in measuring blood pressure. Another explanation for a higher HR in G2 could be that an increased anesthesia depth caused by the addition of isoflurane could have promoted respiratory depression and, consequently, an increase in $PaCO_2$, which has indirectly stimulated the circulatory function through a sympathetic action (Steffey et al. 2015).

Rectal temperature decreased with time in G2, which could also be related to a peripheral vasodilation phenomenon (Steffey et al. 2015) or to the increase in anesthesia depth due to the addition of the inhalant. Also, isoflurane decreases the thresholds for thermoregulatory responses (Xiong et al. 1996), which could be responsible for temperature decrease in G_2.

RT_1 and RT_2 were recorded soon after atipamezole administration in both groups, which is desirable in field immobilization of wild species that have to be released into nature as soon as possible, although both RT_1 and RT_2 were significantly higher in G2. The exact complete recovery times were difficult to assess as animals were under capture stress and they exhibited their "death feigning" behavior. Therefore, all animals were released a minimum of 7 h after RT_2; they could all leave their cages and return to their habitat.

The difficulty in data collection given by field conditions and by equipment that is not designed for the species reduced the amount of information that could be gathered, which, together with the small sample size, makes it ambitious to draw conclusions. Nevertheless, the authors consider the information provided in this work could be useful as very little is published on field anesthesia of wild opossums.

In conclusion, 15 mg kg^{-1} of ketamine and 0.015 mg kg^{-1} of dexmedetomidine IM resulted in loss of righting reflex in a mean of 4.7 min. Both protocols were adequate for short-term field immobilization of white-eared opossums, with minimal differences in HR and T, and relatively short recovery times, although slightly longer in the group receiving isoflurane. With the administered doses of ketamine-dexmedetomidine, a surgical plane of anesthesia could not be achieved since four of nine animals in G1 showed movements during the procedure. The addition of isoflurane eased handling and performance of maneuvers, probably by an increase in anesthesia depth and muscle relaxation, which is desirable to reduce stress of wild animals. However, field inhalation anesthesia is more complicated to perform due to the equipment required. Both protocols allowed safe manipulation of these animals, thus, at capture site, if complete immobility is not a requirement, the administered doses of ketamine-dexmedetomidine could be an option.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest

Ethical approval All procedures performed involving animals were in accordance with the ethical standards of the Institution (Veterinary Sciences School, University of Buenos Aires) and were approved by its Institutional Animal Care and Use Committee (protocol no. 2012/36).

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