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Comparison between a Tail Clamp and Electrical Stimulation for Sevoflurane Minimum Anesthetic Concentration Determination in Green Iguanas (*Iguana iguana*)

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This study aimed to compare the minimum anesthetic concentration (MAC) of sevoflurane in green iguanas using electrical stimulation and tail clamping as noxious stimuli. Seven adult green iguanas (*Iguana iguana*) weighing 580 to 1,300 g were enrolled. Each iguana was anesthetized twice after a 1-week washout period, with MAC being determined using a tail clamp (MACT) or electrical stimulation (MACE) techniques. After sevoflurane mask induction and endotracheal intubation, the fraction of expired sevoflurane (FE'Sevo) was maintained at 3.1% for 15 min before noxious stimulation. In a bracketing design, the subsequent FE'Sevo values were increased or decreased by 10% after positive or negative responses, respectively. Each targeted FE'Sevo was kept constant for 15 min before stimulation. In MACT, the noxious stimulus involved closing a Kelly hemostatic curved forceps to the first ratchet at the base of the tail. At the same site, in MACE, 2 30 × 0.8–mm hypodermic needles inserted 1 cm apart were connected to an electrical stimulator set to deliver 30 mA at 50 Hz at a 6.5-ms interval. The hemostat and the needles were repositioned 2 cm distally and on alternate tail sides at each stimulation round. Individual MAC was obtained when 2 consecutive crossover events occurred (a positive response preceding a negative response or vice versa), with the MAC of each group represented by the average of the individual MAC values. Median (interquartile range) values for the sevoflurane MAC did not differ significantly between groups (2.2 [2.2 to 2.8%] in MACE and 2.2 [1.8 to 3.5%] in MACT; $P = 0.812$). Time to anesthesia induction, time to MAC measurement, heart rate (HR), end-tidal carbon dioxide ($ET'CO_2$), and cloacal temperature were not different between groups. Both the tail-clamping and the electrical stimulation techniques yielded resembling sevoflurane MAC values in green iguanas, which makes the tail clamp a reliable alternative to electrical stimulation-based MAC research in this species.

Abbreviations and Acronyms: $ET'CO_2$, end-tidal carbon dioxide; FE'Sevo, fraction of expired sevoflurane; HR, heart rate; MAC, minimum anesthetic concentration; MACE, minimum anesthetic concentration using electrical stimulation; MACT, minimum anesthetic concentration using a tail clamp

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Introduction

The growing trend of keeping reptiles as pets[15] has increased interest in refining methods of anesthesia for clinical and research purposes. In lizards, the escalating use of inhalation anesthesia has prompted the establishment of species-specific minimum anesthetic concentration (MAC) values,[4-6,9,12] where MAC is described as the concentration abolishing purposeful movements in 50% of the individuals of a population submitted to a supramaximal noxious stimulus.[7] In addition to guiding the maintenance of a surgical plane of anesthesia, the MAC has proved to be key to ensuring safe lizard anesthesia as cardiovascular depression was demonstrated at increasing isoflurane concentrations in green iguanas (*Iguana iguana*).[13]

Traditionally, lizard MAC studies have relied on electrical currents applied to the tail for noxious stimulation.[4-6,9,12] In green iguanas, it has been suggested that electrical stimulation-induced tissue desensitization could be responsible for the observed decreased MAC values when repeated exposure to electrical stimulation at a single tail site is used as noxious stimuli.[4] Indeed, such phenomenon was documented in rats undergoing high-voltage electrical stimulation,[11] thus raising the question of whether the electrical stimulation-based methods used in lizard MAC research actually achieve supramaximal noxious stimuli.

Alternatively, the tail-clamping technique has been applied in other animals,[1,8] representing a more accessible and user-friendly method for MAC determination. The MAC of halogenated anesthetics obtained with electrical currents and a tail clamp were similar in rats[11] and dogs.[7] To the authors' knowledge, it has not been addressed whether electrical stimulation and tail-clamping techniques provide comparable MAC values in lizards. Therefore, this study aimed to investigate the sevoflurane MAC in green iguanas as determined by electrical noxious stimulation and a tail-clamp test. It was hypothesized that the sevoflurane MAC obtained by electrical stimulation would be equivalent to that measured using

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the tail-clamping technique in green iguanas after noxious stimulation at varying tail sites.

Materials and Methods

This study was approved by the Animal Care and Use Committee of the Faculty of Animal Science and Food Engineering, University of São Paulo (protocol Number 8969311022), and by the Brazilian Biodiversity Information and Authorization System (SISBIO Number 79652 to 2).

Animals. Seven adult green iguanas (580 to 1,300 g) of unknown sex were included. A subcutaneous microchip (Destron Fearing, Dallas, TX) identified individuals. Animals were housed at a lizard colony set up to keep a temperature- (25 to 30 °C), humidity- (50 to 60%) and photoperiod-controlled (12:12-h light:darkness) environment. The individuals were housed in pairs in fiberglass tanks (1.35 × 0.73 m) with hay as substrate, plastic containers for food and water, and tree branches as perches. Iguanas were fed a balanced diet composed of dark leafy greens, vegetables and fruits enriched with a calcium carbonate supplement (Homeopatia Ouro Preto, Pirassununga, Brazil). Water was provided ad libitum.

A 12-h-light cycle was maintained by 30-W UVA/UVB fluorescent lamps (Lucky Herp, Changzhou Jiangsu, China). In addition, at basking spots, high-temperature areas (40 to 45 °C) were created by 250-W incandescent lamps (Lumanti Lighting for Life, Erechim, Brazil), remaining switched on for 2 h twice daily. Animals were deemed healthy based on clinical examinations, feeding behavior, and weight gain over time.

Study design. This study used a prospective, crossover, nonblinded, and nonrandomized design. Animals were assigned depending on the technique used for sevoflurane MAC determination to either the tail-clamp (MACT; n = 7) or electrical stimulation (MACE; n = 7) groups. First MACT, then MACE, was conducted, after a 1-week washout period was implemented. All data were collected during summer (December 2022 to March 2023), with anesthesia induction performed at 12:00 to avoid the influence of circadian rhythms.

Instrumentation and variables monitored. Food, but not water, was withheld 24 h before anesthesia. At the anesthesia facility, temperature of ambient air was prewarmed to 28 to 30 °C. Animals were manually restrained in sternal recumbency and anesthesia induction was carried out with sevoflurane (Sevocris, Cristália, Itapira, Brazil) in oxygen (3 L min^{-1}), delivered through a face mask connected to a nonrebreathing Mapleson B circuit modified to contain one-way valves on the inspiratory and expiratory limbs of the circuit. The dial of the vaporizer (V60; Mindray, Shenzhen, China) was initially set to 8 Vol% until mandibular tone and righting reflex were abolished, as assessed at 60-s intervals. Orotracheal intubation was performed with a 2.0-mm uncuffed endotracheal tube (Rusch, Duluth, GA), fresh gas flow rate was reduced to 500 mL min^{-1} and the dial of the vaporizer adjusted to achieve a 3.1% fraction of expired sevoflurane (FE'Sevo). This FE'Sevo neared the sevoflurane MAC established for this species.[4]

The animals underwent pressure-controlled mechanical ventilation (Wato EX-35Vet; Mindray, Shenzhen, China), with peak inspiratory pressure, respiratory rate and inspiration-to-expiration ratio set at 5 cm H_2O, 4 breaths min^{-1} and 1:2, respectively. A 22-gauge catheter (Solidor, India) was inserted through the endotracheal tube and its tip advanced halfway down the tube. The catheter was attached to a sidestream sampling line (aspiration rate: 200 mL min^{-1}) of an infrared gas analyzer (Wato EX-35Vet, Mindray, Shenzhen, China) for end-tidal carbon dioxide (ET'CO_2) and FE'Sevo measurements.

Cardiorespiratory variables were recorded every 10 min by a multiparameter monitor (ePM 12M Vet; Mindray, Shenzhen, China). A lead II electrocardiogram registered HR and rhythm, with the electrodes coupled to the skin folds on both forelimbs and left hindlimb. Body temperature, as assessed with a probe inserted 10 cm into the esophagus, was maintained between 31 and 32 °C by using an electrical heating mattress (Infrared Heat Mattress; Sonobel, Pelotas, Brazil). Animals remained in sternal recumbency during anesthesia.

MAC determination. Before each MAC determination, the gas analyzer was calibrated using room air and 3 standards of known sevoflurane concentrations (0.5, 1.5, and 3%) (White Martins Gases Industriais SA, Rio de Janeiro, Brazil). FE'Sevo values obtained after anesthesia were corrected using linear regression equations obtained from the measurements of the calibration gas standards. After reaching the initial target FE'Sevo (3.1%), this value was kept constant for 15 min before commencing noxious stimuli delivery. In MACT, a Kelly hemostatic curved forceps was closed to the first ratchet at the base of the tail, caudolateral to the cloaca. In MACE, 2 30 × 0.8–mm hypodermic needles inserted 1 cm apart at the same site as in MACT were connected to the electrodes of an electrical stimulator (MT 10-BR, Medcir, Brazil) preset to deliver 30 mA at 50 Hz in 6.5-ms intervals. Furthermore, the hemostat and the needles were repositioned 2 cm distally and on alternate tail sides at each stimulation round.[5,11] Noxious stimulation was continuously delivered for up to 1 min in MACE[12] or for 2 s in MACT,[10] unless the iguana showed positive motor responses before this (gross movements of the limbs and/or lifting of the head or neck). Discrete muscle spasms at the stimulated site were considered negative responses.

A bracketing design was used to establish the sevoflurane MAC. Briefly, the FE'Sevo was increased or decreased by 10% after a positive or negative response to the stimulus, respectively. At every new target FE'Sevo, 15 min were considered before the noxious stimulus was reapplied. These steps were performed until 2 consecutive crossover events were observed, that is, a positive response preceding a negative response or vice versa. Individual MAC values were determined as the arithmetic mean of the highest FE'Sevo allowing movement and the lowest FE'Sevo preventing movement.

After MAC determination, the vaporizer dial was reset to zero and time to MAC measurement was recorded. The aforementioned ventilatory mode was maintained until spontaneous ventilation was observed by capnography, and extubation was performed after endotracheal tube chewing. Animals were returned to their original tanks no earlier than 60 min postextubation and were closely monitored on the following day after anesthesia to ensure normal feeding behavior.

Statistical analysis. Based on pilot studies, the sample size (n = 7) was estimated considering standard deviation, α error, β error, and effect size values of 0.13, 0.05, 0.20, and 0.4, respectively (http://estatistica.bauru.usp.br/calculoamostral/). GraphPad Prism (GraphPad Software, San Diego, CA) was used for all analyses. The symmetry of the data distribution was verified by the Shapiro-Wilk test, and the coefficient of variation helped determine the extent of variability of data. Normally distributed data were expressed as mean ± SD; otherwise, they were expressed as median (interquartile range). Time to sevoflurane induction, MAC, and time to MAC measurement were compared between groups using paired t (parametric) or Wilcoxon (nonparametric) tests. Cardiorespiratory variables were analyzed between treatments by a 2-way repeated measures ANOVA followed by the Sidak multiple comparisons test. Differences were considered to be statistically significant at $P < 0.05$.

Results

Acceptance of restraint during sevoflurane delivery by mask was satisfactory, and time to induction did not differ between groups (9.1 [5.7 to 9.8] min in MACE and 11.1 [9.0 to 12.5] in MACT; $P = 0.375$). Median (interquartile range) values for the sevoflurane MAC were not significantly different between the electrical and tail-clamping techniques, being 2.2 (2.2 to 2.8%) in MACE and 2.2 (1.8 to 3.5%) in MACT ($P = 0.812$) (Figure 1). Time to MAC measurement was 186 (147 to 279) min in MACE and 211 (194 to 292) min in MACT, which were not different between groups ($P = 0.468$). No statistically significant between-treatment differences were found for HR, ET'CO_2 and cloacal temperature. Overall HR was 36 ± 6 bpm in MACE and 35 ± 5 bpm in MACT, while ET'CO_2 was 14 (10 to 16) mm Hg in MACE and 12 (11 to 14) mm Hg in MACT. Cloacal temperature was 31 ± 0.8 °C across treatments. All animals recovered uneventfully.

Discussion

This study provides a comparison of 2 methods for assessing MAC in green iguanas and indicates that tail-clamping and electrical stimulation techniques could be used interchangeably to determine the sevoflurane MAC in this species. This finding bears relevant practical effects for future MAC experiments in green iguanas, especially considering the cost of an electrostimulator compared with a hemostat forceps. Besides, in electrostimulation-based MAC studies, correct needle placement implies tail puncturing, which may be bruising or predispose the skin to infection. This may be particularly concerning when the needles are constantly repositioned to avoid desensitization and thermal injury, as were in the current and in past studies.[5,9,11]

MAC research is grounded on the assumption that the noxious stimulus emulates a surgical incision, which is the one deemed supramaximal.[11] Although lizard MAC studies have widely used electrical stimulation, the ideal electrostimulator settings, that is, the one producing supramaximal stimuli, have not been established. The settings applied in the current study followed a tegu (*Salvator merianae*) experiment,[6] yet the electrical intensity required to attain supramaximal stimuli may vary on a species-specific basis.[5,12] As for the tail clamp, there is a paucity of evidence supporting its use for lizard MAC determination. The protocol reported here was based off of a ball python (*Python regius*) study,[10] though it is unknown whether grasping the tail, and no other body parts (for example, toe or vent), reflects a supramaximal stimulus in lizards. Therefore, whereas present data show that a tail clamp and electrical stimuli, as applied according to current descriptions, yield equivalent levels of noxious stimulation in green iguanas, it remains to be confirmed whether both stimuli are indeed supramaximal.

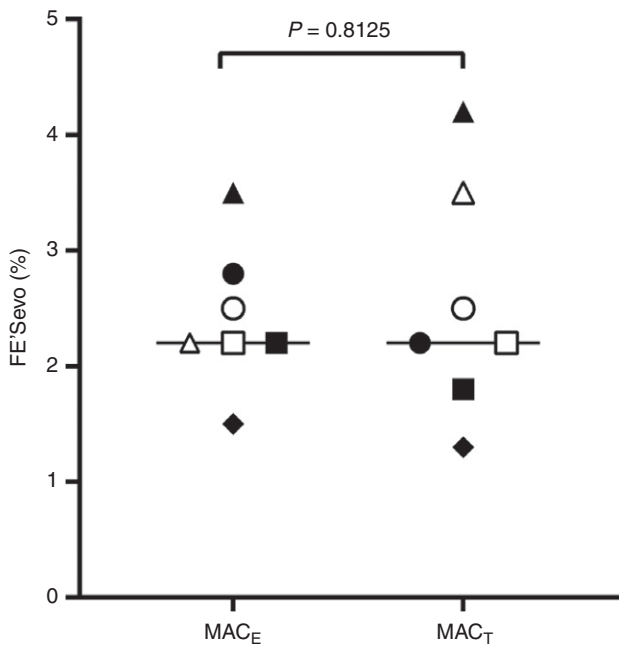

Figure 1. MAC of sevoflurane obtained in green iguanas after electrical stimulation (MACE; $n = 7$) and tail-clamping (MACT; $n = 7$) techniques in a nonrandomized, complete crossover design, with a minimum washout period between treatments of 1 wk. Each symbol represents an animal and the median MAC is indicated by horizontal bars.

MAC underestimation in green iguanas has been attributed to tissue desensitization after repeated application of electrical stimuli at a single site.[4] In this sense, moving the electrodes at each round of stimulation, as compared with using the base of the tail alone,[4] would presumably lead to higher—and more accurate—MAC values. However, in MACE, the sevoflurane MAC (2.2 [2.2 to 2.8%]) was lower than that previously reported for this species (3.1 ± 1%).[4] Such a disparity could be explained by methodology- and physiologic-related factors.

First, in lizard MAC research, contamination with inspired gas may be avoided by collecting end-tidal gases through a sampling line fed down the endotracheal tube up to its tip.[5,12] In view of the small luminal diameter of the endotracheal tube used, this maneuver proved to be impractical for the iguanas of the current study; instead, a catheter was used for end-expired gas measurements, its tip resting on the midpoint between the proximal and distal ends of the endotracheal tube. Although airway gas dilution could still have been present at this sampling site, it likely occurred to a decreased magnitude than that in the above-mentioned experiment,[4] where the sample collection line was placed between the endotracheal tube and the breathing circuit. Therefore, the previously documented sevoflurane MAC of green iguanas could have been abnormally high because equipment dead space contributes with dilution of expired gases with fresh gas flow,[3] which is high in anesthetic and low in carbon dioxide partial pressures. Second, in the present study, the body temperature of the green iguanas (31 ± 0.8 °C) fell within the temperature interval of husbandry recommendations for this species (30 to 32 °C),[14] though largely contrasted to that previously published (35.5 ± 0.2 °C).[4] Considering the well-known linear relationship between MAC and body temperature,[2] MAC values similar to those described here should be expected only in green iguanas maintained within the current reported body temperature range. Such a statement is corroborated by an early study in isoflurane-anesthetized rats, where MAC decreased by as much as 5.28% at the rate of 1 °C Decrease in body temperature.[16] If a similar relationship also holds true for iguanas, then the observed 2.2% sevoflurane MAC at 31 °C in the present study would be near 2.6% at 35 °C. Third, previous studies have pondered whether the extent to which right-to-left intracardiac shunting occurs during lizard gas anesthesia could impair MAC estimates by altering pulmonary dead space.[12] For example, if faveolar-arterial equilibration of sevoflurane partial pressures is delayed because of intracardiac shunting during green iguana anesthesia, then the 15-min equilibration time reported here would have been insufficient for a steady state to be reached prior to MAC measurements. Equilibration times reported in the literature range from 15 to 30 min for

lizards[4-6,9,12] yet merit a more in-depth investigation because it could provide insights into the influence of time elapsed until MAC assessment on interstudy MAC discrepancies.

In summary, the tail clamp and the electrical stimuli produced comparable sevoflurane MAC values in green iguanas, thus making the tail-clamping technique a promising one for MAC determination in other lizard species. Further research is warranted to establish whether the tail-clamp stimulus reflects a supramaximal noxious stimulus in green iguanas.

Conflict of interest

The author(s) have no conflict(s) of interest to declare.

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