Isoflurane, sevoflurane and desflurane use in cane toads (Rhinella marina)

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ABSTRACT

Anesthetic chamber concentrations of isoflurane, sevoflurane and desflurane that resulted in loss of righting reflex within 15 minutes in 50 per cent of toads (Rhinella marina) exposed (ED_{50-LRR<15MIN}) were identified. The median and range ED_{50-LRR<15MIN} was 1.4 (0.9–1.4) per cent for isoflurane, 1.75 (1.1–1.9) per cent for sevoflurane and 4.4 (4.3–5.5) per cent for desflurane. Subsequently, toads were exposed to 1.5 times the ED_{50-LRR<15MIN} and times to loss and return of righting reflex were identified. All toads for all anaesthetics lost righting reflex. The median and range of righting reflex were identified. Time to return of righting reflex was 175 (123–211) minutes for isoflurane, 192 (116–383) minutes for sevoflurane and 74 (52–220) minutes for desflurane. Time to return of righting reflex was significantly shorter for desflurane compared with isoflurane or sevoflurane. The use of isoflurane, sevoflurane or desflurane can be used to provide immobilisation to cane toads and potentially other anurans. Induction times are likely similar when using an anesthetic chamber to provide anaesthesia. However recovery time may take twice as long when utilising isoflurane or sevoflurane over desflurane.

INTRODUCTION

Amphibians continue to increase in popularity as household pets and research species of interest. Exact numbers are unknown, however the American Pet Product Association estimated that in 2011, there were 13.6 million reptiles and amphibians kept as pets in the USA (American Pet Product Association 2011). The Pet Food Manufacturers’ Association estimated that in 2014, 100,000 frogs and toads were kept as pets in the UK (Pet Food Manufacturers’ Association 2014). This growing popularity has amplified the need for veterinarians to have a greater understanding of anaesthesia as it relates to these species. Various sedatives and anaesthetics have been evaluated in amphibian species. Research has revolved around topical and injectable anaesthetics such as tricaine methanesulfonate (MS-222), benzocaine, eugenol, topical compounded formulation of volatile anaesthetics, α-2 agonists, dissociative anaesthetics, propofol, ethanol, alfaxalone and barbiturates (Brenner and others 1994, Cakir and others 2005, Guénette and others 2013, Posner and others 2013, Stone and others 2013, Zec and others 2014). Although volatile anaesthetics have been delivered to amphibians via immersion and topical routes, only a few studies investigating the use of the volatile anaesthetics methoxyflurane, halothane, isoflurane and desflurane delivered via the lungs have been completed (Shim and Andersen 1971, Wass and Kaplan 1974, Smith and Stump 2000, Barter and Antognini 2008).

The standard index of anaesthetic potency in mammals for inhalation anaesthetics is the effective dose 50 per cent (ED_{50}) which is the amount of anaesthetic at 1 atmosphere which produces immobility in 50 per cent of subjects exposed to a supramaximal noxious stimulus (Steffey and others 2015). The use of ED_{50} values in clinical practice of anaesthesia is important in that it gives a practitioner useful information on appropriate dosing to minimise adverse effects. In mammals, ED_{50} values tend to be similar between species, thus extrapolation between mammalian species can be easily done (Steffey and others 2015). Interestingly, the known ED_{50} values for halothane, isoflurane and desflurane in frogs is similar to values in dogs suggesting that ED_{50} values may be conserved across non-mammalian species (Barter and Antognini 2008). However, these ED_{50} values for anaesthesia in amphibians and particularly anurans may be of minimal practical value in clinical practice. This is due to long equilibration times ranging from three hours for desflurane to as much as six hours for isoflurane (Barter and Antognini 2008). As a result, when using ED_{50} values to guide inhalant anaesthetic use, anurans may
require prolonged exposure to anaesthetics to achieve immobility and loss of response to noxious stimuli. Therefore, anaesthetic values that correspond to short induction times may be more clinically relevant in these species.

For evaluation of anaesthetic effect, loss of the righting reflex is a commonly used anaesthetic end point in studies using laboratory or exotic animals for assessment and comparison of potency of various anaesthetic agents (Joo and others 2001, Heaton-Jones and others 2002, Diaz-Gil and others 2015, Zachariah and others 2014). This is performed by manually placing the animal in dorsal recumbency and then observing for the animal to spontaneously return to sternal recumbency. Animals that cannot spontaneously return to a sternal position are considered immobilised.

The aims of this study were (1) to determine the effective dose at which 50 per cent of cane toads, exposed to isoflurane, sevoflurane or desflurane, lost righting reflex in <15 minutes (ED_{50-LRR<15MIN}) and (2) to determine induction and recovery times of cane toads exposed to 1.5 times ED_{50-LRR<15MIN}.

**MATERIALS AND METHODS**

**Determination of ED_{50-LRR<15MIN}**

This portion of the study utilised eight captive-bred, six-month-old cane toads (*Rhinella marina*) of undetermined sex and a mean weight of 95.8±24.4 g. Toads were housed individually in 48 l glass tanks in the same room that all procedures were performed. The room was maintained at a temperature of approximately 26°C (78.8°F). Toads were kept on a substrate of shaved coconut hulls and provided a stainless steel bowl with dechlorinated tap water with sufficient depth to allow the toads to completely submerge. A 12-hour photoperiod was provided with fluorescent lights, and a diet of commercially raised, calcium dusted crickets and night crawlers were fed once weekly. Toads were determined to be healthy based on physical examination, activity level and normal feeding behaviour. The toads were allowed to acclimate to their environment for a period of 14 days before the initiation of the study. Feed was withheld from toads a minimum of three days before all anaesthetic events. All phases of the study were performed in the same room under the same environmental conditions.

A custom anaesthetic chamber that was based on an invertebrate surgery chamber and successfully used in previous amphibian studies was used in this study (Melidone and Mayer 2005, Stone and others 2013, Zec and others 2014). The chamber was built with a 6.9 l, clear, airtight container (Container, Snapware). Two 10.2-cm-diameter round holes were cut into opposite sides of the container, and a 10.2-cm-diameter polyvinyl chloride male pipe connector was inserted into each hole. Silicone sealant was applied around the outside junction of the pipe connectors and the chamber to achieve an airtight seal. A 0.3-cm-diameter hole was made near the top of the chamber on one side, and a 14-gauge intravenous catheter (BD Angiocath, BD Medical), trimmed to 2.5 cm in length, was inserted from the outside with rubber washers to achieve an airtight seal. An airway gas sampling line was attached between the hub of the catheter and an airway gas monitor (Expert DR-5300W, Datascope) for evaluating chamber anaesthetic concentrations. Two 0.5-cm-diameter holes were made in opposite sides of the chamber and plastic adapters were inserted for attachment of a fresh gas flow line from an anaesthesia machine with an agent-specific vaporiser and a waste-gas scavenger hose for introduction and removal of anaesthetic gases within the anaesthetic chamber. Foam-lined kitchen gloves were placed through the PVC pipe connector openings, and the glove openings were stretched inversely around the outside of the PVC connectors and secured with 1-inch medical tape to achieve an airtight seal. This allowed for manipulation of the toads without opening the anaesthetic chamber (Fig 1).

A cross-over design was used for this study. All toads were subjected to an anaesthetic trial with isoflurane, sevoflurane and desflurane. A washout period of a minimum of two weeks was allowed between each anaesthetic trial. For each trial, a toad was placed in the anaesthetic chamber and anaesthetic was delivered in oxygen at a rate of 21/minute. An initial concentration of approximately 0.25 MAC for canines of isoflurane (IsoFlo, Abbott Laboratories), sevoflurane (SevoFlo, Abbott Laboratories) or desflurane (Suprone, Baxter) was delivered and maintained for 15 minutes. Loss of righting reflex was evaluated by manually placing the toad in dorsal recumbency. If a toad demonstrated righting reflex, the ability to return to sternal recumbency, it was deemed to have a positive righting reflex and the trial continued. The anaesthetic concentration within the chamber was increased by 0.2–0.5 per cent and an additional 15 minutes was allowed to elapse. Evaluation for loss of righting reflex was repeated. The trial ended when a toad demonstrated loss of righting reflex by being unable to spontaneously return to sternal recumbency. The anaesthetic concentration was recorded and the toad was removed from the chamber and allowed to recover. The same procedure was repeated for each toad and each volatile anaesthetic. The median and range was calculated for each anaesthetic.

**Determination of loss and return of righting reflex after exposure to 1.5 times ED_{50-LRR<15MIN}**

This portion of the study utilised the same 8 cane toads plus an additional 2 for a total of 10 toads. Toads were kept under the same housing conditions as previously described. The same custom anaesthetic chamber was used.

A cross-over design was used for this portion of the study as well. All toads were again subject to an
anaesthetic trial with isoflurane, sevoflurane and desflurane. A washout period of a minimum of two weeks was allowed between each anaesthetic trial. For each trial, a toad was placed in the anaesthetic chamber and anaesthetic was delivered in oxygen at a rate of 2 L/minute. Each anaesthetic was delivered at 1.5 times the ED$_{50}$-LRR$<$15MIN. Toads were manually placed into dorsal recumbency at 30-second intervals until loss of righting reflex was noted. Time to loss of righting reflex was recorded. Once loss of righting reflex was noted, toads were maintained with anaesthetic for an additional 15 minutes. At the end of 15 minutes, toads were removed from the anaesthetic chamber and placed on a cotton towel in dorsal recumbency. The probe from a Doppler flow detector was placed over the heart and heart rate was recorded at 5-minute intervals. The rate of gular movement was also recorded at 5-minute intervals. When toads spontaneously returned to sternal recumbency, heart rate recording stopped and the time was noted. Data were analysed for normality with a Kolmogorov-Smirnov test. Loss and return of righting reflex times were analysed with a Friedman test and a post hoc Dunn’s multiple comparison test. Heart rate data were assessed with a Kruskal-Wallis test. Mean gular rate data was assessed with a Mann-Whitney test. A commercial statistical software program was used for analysis (InStat 3.10, GraphPad Software). A P<0.05 was used for statistical significance.

RESULTS
The ED$_{50}$-LRR$<$15MIN, the loss and return of righting for each anaesthetic, and the gular rate data are reported as median and range. Heart rate data are reported as mean±sd. The ED$_{50}$-LRR$<$15MIN was 1.4 (0.9–1.4) per cent for isoflurane, 1.75 (1.1–1.9) per cent for sevoflurane and 4.4 (4.3–5.5) per cent for desflurane. For toads exposed to 1.5 times ED$_{50}$-LRR$<$15MIN (2.1 per cent isoflurane, 2.6 per cent sevoflurane and 6.6 per cent desflurane), loss of righting reflex was 4 (3:00–5:30) minutes for isoflurane, 4:45 (3:30–7:00) minutes for sevoflurane, and 4:15 (4:00–5:30) minutes for desflurane. Time to loss of righting reflex did not differ between groups (P=0.30). Time to return of righting reflex was 175 (123–211) minutes for isoflurane, 192 (116–383) minutes for sevoflurane and 74 (52–220) minutes for desflurane. There was a significant difference overall for time to return of righting reflex (P=0.0063). There was no difference in time to return of righting reflex between isoflurane and sevoflurane (P>0.05), however desflurane time was significantly shorter than isoflurane and sevoflurane (P<0.05 for both). Data for individual toads can be found in Table 1. Mean heart rate was 29.2±7.1 bpm for isoflurane, 28.6±7.3 bpm for sevoflurane and 29.8±8.1 bpm for desflurane and differences between groups were not identified (P=0.05). Mean gular rates were 23.2 (4.0–52.3) movements per minute for isoflurane,

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15.0 (5.8–54.1) movements per minute for sevoflurane and 56.8 (8.6–78.2) movements per minute for desflurane. There was no difference in mean gular rates between isoflurane and sevoflurane (P>0.05). Desflurane mean gular rates were significantly higher compared with mean isoflurane gular rates (P=0.0089) and mean sevoflurane gular rates (P=0.0052).

DISCUSSION
Anaesthetic chamber concentrations of isoflurane, sevoflurane and desflurane that resulted in loss of righting reflex within 15 minutes of exposure in 50 per cent of the toads were identified. These values likely do not represent a true ED50 of these anaesthetics as it has been estimated that to achieve equilibration in anurans between the lung and the effect site (the CNS) requires 480 minutes for isoflurane and 180 minutes for desflurane (Barter and Antognini 2008). The ED50 for sevoflurane has not been determined in anurans. The time required for volatile anaesthetic equilibration in mammals is considerably shorter (approximately 15 minutes) and is based on cerebral blood flow and the brain/blood partition coefficient of the anaesthetic agent (Eger and others 1965). Anurans possess physiological and anatomical differences that cause prolonged equilibration time. Additionally, anurans also have gas exchange across the skin and buccal cavity and these can be manipulated for anaesthetic delivery (Huchison and others 1968, Stone and others 2013, Zec and others 2014). After exposure to inhalant anaesthetics, if pulmonary ventilation is reduced or ceases, simple cutaneous diffusion dictates movement of anaesthetics and is much less efficient than diffusion that occurs in the lung (Püper and Scheid 1975). Additionally, the heart of anurans has only a single ventricle that results in intraventricular shunting. As a consequence, distribution of anaesthetic agents may be impaired because only a fraction of blood is participating in gas exchange and cardiac output is reduced (Moalli and others 1980).

Exposure to 1.5 times the ED50 of isoflurane, sevoflurane and desflurane resulted in loss of righting reflex in all toads. This is of clinical relevance to practitioners as it gives an estimate of the amount of anaesthetic necessary for chamber inductions of anurans. Times to loss of righting reflex were similar for all anaesthetics at approximately four minutes. It is important to note that testing for loss of righting reflex was performed at 30-second intervals and therefore more exact times are not known. Exact determination would likely decrease the range of times for loss of righting reflex but would have been difficult to determine as it would require continuous manipulation of the toads. The values reported here are likely to be considered clinically acceptable and subjectively, all toads appeared to be completely immobilised. It is unknown if a procedure that involved pain, such as a skin incision would be tolerated, as response to noxious stimuli was not tested. Noxious stimuli used in amphibians include leg/foot clamps, electrical current and acetic acid (Shim and Andersen 1971, Stevens 1992, Barter and Antognini 2008). Noxious stimuli testing was not an option for this study as permanent lesions could result and these toads were only available for non-invasive use.

In this study, toads were not intubated and therefore end expiratory measurement of anaesthetic concentration in the toad’s lung sacs could not be determined. End expiratory gases are used to determine ED50 values in mammals (Steffey and others 2015). However, because of cutaneous gas exchange, measurement of gases solely from an endotracheal tube would be unreliable and expose personnel to cutaneous waste gas. The
use of the airtight anaesthetic chamber in this study allowed for manipulation of toads as well as measurement of anaesthetic while minimising waste gas exposure. The chamber used in this study has a volume of 6.9 l and anaesthetic was delivered into the chamber in 100 per cent oxygen at a rate of 2 l/minute. The time constant for this chamber (the time required for the concentration of anaesthetic in the chamber to rise exponentially approximately 63 per cent) is calculated as volume/flow. Therefore, a single time constant for the anaesthetic chamber used in this study and a fresh gas flow rate of 2 l/minute is 3:45 minutes. Three time constants are required to achieve greater than 95 per cent of the volume of the chamber to achieve the desired anaesthetic concentration. Thus, when a change was made to the vaporiser on the anaesthetic machine, anaesthetic concentration. Thus, when a change was made to the vaporiser on the anaesthetic machine, during the determination of the ED50-LRR<15MIN values, it took approximately 10 minutes for chamber anaesthetic concentration to equal vaporiser output. Once the chamber anaesthetic reached the desired concentration, toads were allowed an additional 15 minutes of exposure time before testing for loss of righting reflex.

Volatile anaesthetic dose is frequently determined in terms of multiples of ED50 that is, 1.5 or 2 times ED50. In general, 1.2 to 1.5 times ED50 provides a surgical depth of anaesthesia in mammals and 2 times ED50 results in autonomic depression and potential overdose (Steffey and others 2015). The authors chose to use 1.5 times the derived ED50-LRR<15MIN values to ensure that 100 per cent of the subjects would lose their righting reflex. In mammals, adverse effects of volatile anaesthetics include decreased sympathetic tone, depressed respiratory function, vasodilation and bradycardia that are dose-dependent and can lead to decreased effective circulating blood flow, organ damage and death (Steffey and others 2015). Heart rate was assessed in this study as an indirect marker of anaesthetic depth and was not different between anaesthetics. This may indicate that toads were at similar anaesthetic depths and that cardiac suppression between the anaesthetics was not different. However, the difference in rates of gular movement was an unexpected finding in this study and may have impacted the times to return of righting reflex. The buccal cavity in anurans can be expanded by movement of the floor of the cavity (gular movement). Because of a lack of diaphragm, anurans use gular (or buccal) pumping to complete both inspiration and expiration (Brainerd 1999). Thus, in anurans, gular movement can be observed for respiratory effort. Because the gular rate was higher in toads exposed to desflurane, elimination of the volatile anaesthetic may have occurred at a higher rate compared with isoflurane and sevoflurane because of the greater efficiency of pulmonary gas exchange compared with cutaneous diffusion resulting in a quicker return of righting reflex. Perhaps, desflurane causes less respiratory depression in toads or gular movement was better maintained because toads exposed to desflurane were at a lighter plane of anaesthesia. The lethal dose of isoflurane, sevoflurane and desflurane in cane toads is unknown.

In mammals, ED50 values are derived from a supramaximal noxious stimulus and in dogs are 1.28 per cent for isoflurane, 2.36 per cent for sevoflurane and 7.2 per cent for desflurane (Steffey and Howland 1977, Doorley and others 1988, Kazama and Ikeda 1988). The ED50-LRR<15MIN values derived here are similar to the canine value for isoflurane but less for sevoflurane and desflurane. However the values derived in this study are from loss of righting reflex and not a noxious stimulus and may not be suitable for surgical procedures.

Induction and recovery time differences between inhalant anaesthetics in mammals is related to the physiochemical properties of each agent. Solubility in different tissues is one of the main properties that governs speed of induction and recovery and less soluble agents have a more rapid onset and termination of effect (Steffey and others 2015). Desflurane has a lower solubility than sevoflurane and isoflurane. Therefore, in mammals, induction and recovery times tend to be quicker with desflurane when compared with isoflurane and sevoflurane, whereas the difference between isoflurane and sevoflurane is clinically negligible (Lopez and others 2009, Lozano and other 2009, Steffey and others 2015). In this study, time to loss of righting reflex did not differ between the anaesthetics, however righting reflex returned in desflurane toads more than twice as fast than with either isoflurane or sevoflurane.

CONCLUSIONS

In conclusion, the use of isoflurane, sevoflurane or desflurane can be used to provide immobilisation to cane toads and potentially other anurans. Induction times are likely similar when using an anaesthetic chamber to provide anaesthesia. However recovery time may take twice as long when utilising isoflurane or sevoflurane over desflurane.

Contributors All authors contributed to study design, data collection and interpretation, and manuscript preparation.

Competing interests None declared.

Ethics approval This study was approved by the Institutional Animal Care and Use committee (protocol #s 14221 and 15092).

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