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Optimization of inhaled anesthesia for *Octodon degus* using electroencephalography

Yuki IKAI[1,2], Goro A. NAGURA-KATO[1], Shinsuke H. SAKAMOTO[3], Akio SHINOHARA[1] and Chihiro KOSHIMOTO[1]

[1]*Division of Bio-resources, Department of Biotechnology, Frontier Science Research Center, University of Miyazaki, Kihara 5200, Kiyotake, Miyazaki 889-1692, Japan*
[2]*Graduate School of Medicine and Veterinary Medicine, University of Miyazaki, Kihara 5200, Kiyotake, Miyazaki 889-1692, Japan*
[3]*Department of Animal and Grassland Sciences, Faculty of Agriculture, University of Miyazaki, 1-1 Gakuen Kibanadai-nishi, Miyazaki 889-2192, Japan*

Abstract: Physiological responses to inhaled anesthetics vary among species. Therefore, a precise anesthetic technique is important for each individual species. In this study, we focused on the degu (*Octodon degus*), a small herbivorous rodent. Degus have recently begun to be used as laboratory models for brain research because of certain human-like characteristics, such as spontaneous development of Alzheimer's disease. In this study, we evaluated appropriate induction and maintenance anesthesia conditions for isoflurane and sevoflurane in degus by a stimulation test, electroencephalography (EEG), minimum alveolar concentration (MAC), and vital signs. During induction, more rapid time to loss of the righting reflex and deeper anesthesia in degus were observed in isoflurane. The MAC value for degus were 1.75 ± 0.0% in isoflurane and 2.25 ± 0.27% in sevoflurane. Whereas some degus were awake during maintenance anesthesia using both anesthetics at concentrations of ≤2%, no rats were awake when using sevoflurane at a concentration of 2%. The duration of the total flat EEG, a measure of the depth of maintenance anesthesia, was longer for isoflurane than for sevoflurane. Furthermore, higher concentrations of both anesthetics suppressed the respiratory rate in degus. These new findings regarding inhalation anesthesia in degus will contribute to future developments in the fields of laboratory animals and veterinary medicine.
Key words: animal welfare, degu, isoflurane, refinement, sevoflurane

Introduction

Anesthetic techniques for experimental animals can be broadly classified into injection and inhalation anesthesia [1]. Injectable anesthesia requires careful handling of animals and securement of the injection route. By contrast, inhalation anesthesia is much simpler to perform; the anesthetic state is quickly achieved by simply moving the animal into a chamber filled with a mixture of anesthetic and air (oxygen). In addition, the depth of inhalation anesthesia during the operation can be precisely adjusted using a dedicated inhalation vaporizer to achieve the appropriate concentration of anesthetic agent. Furthermore, awakening and recovery after inhalation anesthesia is usually quicker than that after injectable anesthesia. Because of these advantages, inhalation anesthesia has been used in numerous experimental species ranging from small- to medium-sized animals.

Isoflurane and sevoflurane are representative inhaled anesthetics and have been used for a variety of applications in animals, including short-term sedation, deep anesthesia, and euthanasia [2–5]. Especially for small

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Corresponding authors: C. Koshimoto. email: ckos@med.miyazaki-u.ac.jp
A. Shinohara. email: mogura3@med.miyazaki-u.ac.jp
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mammals, dosages and concentrations have been established in laboratory animals such as mice and rats [6]. Studies are still underway to determine the application of these anesthetic agents in other laboratory rodents, such as hamsters and gerbils [7]. Although the methods for using isoflurane and sevoflurane in mice and rats have been applied to other small mammals, the optimal concentrations and exposure times of these anesthetics are unknown [8]. Notably, the physiological responses to inhaled anesthetics differ among animals. For example, isoflurane anesthesia in guinea pigs stimulates the mucous membranes of the body and induces tear flow and salivation [8]. Additionally, the concentration required to maintain anesthesia is higher in gerbils than in mice and rats [9]. Therefore, it is important to determine the most appropriate concentrations of anesthetics and exposure conditions for each species.

The degu (*Octodon degus* (Molina 1782)) is a highly social, herbivorous small rodent native to South America [10, 11]. This species is characterized by a long lifespan among rodents (up to 8 years in laboratory conditions), diurnal habits, and early maturity, allowing it to walk independently from birth [12]. These characteristics have enhanced the use of this species as a laboratory animal. For example, circadian rhythms have been studied in degus [13], and the effects of early postnatal stress have been studied in individual juvenile degus [14]. Most notably, degus exhibit certain human-like characteristics in terms of brain function. This species has a high level of intelligence with respect to understanding the function of tools [15]. Degus can also spontaneously develop Alzheimer's disease pathology [16, 17], have autism-related genes similar to those of humans [18], and may spontaneously develop seizures similar to epilepsy [19]. These characteristics distinguish degus from mice and rats and have attracted attention toward the use of degus in research of brain function, brain diseases, and aging [20].

Surgical procedures, including the installation of electrodes for electroencephalography (EEG), are required to use laboratory rodents in brain research. However, the literature on the anesthetic properties of degus is very limited [21]. Recent studies on degus have used both sevoflurane and isoflurane, but their concentration were very varied in both induction and/or maintenance [22–27], indicating that these anesthetic properties in this species continue to be used without being investigated. Therefore, in this study, we examined the induction and maintenance conditions of deep anesthesia using sevoflurane and isoflurane in degus. The usual method for evaluating the depth of anesthesia in small rodents is to examine the righting reflex (i.e., the ability of the animal to get up on its own), the retraction reflex in response to stimulation of the limbs, and the eyelid opening and closing reflexes [28, 29]. However, because the degrees of loss of consciousness and muscle tone vary with the type of anesthetic, unresponsiveness to physical stimuli does not necessarily reflect a state of deep anesthesia [30]. Therefore, we also used EEG measurements to assess the depth of anesthesia in degus in the present study. In mammals under deep anesthesia, the electrical activity in the brain is suppressed, resulting in the appearance of a flat EEG waveform with an infinitesimally small amplitude [29]. A greater depth of anesthesia is associated with a longer duration of this flat EEG waveform, and the rate of its appearance is used as an index of the depth of anesthesia [29, 31–34]. The relationship between flat waveforms and minimum alveolar concentration (MAC; 50% of animals become immobilized) is also known; flat waveforms account for about 10% of the EEG at 1 MAC and 80% at 1.4 MAC in pigs with Isoflurane, for more than 90% of the EEG at 1.25 MAC in rats with Isoflurane, and for about 70% at 1.25 MAC in rats with sevoflurane [32, 33]. We thus evaluated the appropriate induction anesthesia conditions in degus by comparing the concentration and exposure time of anesthetics required for sedation and deep anesthesia with those of rats. Furthermore, we examined the time of appearance of flat waveforms on the EEG during anesthesia and MAC values of degus.

Materials and Methods

Animals and housing

This study involved the use of degus maintained by the Department of Bio-resources, Frontier Sciences Research Center, University of Miyazaki. They were kept in cages with sufficient nesting material, containing either one or two animals each [CL-0125 (282 × 451 × 157 mm) or CL-0126 (345 × 403 × 177 mm); CLEA Japan, Inc., Tokyo, Japan]. The degus were fed hay cubes (Japan Agricultural Cooperatives, Miyazaki, Japan), a guinea pig diet (5025; Japan SLC, Inc., Shizuoka, Japan), and a commercial diet for mice and rats (CE-2; CLEA Japan, Inc.). The weight of the degus was 210.3 ± 18.3 g. In addition, we used the rat as comparison because their anesthetic strategy is well established [3, 6]. In this study, Wistar rats (strain Crlj:WI) purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan) were selected because their body size is similar to that of degus than SD rat. The weight of the rats was 480.9 ± 164.0 g. The rats were also fed a commercial diet (CE-2; CLEA Japan, Inc.). For all animals, the diets and water were supplied *ad libitum*. The laboratory en-

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vironment had a light:dark period of 12:12 h (lights on at 8:00 A.M.), room temperature of 23 ± 1°C, and humidity of 50–65%. Surgically treated animals were given an adequate recovery period and checked daily for normalcy.

Ethics

All animal experiments conducted in this study were reviewed and approved by the University of Miyazaki Animal Experiment Committee (approval number: 2017-517 and 2023-514).

Experiment 1: Introduction to anesthesia

In Experiment 1, we investigated the effects of different concentrations of isoflurane or sevoflurane on the time required for the animals to become sedated and reach deep anesthesia. The experiment involved 12 degus (female:male = 6:6, age of 1–4 years) and 12 rats (female:male = 6:6, retired animals). The experiments were conducted on randomly selected individuals and could be repeated up to two times, but no repetition was done within the same day. The experimental design is shown in Fig. 1A.

The animals were placed in a chamber (250 × 130 × 130 mm) filled with isoflurane or sevoflurane (Mylan EPD G.K., Tokyo, Japan) at a concentration of 3%, 4%, or 5%, and the lid was closed. Isoflurane or sevoflurane continued to be supplied into the chamber at pre-set concentrations using a dedicated vaporizer (KN-1070 NARCOBIT-E, KN-1071 NARCOBIT-E(II) Natsume Seisakusho Co., Ltd., Tokyo, Japan) at approximately 5 l/min. The chamber was then turned sideways every 30 s to evaluate the animal's righting reflex. At 120, 180, and 240 s after the animal had been placed in the chamber, the lid of the chamber was opened, and the presence of deep anesthesia was evaluated. This involved use of a stimulation test commonly used in laboratory rodents to evaluate whether deep anesthesia has been reached (loss of pain reflexes in the right forelimb, left hindlimb, and caudal region; loss of eyelid reflex; and loss of righting reflex) [28]. The animal was considered to have reached deep anesthesia when reflexes were lost in at least four of these body parts.

Experiment 2: Use of EEG patterns to evaluate maintenance of deep anesthesia

The purpose of Experiment 2 was to infer from the EEG patterns the anesthetic concentrations necessary to maintain a state of deep anesthesia. The experiment involved 12 degus (female:male = 6:6, age of 1–3 years) and 4 rats (female:male = 2:2, retired animals, all aged >1 year). Among these animals, the experiments were performed with randomly selected individuals as in Experiment 1. The experimental design is shown in Fig. 1B.

Placement of electrodes for EEG measurement

After the animals achieved deep anesthesia with a concentration of 4% or 5% isoflurane for >120 s, as described in Experiment 1, a subcutaneous incision was made in the cranium, and the cranial connective tissue was completely exfoliated with hydrogen peroxide (Taisei Yakuhin Inc., Kagawa, Japan). With reference to past literature, EEG electrode screws (1.57 mm in diameter, 0–80 × 1/8; BRC, Aichi, Japan) were placed at four locations on the cranium (one on each side on the brain cortex nasal to the bregma and one on each side between the bregma and lambda [35]). These electrodes were fixed on the cranium using dental resin (UNIFAST II; GC Corp., Tokyo, Japan). Two electrode wires were also inserted over the cervical muscles for electromyography (EMG) measurements [35]. Butorphanol tartrate (5 mg/kg: Meiji Seika Pharma Co., Ltd., (presently Meiji Animal Health Co., Ltd.), Tokyo, Japan) was administered

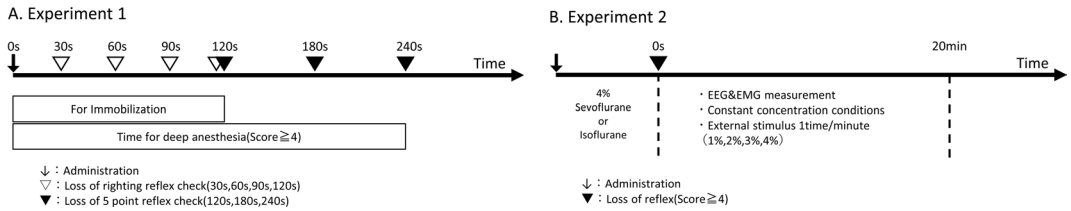

Fig. 1. Experimental design. (A) Experiment 1 was performed to investigate the effects of different concentrations of isoflurane or sevoflurane on the time of loss of the righting reflex after the introduction to anesthesia (checked at 30, 60, 90 and 120 s; n=18 for each concentration of both anesthetic drugs) and of loss of five-point reflex after deep anesthesia (checked at 120, 180, and 240 s; n=6 at each time point for each concentration of both anesthetic drugs). (B) Experiment 2 was performed to evaluate the effects of different concentrations of isoflurane or sevoflurane on the maintenance of deep anesthesia for 20 min based on EEG patterns, with an external stimulus given every minute. EEG, electroencephalography; EMG, electromyography.

subcutaneously once after surgery as postoperative pain treatment. The postoperative recovery period was 7 days in duration.

EEG and EMG measurements were performed via a wired cable attached to the animals only during measurement. The measurements were taken in an environment surrounded by a copper mesh net (mesh size of 0.3 mm) to reduce environmental noise. EEG and EMG recordings were obtained using a PowerLab 26T device (ADInstruments Pty Ltd., New South Wales, Australia). The EEG recording was representative of the electrical activity potential on the brain cortex between the bregma and lambda. The sampling rate (number of sampling processes per second) was set to 1 kHz. A 0.5-Hz high-pass filter and a 50-Hz low-pass filter were used to remove noise from the collected EEG data, and the absence of significant baseline deviations was visually confirmed.

EEG measurements during maintenance anesthesia

The animals were placed under deep anesthesia using isoflurane or sevoflurane (flow rate of approximately 5 l/min at 4% concentration for ≥5 min; this condition was sufficient, as shown by the results of Experiment 1). The animals were then removed from the chamber and fitted with a face mask (KN-1019-2; Natsume Seisakusho Co., Ltd., Tokyo, Japan) for maintenance anesthesia. During EEG measurements, a 37°C thermal pad was placed under the animals. EEG and EMG were recorded during anesthesia, which was maintained for 20 min at a constant concentration of 1%, 2%, 3%, or 4% and a constant flow rate of 1 l/min (Fig. 1B). During this time, physical stimuli were also given to assess the animals' reflexes. A wire was wrapped around the left hindlimb of the degus and pulled every minute to visually observe the appearance of the retraction reflex. The animal was considered awake if it showed a reflex response to the stimulus, at which time point the measurements were stopped.

The EEG recording was analyzed to determine the time point at which the flat waveform appeared [29, 36], indicating the achievement of deeper anesthesia. A flat waveform was defined as a waveform with an amplitude of ≤100 μV and duration of ≥1 s (Fig. 2). The appearance time per minute at each concentration was visually checked, and the total flat waveform appearance time was calculated. Power spectral density analysis was also performed using Labchart (ADInstruments Pty Ltd., New South Wales, Australia) for 20 min during maintenance anesthesia. In this study, the dominant peak frequency distribution was examined for δ (0.5–3 Hz), θ (4–7 Hz), α (8–12 Hz), and β (13–30 Hz) waves. EMG recordings were also set up to record muscle activity when epilepsy occurred. However, no seizures were observed during this experiment; therefore, these results were not used in the analysis.

Experiment 3: MAC and vital assessment during maintenance anesthesia

Estimation of MAC value for degus

The tail clamp method [34, 37] was used to evaluate MAC values of isoflurane and sevoflurane for degus. This method evaluates the presence or absence of a reaction when the base of the tail of anesthetized animals is clamped with forceps. We used 8 (female:male = 4:4, age of 2–4 years) degus per type of anesthesia. First, the degus were deeply anesthetized in a plastic container (155 × 105 × 60 mm) under a concentration of 4% at 1 l/min. The starting concentrations were 1.5% and 3.0% for isoflurane and sevoflurane, respectively, based on the results from the Experiment 1 and 2. After keeping at the starting concentration for 20 min for equilibration, the base of the tail was clipped with forceps for up to 1 min and the reaction of animals was observed. If intentional movement of the head, limbs, and/or entire body was observed, it was considered as positive response; if no movement or ear movement was observed, it was considered as negative response. In the case of a positive response, the concentration of anesthesia was increased by 0.5% during the next 20 min. Conversely, if there was a negative response, the concentration was lowered by 0.5%. When the response to the stimulus on four consecutive trials was "positive-negative-positive-negative" or the reverse (negative-positive-negative-positive), the average of the mean values between two consecutive concentrations was judged as the MAC value [34].

Vital assessment (heart rate, respiratory rate, and rectal temperature)

To further evaluate the two anesthesia on degus, we measured the vital signs during deep anesthesia. Nineteen degus (female:male = 11:8, age of 1–4 years, randomly selected individuals were used as in Experiment 1)

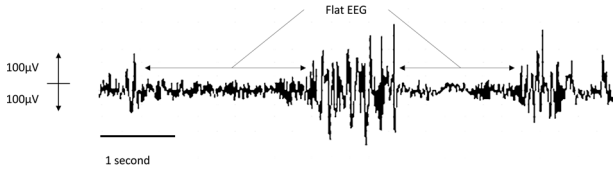

Fig. 2. Analysis of deep anesthesia by EEG. Flat EEGs increased with deeper anesthesia [29]. We defined flat waveforms as those with amplitudes of ≤100 μV and durations of ≥1 s. EEG, electroencephalography

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were used. After deep anesthesia with 4% sevoflurane or 4% isoflurane, the animals were masked and then the heart rate, respiratory rate, and rectal temperature of the animals under constant concentration were recorded for 20 min using vital sign monitor (Rodent Surgical Monitor +, Indus Instruments, Webster, TX, USA) according to the manufacturer protocol. Based on the results of the Experiments 2 and the MAC values, concentration of anesthesia was set at 2%, 3%, and 4% for isoflurane and 3% and 4% for sevoflurane. The respiratory rate of the degus under unanesthetized conditions was visually measured for 10 s and converted to breaths per minute (n=19).

Data handling and statistical analysis

In Experiment 1, we aimed to elucidate the impact of the anesthetic type (isoflurane or sevoflurane) and concentration (3%, 4%, and 5%) on the effects of induction anesthesia for each animal species (degus and rats). We analyzed the number of individual animals exhibiting a righting reflex at 30, 60, 90, and 120 s after placement in the induction anesthesia chamber. This was achieved by performing a survival time analysis with the Kaplan–Meier method, and inter-treatment differences were assessed using the log-rank test. Based on the results of the stimulation test [28], we assigned each case in which an animal reached deep anesthesia as "1" and that in which it did not reach deep anesthesia as "0" at 120, 180, and 240 s after the animal was placed in the induction anesthesia chamber. Using this raw rate as the response variable, the effects of anesthetic type (isoflurane or sevoflurane) on the rate of achievement of deep anesthesia over time were analyzed by three-way analysis of variance (ANOVA) for each animal species (degus and rats) and each anesthetic concentration (3%, 4%, and 5%).

In Experiment 2, EEG analysis was used to investigate the time point at which the flat waveform appeared (Fig. 2). For each animal species (degus and rats), the effects of the anesthetic type (isoflurane or sevoflurane) and concentration (3% and 4%) on the total flat waveform appearance time were analyzed using two-way ANOVA.

In Experiment 3, the mean values of heart rate, respiratory rate, and rectal temperature per min during anesthesia were taken as followings: we first calculated the average values per minute as representative values of one minute, and then averaged them for a total of 20 min. In same way, the maximum and minimum values were also taken. The Tukey-Kramer method and ANOVA were used to compare them among concentrations of isoflurane and sevoflurane, respectively.

All data were analyzed using JMP version 15 (SAS Institute Japan, Tokyo, Japan). *P*-values of <0.05 were considered statistically significant. Data are reported as mean ± SD.

Results

Experiment 1: Introduction to anesthesia

Immobilization (loss of righting reflex): The righting reflex was lost in most individual degus at 60–90 s with a concentration of 3% isoflurane, whereas the righting reflex was not lost in some individuals even after 120 s with 3% sevoflurane (Fig. 3A). At concentrations of ≥4% isoflurane and sevoflurane, the righting reflex was lost within 60 s in most individuals (Fig. 3B and C). In degus, isoflurane produced significantly faster loss of the righting reflex than sevoflurane at 3% and 5% ($P<0.05$, log-rank test), although no difference was observed at 4% ($P=0.15$). In rats, isoflurane induced faster loss of the righting reflex than sevoflurane at all concentrations ($P<0.05$, log-rank test) (Figs. 3D–F). Like the degus, not all individual rats lost their righting reflex even after 120 s at a sevoflurane concentration of 3%.

Isoflurane at a concentration of 4% and 5% produced faster immobilization in rats than in degus ($P<0.05$, log-rank test), but the difference was not statistically significant at 3% ($P>0.05$). By contrast, sevoflurane at a concentration of 3% produced significantly faster immobilization in degus than in rats ($P<0.05$, log-rank test), but the difference was not statistically significant at 4% ($P=0.38$, log-rank test). Immobilization was significantly slower in degus than in rats at a concentration of 5% ($P<0.05$, log-rank test).

Thus, a sevoflurane concentration of 3% appeared to be insufficient for loss of the righting reflex in both species. In addition, the righting reflex was lost more slowly in degus than in rats when using isoflurane.

Deep anesthesia: With 3% isoflurane, two of the six individual degus were awake at 120 s, but all individuals tested reached deep anesthesia after 180 s (Fig. 4A). Isoflurane at >4% successfully induced deep anesthesia at 120 s (Figs. 4B and C). By contrast, only one condition of sevoflurane (5% concentration and 240 s) induced deep anesthesia for all individuals tested (Figs. 4D–F). Notably, none of the other sevoflurane conditions successfully induced deep anesthesia for all animals. Isoflurane had a significantly stronger anesthetic effect than sevoflurane in degus ($P<0.05$, three-way ANOVA).

In rats, isoflurane effectively induced deep anesthesia at 180 s with concentrations of 3–5% (Figs. 4D–F), although five of six rats (3%) and two of six rats (4%) were awake at 120 s. With sevoflurane, all individual rats

reached deep anesthesia at 240 s with a 4% concentration and at >120 s with a 5% concentration. Comparison of the anesthetic effects on rats between isoflurane and sevoflurane also revealed that isoflurane had a stronger effect (P<0.05, three-way ANOVA).

Based on these results, the anesthetic effects of isoflurane and sevoflurane on degus and rats appeared to be similar: exposure to isoflurane produced more rapid anesthetic effects than exposure to sevoflurane.

Experiment 2: Maintenance of deep anesthesia

Anesthetic maintenance was evaluated by the retraction reflex and EEG. In degus, the retraction reflex was observed throughout the entire 20-min period in 10 of

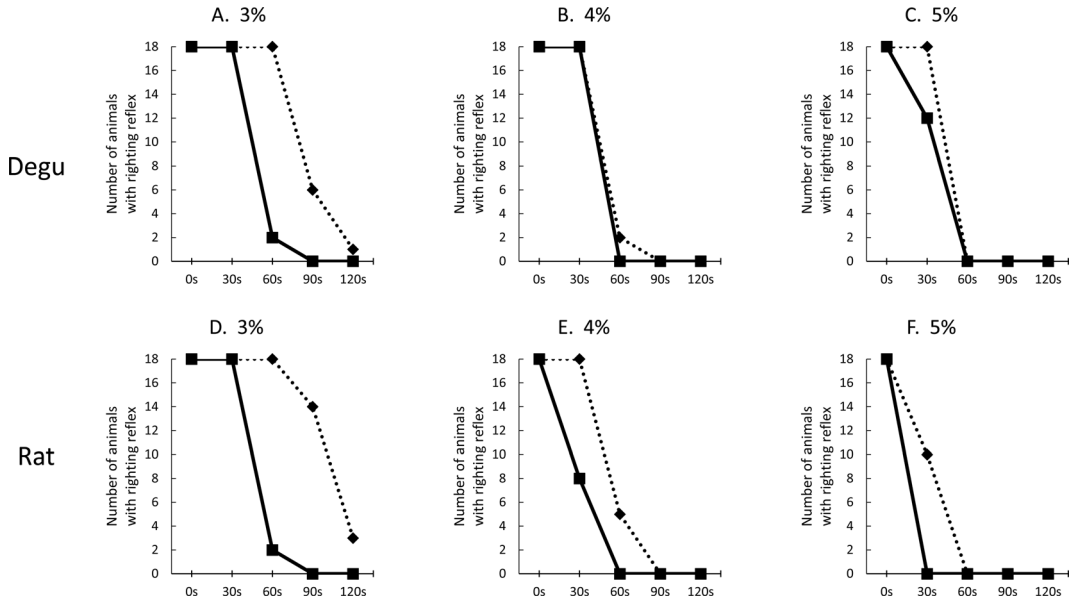

Fig. 3. Time of loss of righting reflex after the introduction to anesthesia (30, 60, 90 and 120 s) at different concentrations (3%, 4%, and 5%) of isoflurane (solid line) or sevoflurane (dotted line) in (A–C) degus (n=18) and (D–F) rats (n=18) in Experiment 1. The Y-axis indicates the number of animals who maintained the righting reflex. Individuals whose reflex disappeared were not evaluated again in the same experiment.

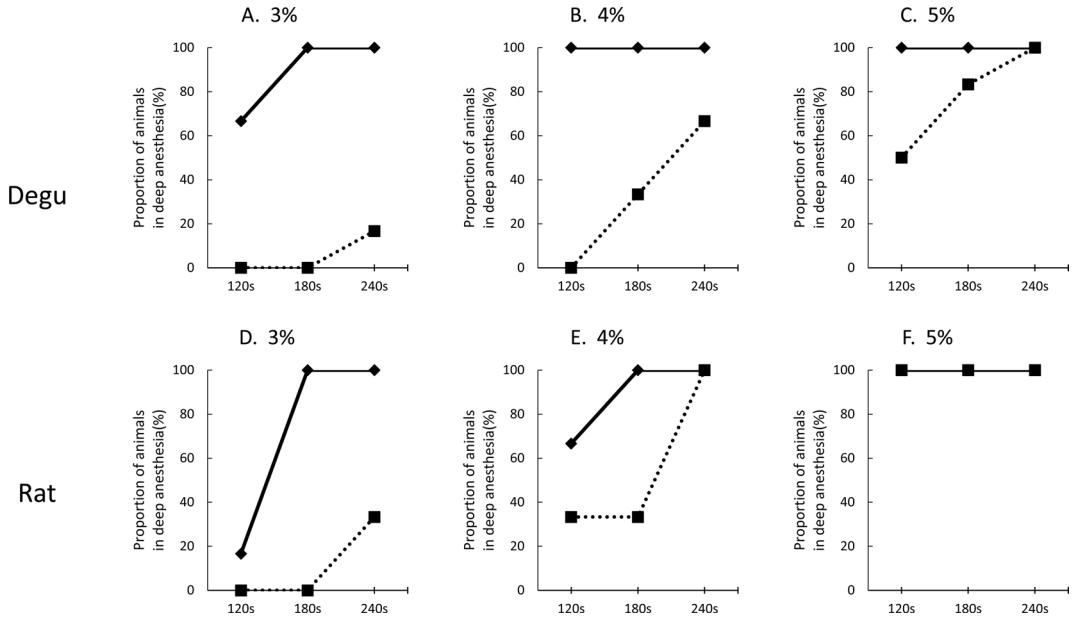

Fig. 4. Time of loss of five-point reflex after the introduction to anesthesia (120, 180, and 240 s) at different concentrations (3%, 4%, and 5%) of isoflurane (solid line) or sevoflurane (dotted line) in (A–C) degus (n=6 at each time point) and (D–F) rats (n=6 at each time point) in Experiment 1. All individual rats reached deep anesthesia at a 5% concentration of both isoflurane and sevoflurane (lines overlapped).

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10 individuals at a 1% isoflurane concentration and in 1 of 9 individuals at a 2% isoflurane concentration. However, at 3% and 4% isoflurane concentrations, no individuals exhibited a retraction reflex during anesthesia. Similar to the results obtained when using isoflurane, all individuals were awake at a 1% sevoflurane concentration, and five of eight individuals were awake at a 2% sevoflurane concentration; none were awake during anesthesia using 3% or 4% sevoflurane. The rats showed the same arousal status as the degus at concentrations of 1% isoflurane (two of two individuals were awake), 2% isoflurane (one of three individuals were awake), and 1% sevoflurane (three of three individuals were awake); at 2% sevoflurane, however, no rats were aroused. At concentrations exceeding 3%, both isoflurane and sevoflurane maintained deep anesthesia in the rats (n=3 at both 3% and 4%). Based on these results, we excluded the 1% and 2% EEG data from subsequent statistical analyses and examined only the 3% and 4% data.

Evaluation of the total flat EEG (Fig. 2) appearance time over a 20-min period showed that the appearance time increased in a concentration-dependent manner for both isoflurane and sevoflurane in both species (Fig. 5). In the comparison between concentrations of 3% and 4%, the flat EEG appearance time was longer at 4% than 3% for both isoflurane and sevoflurane in both degus and rats (P<0.05, two-way ANOVA). In addition, isoflurane induced a longer flat EEG appearance time than sevoflurane in both species (P<0.05, two-way ANOVA). The flat EEG appearance time at concentrations of 3% and 4% was not significantly different between the two animal species (P=0.07 and 0.70 for sevoflurane and isoflurane, respectively). However, the duration of each flat EEG that appeared during the 20-min anesthesia period in degus was relatively short under sevoflurane anesthesia, whereas it tended to be longer with isoflurane (data not shown). Throughout the maintenance anesthesia period in degus, many short flat EEGs appeared under sevoflurane anesthesia but many long flat EEGs appeared under isoflurane anesthesia (Supplementary Fig. 1). In addition, by power spectral density analysis, the amplitude of the EEG decreased with higher concentrations (Supplementary Table 1), and dominant peaks of θ (4–7 Hz) or α (8–12 Hz) waves were found at both 3% and 4% concentrations of both anesthetics (Supplementary Table 2). Our results indicate the need to use a concentration of ≥3% for both sevoflurane and isoflurane to maintain a deeper anesthetic state without awakening degus.

Experiment 3: MAC values and vital assessment

MAC values were estimated as 1.75 ± 0.0% for isoflurane and 2.25 ± 0.27% for sevoflurane for degus. There were no differences in heart rate and rectal temperature among concentrations of each anesthesia (Table 1). The respiratory rate without anesthesia in degus was 211.9 ± 27.2 bpm (n=19), and the rate was strongly suppressed during the anesthetic period (Table 1). In addition, 4% concentrations of both isoflurane and sevoflurane significantly suppressed the respiratory rate than 3% (P<0.05, The Tukey-Kramer method in isoflurane and ANOVA in sevoflurane, Table 1).

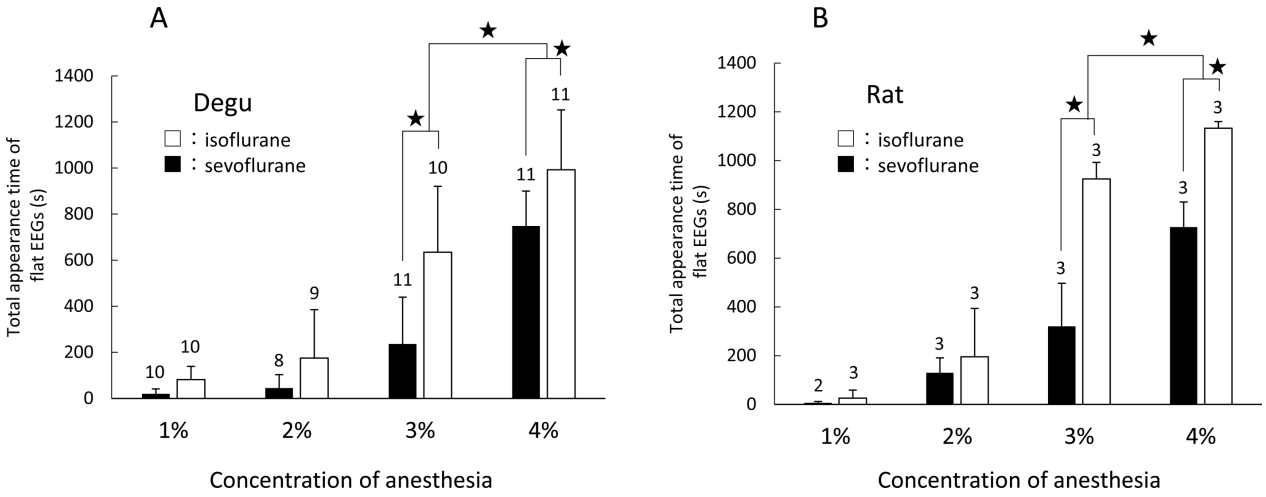

Fig. 5. Total appearance time (s) of flat EEGs at different concentrations of isoflurane (white) or sevoflurane (black) in (A) degus and (B) rats in Experiment 2. Numbers on the bar indicate the numbers of animals used, and the error bar indicates the standard deviation. Because awakened individuals were observed under anesthesia at concentrations of both 1% and 2% (see Results), these data were excluded from the statistical analysis. The star indicates significant differences by two-way analysis of variance (P<0.05). EEG, electroencephalography

Table 1. Vital signs during maintenance anesthesia in degus (mean ± SD)

		N	Heart rate (bpm)			Respiratory rate (bpm)			Rectal temperature (°C)		
			Average	Max	Minimum	Average	Max	Minimum	Average	Max	Minimum
Isoflurane	2%	6	284.3 ± 39.5	323.7 ± 33.7	240.3 ± 49.8	53.6 ± 15.7[a]	83.2 ± 31.0	37.5 ± 7.5	35.6 ± 0.5	36.1 ± 0.7	35.1 ± 0.3
	3%	6	277.1 ± 32.8	315.0 ± 40.8	246.8 ± 24.9	58.5 ± 19.4[a]	79.2 ± 20.2	39.7 ± 17.3	35.5 ± 0.4	36.0 ± 0.5	35.1 ± 0.3
	4%	7	277.2 ± 37.9	320.1 ± 37.0	243.3 ± 36.4	39.9 ± 17.8[b]	76.3 ± 31.5	24.1 ± 10.7	35.6 ± 0.7	36.2 ± 0.7	35.1 ± 0.6
Sevoflurane	3%	6	246.2 ± 35.6	311.3 ± 33.2	220.7 ± 26.7	58.4 ± 19.4*	83.2 ± 13.6	38.2 ± 17.0	35.5 ± 0.7	36.4 ± 0.5	34.8 ± 0.5
	4%	6	239.2 ± 26.7	284.7 ± 36.3	214.2 ± 12.3	50.1 ± 19.0	90.7 ± 19.1	29.3 ± 7.0	35.5 ± 0.7	36.4 ± 0.7	34.8 ± 0.4

*P<0.05 between 3% and 4% sevoflurane (ANOVA). [a,b]P<0.05 among different letters in isoflurane (Tukey-HSD test).

Discussion

This study was performed to establish an appropriate anesthetic technique in degus. During the introduction to anesthetic immobilization, the righting reflex disappeared within 60 s in most individual degus at concentrations of ≥4% for both isoflurane and sevoflurane, and no effect of the anesthetic type was observed (Fig. 3). Isoflurane generally has a stronger anesthetic effect than sevoflurane in a variety of animal species [38–40]. This was also apparent in degus from the MAC values estimated in Experiment 3. Our result is also consistent with the study from other species [3]. For example, in adult rats, MAC value is higher for sevoflurane (1.97%) than isoflurane (1.12%) [41]. Consistent with this, we found that at a 3% concentration, most individual degus that received isoflurane lost their righting reflex at 60–90 s, whereas some individuals that received sevoflurane did not lose their righting reflex even after 120 s (Fig. 3). Notably, a sevoflurane concentration of 3% is not sufficient for loss of the righting reflex in degus. Thus, concentrations of ≥4% for both isoflurane and sevoflurane are recommended for quick induction and immobilization of degus.

Concentrations of ≥3% isoflurane and ≥5% sevoflurane are considered necessary to achieve deep anesthesia in degus. When using isoflurane, deep anesthesia was achieved in all animals after 180 s at a 3% concentration and after 120 s at a ≥4% concentration (Fig. 4). By contrast, when using sevoflurane, some individuals did not reach deep anesthesia after 240 s at a 4% concentration; even at a 5% concentration, all degus required 240 s to reach deep anesthesia (Fig. 4). In humans, it is known that the blood/gas partition coefficient, which indicates the speed of anesthesia dissolution into the blood and transfer to the brain, is lower in sevoflurane than isoflurane [42]. The lower blood/gas partition coefficient has advantages to modulate anesthesia since that allows rapid regulation of alveolar concentrations [42]. We did not analyze the blood/gas partition coefficient in this study, thus details regarding modulating properties were unknown. This may be clarified by future studies.

Neither isoflurane nor sevoflurane at a concentration of ≤2% is appropriate for maintenance of deep anesthesia in degus. At this concentration, degus in the present study awakened in the middle of the 20-min anesthesia period. It is generally assumed that 95% of animals become unresponsive to painful stimuli at 1.4 MAC doses [43]. The estimated MAC value in degus is 1.75 ± 0.0% in isoflurane and 2.25 ± 0.27% in sevoflurane, and thus our results are compatible. At concentrations of >3%, isoflurane was much more effective in maintaining deep anesthesia in degus. This was supported by the fact that the duration of a flat EEG during anesthesia in degus was longer for isoflurane than for sevoflurane at all concentrations (Fig. 5). Flat EEGs appeared more frequently at 3% than 4% isoflurane and less frequently at 3% than 4% sevoflurane (Supplementary Fig. 1). In other words, with sevoflurane, both the number of flat EEGs and their duration increased in a concentration-dependent manner throughout the range examined; with isoflurane, however, the duration of flat EEGs was longer at the 4% concentration, and flat EEGs appeared less frequently at the 4% than 3% concentration. Thus, the duration of each flat EEG was longer at 4% than 3% isoflurane and showed a different pattern of appearance. A flat EEG indicates cessation or suppression of electrical activity in the brain and is used to determine brain death and the depth of anesthesia [29]. At the 4% isoflurane concentration in this study, brain activity was suppressed for a longer period of time. This is further supported by power spectrum density analyses. The dominant peak frequencies on the EEG of degus during maintenance anesthesia at concentrations of 3% or higher were mostly in the low frequency bands (Supplementary Table 2). It is known that the power of the low frequency bands on the EEG increases as anesthesia causes loss of consciousness. With inhaled anesthetics, the EEG recorded below the MAC value is dominated by α and δ waves, while θ waves are more prominent at higher concentrations above the MAC value, showing that the appearance of θ waves indicates a deeper anesthetic state [44]. Our result is consistent with this theory.

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The heart rate of the degus under both anesthetics did not change at the higher concentrations (Table1). A previous study [24] reported the heart rates of 220–270 bpm with 4% sevoflurane in 3-month-old degus, which was mostly consistent with our results. Cuenca-Bermejo *et al.* [45] also examined the heart rate of various aged degus under 20.0 mg/kg of ketamine and 0.20 mg/kg of medetomidine anesthesia and reported the average value as 207.2 ± 41.3 bpm (ranging from 110 to 340 bpm). The rectal temperatures during isoflurane and sevoflurane anesthesia of the degus in this study (34–36°C: Table 1) were slightly lower than the body temperature of degus under normal conditions which is reported to be 36–38°C [46, 47]. It may be a good idea to use thermal blankets as well as thermal pads to prevent lowering body temperature. Respiratory rate during anesthesia was suppressed as increasing the concentration of both anesthesia (Table1). Under the 4% concentration, the minimum value was observed below 30 bpm, indicating apparent respiratory suppression. Mancinelli [24] also reported similar respiratory rates of degus ranging from 20 to 60 bpm under 4% sevoflurane. In general, the effects of inhaled anesthetics on respiratory function are well known [3], thus monitoring respiratory rate is very important to maintain safe anesthesia when using isoflurane or sevoflurane in degus.

In the present study, the righting reflex was lost more slowly in degus than in rats (Fig. 3). In addition, induction of deep anesthesia was slower in degus than in rats (Fig. 4). Furthermore, whereas no rats woke during maintenance anesthesia at a sevoflurane concentration of 2%, some individual degus woke at this concentration. These results suggest that the anesthetic effects are weaker in degus than in rats, although it is also known that the anesthetic effect is different in the strains of rat [48, 49]. Age is also a known factor affecting anesthetic effects. In rats, anesthesia is more effective in older animals [41]. Although a rigorous comparison between rats and degus is difficult to perform because of their different life spans, the differences in anesthetic effects are generally influenced by the species themselves. For example, the MAC values of both isoflurane and sevoflurane anesthesia is higher in gerbils than in mice and rats [9]. Therefore, at least when using sevoflurane, we should consider that deep anesthesia for degus requires higher concentrations than for rats. Importantly, because the use of sevoflurane in epileptic patients can reportedly induce convulsions [50, 51], we prepared to obtain EMG recordings. However, no degus in this study developed convulsions throughout the experiment. Some spinal slow-wave complex–like waveforms were observed by EEG (data not shown); thus, further investigation is needed.

Conclusion

We examined the optimal technique for inhalation anesthesia in degus. Isoflurane had a stronger anesthetic effect on degus than did sevoflurane, and species-related differences in the anesthetic effects were observed. The EEG and vital data show that sevoflurane and isoflurane maintenance anesthesia require more than 3% to maintain anesthesia in degus, but higher concentrations may result in hyperanesthesia with suppression of brain activity and respiratory rate. The new findings obtained in this study will be useful not only in laboratory animal science but also in veterinary medicine and field research.

Conflict of Interest

The authors declare no competing financial interests.

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