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Comparison of anesthetic protocols indicates Japanese grass lizards (*Takydromus tachydromoides*) are insensitive to medetomidine

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ニホンカナヘビ（*Takydromus tachydromoides*）が
メデトミジン非感受性であることを示した麻酔薬の比較検討

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Abstract

The systematic effects of anesthetics on reptiles remain obscure, and the present study evaluated the effects of intraperitoneal injections of 1) alfaxalone alone, 2) a combination of alfaxalone and midazolam, 3) a combination of medetomidine chloride, midazolam and butorphanol tartrate, 4) medetomidine chloride alone and 5) midazolam alone in Japanese grass lizards. The present findings indicated that a combination of alfaxalone (15 mg/kg) and midazolam (1 mg/kg) anesthetizes Japanese grass lizards for 1.5–3 h, whereas these lizards are apparently medetomidine-insensitive unlike some other reptiles. The effects of anesthetic and sedative agents differ among reptile species, and thus further studies should investigate the effects of anesthetics among reptiles.

Keywords: alfaxalone, butorphanol, midazolam, reptiles, squamates

Introduction

The Class Reptilia consists of over 7,300 species and the number of domestic reptiles such as pets, zoo animals and laboratory animals has increased, but the systematic effects of anesthetics on reptiles remain obscure. The effects of anesthesia have been investigated in only a few species of reptiles, but the effects of some anesthetic agents differ among reptiles even within the same family (Bennett 1998; Mosley 2005). This means that the effects of anesthetics should be carefully evaluated for each reptile species.

Japanese grass lizards (*Takydromus tachydromoides*) belong to the Scincidae family and are native to Japan, where they comprise one of the most common species of lizard. The lizards reach sexual mature after about 1 year and lay eggs 1–6 times per year, and their duration of life is about 7 years. They have been included in various biological studies, yet a suitable anesthesia protocol has not been established.

Managing inhalation anesthesia is more challenging in reptiles than in mammals, because some reptiles can

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stop respiration and blood circulation to the lungs for long periods (Girling and Raiti 2004). Intravenous, intramuscular and subcutaneous injections of anesthetics also do not seem suitable for Japanese grass lizards because even adults are very small, weighing approximately 3.0 g. Therefore, the present study evaluated the effects of intraperitoneal injections of individual and combined general anesthetics and sedatives in Japanese grass lizards.

Materials and Methods

Animals

This study included 18 Japanese grass lizards (body weight, 1.9–4.8 g; Hokudo Co. Ltd., Sapporo, Japan) of unknown sex that were caught in the wild. These lizards were considered to be young adults or adults based on an average body weight at maturity (Takeishi 1987), and they were randomly selected for each experiment. The degree of anesthesia changes according to body temperature in reptiles (Olsson and Phalen 2013; Olsson et al. 2013), and thus the effects of anesthesia were evaluated at a constant room temperature (26–28 °C). This study proceeded according to the Regulations on the Management and Operation of Animal Experiments, and the Animal Care and Use Committee of Obihiro University of Agriculture and Veterinary Medicine approved the experimental protocol (no. 29-161 and no. 18-136).

Anesthetic agents

All agents described below were injected intraperitoneally into the lizards by a single experimenter. Animals were held by a hand, and 0.1–0.2 ml of saline containing each concentration of anesthetic agents was injected at the paramedian region of abdominal wall by using a 27G needle. Alfaxalone (15.0 mg/kg; Alfaxan; Jurox Pty Ltd, NSW, Australia) and a mixture of alfaxalone (15.0 mg/kg) and midazolam (1.0 mg/kg; Dormicum®, Astellas Pharma Inc., Tokyo, Japan) were injected into three Japanese grass lizards each. This concentration of alfaxalone combined with midazolam can appropriately anesthetize leopard geckos (Doss et al. 2017).

Our preliminary findings indicated that the concentration of each component of the mixture (medetomidine chloride, 0.3 mg/kg; midazolam, 4.0 mg/kg; butorphanol tartrate 5.0 mg/kg) that is generally used for anesthetizing mice (Kirihara et al. 2013) cannot sedate Japanese grass lizards, and the two- and four-fold higher concentrations does not anesthetize these lizards. Here, an eight-fold higher concentration of each component, i.e. medetomidine chloride (2.4 mg/kg; Dorbene® vet, Kyoritsu Seiyaku Co. Ltd., Tokyo, Japan), midazolam (32.0 mg/kg) and butorphanol tartrate (40.0 mg/kg; Vetorphale®, Meiji Seika Pharma Co., Ltd., Tokyo, Japan), was injected into six lizards. Separately, medetomidine chloride (3.0 mg/kg) alone and midazolam (10.0 mg/kg) alone were injected in four and two lizards, respectively.

Evaluation of the anesthetic status

The status of anesthesia was evaluated based on the loss of following five reflexes (Kirihara et al. 2013): body-righting reflex, in which animals right themselves after being placed on their backs; corneal reflex, in which animals move their eyelids after gently stimulating the air 10 mm from the eye using a Pasteur pipette; tail reflex, tail movement after pinching the area at the base of the tail using hooked forceps; right front hand and left hind foot reflexes, the hand and foot movements after pinching the skin between the second and third digits with hooked forceps. Animals were gently pinched by a single experimenter using the same Adson tissue forceps in all experiments. Start and end points of anesthesia were relatively defined as the time when animals lost four and recovered two of the five reflexes described above. When agents exerted anesthetic effects, the body-righting reflex was lost first and recovered last. Thereafter,

the elapsed time between the administration of anesthetics to the start of anesthesia (onset), from the onset to the end of anesthesia (duration [of action]) and from the end of anesthesia to recovery of the body-righting reflex (awakening) were recorded. Sedation was assumed when the lizards lowered their heads and closed their eyes or lost only the body-righting reflex.

Results and Discussion

Alfaxalone and a mixture of alfaxalone and midazolam

Alfaxalone sedated, but did not anesthetize all of three lizards, whereas alfaxalone with midazolam stably anesthetized all of three lizards for 1.5–3.0 h (Table 1). These findings indicated that the intraperitoneal injection of alfaxalone with midazolam is an appropriate anesthesia protocol for Japanese grass lizards, like leopard geckos (Doss et al. 2017). However, the number of animals examined in the present study seemed too small to establish correct anesthetic effects (onset, duration and awakening times) and sex- and age-dependent differences of this combination in these lizards, and further studies with a larger sample size are required.

A mixture of medetomidine, midazolam and butorphanol

The effects of a combination of medetomidine, midazolam and butorphanol were assessed. All of six lizards were sedated, but a combination of these agents anesthetized only two (33%) lizards for a long duration followed by a sudden awakening (Table 1). These findings indicated that the effects of a combination of medetomidine, midazolam and butorphanol are unstable in Japanese grass lizards, although dexmedetomidine (0.1 mg/kg) with midazolam (1.0 mg/kg) can anesthetize leopard geckos (Doss et al. 2017).

Medetomidine alone and midazolam alone

According to the findings described above, either medetomidine or midazolam did not seem to affect in Japanese grass lizards, and then the effects of medetomidine chloride and midazolam were evaluated. Both of two lizards with midazolam were sedated, but all of four lizards injected with medetomidine were not (Table 1). In addition,

Table 1. Sedative and anesthetic effects of five intraperitoneal injection protocols in Japanese grass lizards.

Agents	Lizard ID.	Body weight (g)	Sedative effect	Anesthetic effect	Onset (min)	Duration (min)	Awakening (min)
Mixture of alfaxalone and midazolam	A	3.9	✓	✓	3	160	20
Alfaxalone (15.0 mg/kg)	B	2.9	✓	✓	4	153	15
Midazolam (1.0 mg/kg)	C	4.3	✓	✓	13	93	ND [1]
Alfaxalone (15.0 mg/kg)	D	3.2	✓				
	E	2.6	✓				
	F	4.1	✓				
Mixture of medetomidine, midazolam and Butorphanol Medetomidine (2.4 mg/kg) Midazolam (32.0 mg/kg) Butorphanol (40.0 mg/kg)	G	3.9	✓	✓	15	172	0 [2]
	H	4.8	✓				
	I	3.5	✓				
	J	3.7	✓				
	K	3.9	✓	✓	34	364	0 [2]
	L	3.7	✓				
Medetomidine (3.0 mg/kg)	M	2.4					
	N	3.2					
	O	3.0					
	P	3.8					
Midazolam (10.0 mg/kg)	Q	1.9	✓				
	R	2.9	✓				

[1] A correct time could not be determined.

[2] Recovery of a body-righting reflex was same as the end of anesthesia.

medetomidine did not increase the blood glucose level that is increased by effects of medetomidine (pools of each three saline- and medetomidine-administrated lizard; 236 and 124 μg/ml, respectively). These findings suggested that medetomidine does not function in Japanese grass lizards. Medetomidine or dexmedetomidine exerts anesthetic and/or sedative effects in some other reptiles (Sleeman 2000; Olsson and Phalen 2012), whereas the present study found that Japanese grass lizards are apparently medetomidine-insensitive. Midazolam also has sedative effects in some other reptiles (Arnett-Chinn et al. 2016) but not in several turtle species (Harvey-Clark 1993). Taken together, the present and previous findings indicate that the effects of anesthetic and sedative agents differ among reptile species, and thus further studies should investigate the effects of anesthetics among reptiles.

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要　約

本研究では、ニホンカナヘビにおける１）アルファキサロン単体、２）アルファキサロンとミダゾラム、３）メデトミジンとミダゾラム、ブトルファノール、４）メデトミジン単体、５）ミダゾラム単体の腹腔投与の影響を評価した。アルファキサロン（15 ㎎/kg）とミダゾラム（1.0 ㎎/kg）の混合薬によって1.5-3時間の安定した麻酔状態が得られた一方で、これまで報告のある爬虫類種とは異なり、ニホンカナヘビではメデトミジンによる

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鎮静効果は認められなかった。

キーワード: アルファキサロン、ブトルファノール、
ミダゾラム、爬虫類、有鱗目