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Sources of Variability in Recovery Time from Methohexital Sodium Anesthesia in Snakes

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Variability in anesthetic effect is frequently observed in individuals within a species, yet few studies have investigated its causes in reptiles. To evaluate potential sources of variability in reptile anesthesia, we conducted experiments to test for effects of body temperature, body condition, gravidity, and time post-feeding on the recovery time of Red-sided Garter Snakes (*Thamnophis sirtalis parietalis*) anesthetized with methohexital sodium (Brevital Sodium). The mean recovery time of snakes anesthetized at 21°C was 125 min, at 26°C it was 86 min, and at 31°C it was 64 min. There was a significant correlation between body condition and recovery time, with thinner snakes experiencing longer recovery times. Gravid snakes anesthetized within five weeks of parturition had a mean recovery time that was twice as long as nongravid snakes of a similar mass. Time post-feeding did not have a statistically significant effect on the recovery time in snakes that were anesthetized one, three, and ten days after consuming 30% of their body mass in food. Based on our findings, we provide recommendations for producing more predictable results when using methohexital sodium in reptiles.

SURGICAL procedures requiring anesthesia are often necessary to study aspects of reptilian anatomy, physiology, and behavior (Miller and Gutzke, 1999; Blouin-Demers and Weatherhead, 2001; Krohmer et al., 2004). Safe and effective anesthesia protocols are necessary to facilitate surgery and minimize stress and injury to experimental subjects. An ideal anesthetic agent should take effect rapidly, create a sufficient depth of anesthesia for the desired procedure, allow reasonably fast and predictable recovery, and should have a low risk of mortality. A suitable anesthetic for reptile research must also be easy to administer under laboratory and field conditions and should not create side effects that may impact experimental data.

A wide variety of injectable and inhalant anesthetic agents have been used in reptiles (for reviews see Mosley, 2005; Bertelsen, 2007; Schumacher, 2007). Inhalant anesthetics, such as isoflurane, are the agents of choice when anesthesia machines and experienced personnel are available for drug administration (Bertelsen, 2007). However, in research settings, anesthesia machines may be impractical for use in the field and many taxa, such as aquatic turtles, have breath-holding abilities that make inhalants alone ineffective unless the animal is intubated and positive pressure ventilation is used (Schumacher, 2007). Inhalant anesthetics can alternatively be administered without precision vaporizers using the ''open method,'' where an animal is placed into a closed container with a cotton ball that has been soaked in a volatile gas agent (Blouin-Demers et al., 2000). However, it is difficult to control the depth of anesthesia with this technique, the risk of overdose may be high, and better options for anesthesia in reptiles exist.

Injectable agents are simple to administer and are valuable in many circumstances when inhalants cannot be used effectively. Among the commonly used injectable agents in reptiles are ketamine, propofol, medetomidine, and midazolam (Read, 2004). Ketamine, while often used in reptile research, provides poor muscle relaxation at low doses and leads to prolonged recovery periods at high doses (Glenn et al., 1972; Throckmorton, 1981). Propofol is the injectable agent of choice in clinical settings, but has not gained wide use in research (Anderson et al., 1999). Propofol is only effective when administered intravenously, making it challenging or impossible to use in small reptiles or taxa that lack practical venous access. Medetomidine and midazolam are frequently used as sedatives in chelonians, often in combination with ketamine (Schumacher, 2007). These agents are not generally used for maintenance of anesthesia.

Methohexital sodium (Brevital Sodium) is an ultra-short acting barbiturate anesthetic that has been used in numerous reptile taxa, including turtles (Gaztelu et al., 1991; Crocker et al., 1999; Jackson et al., 2000), lizards (Wang et al., 1977; Smith, 1982; Gans et al., 1985; Gaztelu et al., 1991), snakes (Wang et al., 1977; Nichols and Lamirande, 1994; Miller and Gutzke, 1998), and alligators (Gatesy, 1990). Methohexital sodium is associated with rapid induction and recovery times, is effective when administered subcutaneously, and does not cause the tissue irritation that is associated with other barbiturates (Wang et al., 1977; Nichols and Lamirande, 1994). Research conducted by one of us (RTM and collaborators) has utilized methohexital sodium in the field and laboratory for over 20 years to anesthetize Red-sided Garter Snakes (*Thamnophis sirtalis parietalis*) prior to surgical procedures (Nelson et al., 1987; Shine et al., 2001; Lutterschmidt et al., 2006). While methohexital sodium is an effective injectable agent in *T. s. parietalis*, a high degree of variability in the duration of anesthesia and recovery time has been observed among individuals. No studies have been conducted to assess the causes of variability in anesthesia in snakes.

Variable anesthetic effects among reptiles of the same species have been anecdotally attributed to possible differences in health, body temperature, body size, body condition, sex, stress level, stage of ecdysis, gravidity, drug administration route, and elapsed time since previous

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dosings (Karlstrom and Cook, 1955; Cooper, 1974; Wang et al., 1977; Holz and Holz, 1994; Hill and Mackessy, 1997; Miller and Gutzke, 1998; Blouin-Demers et al., 2000). Few studies have experimentally evaluated causes of variability in anesthesia within a species. Body temperature has an effect on ketamine anesthesia in skinks (Arena et al., 1988) and isoflurane anesthesia in desert iguanas (Dohm and Brunson, 1998). Both studies found lower body temperatures resulted in a longer duration of anesthesia. Other potential variables remain unstudied with regard to anesthesia in reptiles.

Identifying sources of variability in the anesthesia of snakes will help improve anesthesia protocols and will increase our understanding of the mechanisms that induce and terminate the action of anesthetics in reptiles. We hypothesized that several physiological parameters may influence recovery time from anesthesia in snakes. Separate experiments were conducted to assess the effects of body temperature, body condition, gravidity, and time post-feeding on recovery time from methohexital sodium anesthesia in *Thamnophis sirtalis parietalis*. Based on our results, recommendations are given for improving anesthesia protocols using methohexital sodium in snakes.

MATERIALS AND METHODS

Collection and captive maintenance of garter snakes.—Thirty male and 45 female *Thamnophis sirtalis parietalis* were collected from over-wintering hibernacula near Inwood, Manitoba, Canada during May of 2007 and 2008. Snakes were transported to Oregon State University in Corvallis, Oregon, and maintained in 38 L glass aquaria in a microprocessor controlled environmental chamber. From May until September the environmental chamber was maintained at 24°C during the day and 14°C at night with 14L:10D photophase. Forty-watt incandescent bulbs provided a thermal gradient within aquaria and allowed snakes to reach a body temperature of 30°C during the day. From October to April, the environmental chamber was maintained at 4°C, 0L:24D to induce hibernation. During the summer months, *T. s. parietalis* were fed an alternating diet of earthworms and trout weekly. Snakes used in the body condition experiment were not fed in captivity prior to being anesthetized in early June. These individuals were fed weekly after this time. Water was provided *ad libitum*.

Methohexital sodium.—Methohexital sodium (Brevital Sodium, JHP Pharmaceuticals, Parsippany, NJ) was diluted to a 1% solution (10 mg/ml) in deionized water. The 1% solution was further diluted to a 0.5% solution in sterile 10 ml vials of 0.9% NaCl. Methohexital sodium was stored at 4°C and was used over a period of several months.

Anesthetic protocol.—The snout–vent length (SVL) and mass of each snake were measured on the day of each experiment. One hour prior to drug administration, snakes were removed from the environmental chamber and placed individually into 38 L glass aquaria lined with newspaper. This allowed the body temperatures of the snakes to reach the desired temperature for each experiment. During anesthesia, snakes were maintained in a coiled position. Dosages of 15 mg/kg (equivalent to 0.003 ml of 0.5% methohexital sodium/g body mass) were administered subcutaneously at the juncture between the dorsal and ventral scales at a distance of approximately 20 cm from the head of the snake. Injections were made with 1 ml syringes and 0.75 in 26 G needles. Snake body temperatures were recorded using a thermocouple thermometer (Digi-Sense ThermoLogR Thermistor, Cole-Parmer, Vernon Hills, IL) inserted 3 cm into the snake's cloaca and were measured every 30 min during anesthesia and recovery. Quantifying anesthetic effect was accomplished by measuring the time after drug administration at which each snake regained its ability to right its entire body when placed on its back (hereafter called recovery time). The righting reflex of each snake was evaluated at intervals of five min or less throughout anesthesia.

Experiment 1: body temperature.—Twenty male *T. s. parietalis* (43–50 cm SVL, 27–47 g body mass) were anesthetized at body temperatures of 21°C, 26°C, and 31°C in a repeated measures experimental design. At each temperature, the 20 snakes were anesthetized over two consecutive days. Ten snakes were randomly assigned to be anesthetized on day one and ten on day two. After being anesthetized at one temperature, all 20 snakes were fed and then anesthetized approximately one week later at the next temperature. This schedule ensured all snakes were anesthetized without food in their gut. Snakes at 21°C received no supplemental heat. Snakes at 26°C and 31°C were warmed with incandescent light bulbs throughout anesthesia and recovery.

Experiment 2: body condition.—Forty-five female *T. s. parietalis* of similar length, but widely varying mass (60–70 cm SVL, 57–118 g body mass) were anesthetized in groups of nine per day. All 45 snakes were anesthetized during five days. Snakes were randomly selected to be anesthetized on each of the five days, and all snakes were maintained at 21°C during anesthesia. Snakes for this experiment were anesthetized in early June, within three weeks of being collected. No significant difference in recovery time between gravid and nongravid snakes was detected at this time of year, and both types of snakes were included in the experiment. To quantify body condition in each snake, we calculated residuals from a simple linear regression of log-transformed mass to snout–vent length.

Experiment 3: gravidity.—Of the 45 female *T. s. parietalis* that were used in the body condition experiment, four became gravid after mating in the wild. The four gravid females (62–66 cm SVL, 96–111 g body mass) and four nongravid females of similar size (64–66 cm SVL, 88–108 g body mass) were anesthetized in August. All gravid snakes gave birth within five weeks of being anesthetized. Body masses of snakes were recorded in June, August, and immediately after parturition in October. Snake body temperatures were maintained at 21°C during anesthesia.

Experiment 4: time post-feeding.—Ten male *T. s. parietalis* (50–55 cm SVL, 43–60 g body mass) and ten female *T. s. parietalis* (61–66 cm SVL, 93–107 g body mass) were anesthetized one, three, and ten days after a single feeding event in a repeated measures experimental design. Snakes were anesthetized in groups of ten and were given as much food as they could consume in one day prior to the first day of anesthesia. The dosage of methohexital sodium administered to each snake was calculated based on the snake's mass one day prior to feeding. Snake masses were measured before feeding and daily for ten days after feeding, and snake body temperatures were maintained at 21°C during anesthesia.

Table 1. Mean Recovery Times per Treatment of *T. s. parietalis* Anesthetized with Methohexital Sodium in Three Separate Experiments. Mean recovery times are in minutes.

Experiment	Treatment	Mean recovery time (SE)	Statistics
Body temperature	21°C	124 (±13)	ANOVA:
	26°C	86 (±4)	$F_{2,17} = 12.71$,
	31°C	64 (±7)	$P < 0.001$
Gravidity	Gravid	111 (±11)	t-test: $t_3 = 3.93$,
	Nongravid	63 (±10)	$P = 0.008$
Time post-feeding	Day 1	74 (±5)	ANOVA:
	Day 3	67 (±5)	$F_{2,17} = 3.09$,
	Day 10	78 (±5)	$P = 0.057$

Statistical analysis.—One-way repeated-measures ANOVA was used to compare differences in recovery time between temperature groups and between time post-feeding groups. Simple linear regression was used to evaluate the relationship between body condition and recovery time. A two-sample t-test was used to compare recovery time between gravid and nongravid snakes. All *P*-values are two-tailed, and recovery time was log transformed for all experiments to normalize data. IBM SPSS Statistics 18 was used for statistical analyses, and Sigma Plot 10.0 was used for graphics.

RESULTS

Experiment 1: body temperature.—Mean snake body temperatures during anesthesia for the three temperature groups were 21.5°C (0.1 SE), 26.3°C (0.1 SE), and 31.5°C (0.1 SE). At 21°C and 26°C all 20 snakes lost righting ability. At 31°C one snake did not lose righting ability and was not included in the analysis. Higher temperatures resulted in significantly shorter recovery times (Table 1). The mean recovery time at 21°C was nearly twice as long as the mean recovery time at 31°C, and differences in recovery time between all temperature treatments were statistically significant.

Experiment 2: body condition.—There was a significant relationship between snake body condition and recovery time, with thinner snakes experiencing longer recovery times (Fig. 1, $r^2 = 0.251$, $P < 0.001$). The mean recovery time of the 15 heaviest snakes was 73 min (7 SE), while for the 15 thinnest snakes it was 121 min (12 SE).

Experiment 3: gravidity.—Gravid snakes took significantly longer to recover from anesthesia than nongravid snakes (Table 1). The mean recovery time of gravid snakes was over 40 min longer than nongravid snakes.

Experiment 4: time post-feeding.—On average, snakes ate 31% (0.012 SE) of their body mass in food during the single feeding event and then gradually decreased within 3% (0.007 SE) of their initial mass by day ten post-feeding. There was not a statistically significant difference in recovery time on day one, three, or ten post-feeding (Table 1).

DISCUSSION

In our experience, methohexital sodium is the most effective anesthetic agent for snakes under many circumstances, especially when anesthesia machines to deliver inhalant anesthetics are unavailable. Other injectable agents, including ketamine, propofol, medetomidine, and midazolam have been used alone and in various combinations in *T. s. parietalis* and none have been determined to be superior to methohexital sodium in terms of ease of administration, depth of anesthesia, and time to recovery (D. Preston, unpubl.).

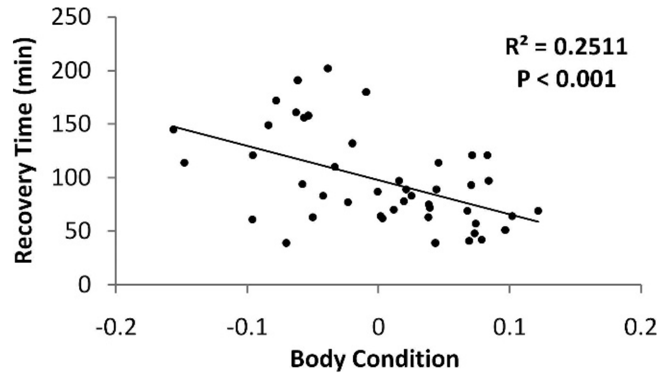

Fig. 1. Simple linear regression of recovery time versus body condition of *T. s. parietalis* anesthetized with methohexital sodium. Body condition values represent residuals from a regression of log-transformed mass against snout–vent length.

A variety of measurements can be taken to assess the effects of an anesthetic, and the most useful measurement may vary depending on the objectives of the research. In this study, our goal was to assess whether several physiological parameters affect the duration of anesthesia in snakes. Recovery time was recorded to quantify anesthetic effect, and we felt this measurement was suitable for several reasons. Fast acting barbiturates are generally associated with relatively short recovery periods, and recovery time correlates with the overall depth and duration of anesthesia (i.e., snakes that have longer recovery times reach a deeper plane of anesthesia and experience a longer duration of anesthesia than do individuals with shorter recovery times). Recovery time, defined as the time when the snake has regained the ability to right itself, is also an unambiguous measure that could be readily measured on nearly every individual in the study. Furthermore, recovery time is a useful measurement because some anesthetics result in a prolonged recovery period which makes them less useful in some situations, especially in a research setting when it is often desirable to have animals behaving ‘normally’ as soon as possible after a surgical procedure. Recovery time, however, is of limited use alone when the overall objective of anesthesia research is to evaluate the efficacy and safety of a particular anesthetic. In these circumstances there are a variety of other informative measurements that are useful to measure, such as induction time, response to nociceptive stimuli, heart rate, respiratory rate, blood gas values, and others.

The shortened duration of methohexital sodium anesthesia at higher temperatures in *T. s. parietalis* is consistent with previous reports in lizards anesthetized with ketamine (Arena et al., 1988) and isoflurane gas (Dohm and Brunson, 1998). Elevated body temperatures likely reduce duration of anesthesia in all reptile species, whether inhalant or injectable anesthetics are used. The respiration and heart rates of reptiles increase with body temperatures (Greenwald, 1971), and this likely increases rates of distribution and metabolism of anesthetics and decreases their duration of action. It is also of note that the differences in recovery time with temperature correspond to a Q_{10} of roughly two, which is within the range of expected values for a physiological rate.

Anesthetic protocols in veterinary medicine recommend maintaining reptiles at or near their preferred body temperature (PBT) for the duration of anesthesia and recovery (Mosley, 2005; Bertelsen, 2007). This practice is used to maintain optimal immune response and elevated metabolic rates in reptiles, which may reduce risks associated with anesthesia and surgery. The PBT of *Thamnophis sirtalis parietalis* ranges from 26°C to 30°C depending on the digestive state of the snake (Lysenko and Gillis, 1980). In our experience, Red-sided Garter Snakes anesthetized near 30°C require over 20 mg/kg of methohexital sodium, compared to 15 mg/kg at 21°C, and rarely reach a level of anesthesia suitable for surgery. We have obtained the most effective anesthesia by maintaining snakes near 21°C from one hour prior to anesthetic administration until the completion of surgical procedures. Snakes are then warmed to their PBT immediately after surgery. This results in a deeper plane of anesthesia, but speeds the recovery process after surgery has ended. We have not observed any detrimental effects from anesthetizing healthy Red-sided Garter Snakes at 21°C, and this allows using smaller doses of anesthetic. Anesthetizing snakes at much lower body temperatures, however, is not recommended, and the practice of cooling animals in a freezer prior to anesthesia or using cold narcosis alone should be avoided entirely as far more ethical and effective options for reptile anesthesia and analgesia exist.

On average, thinner snakes took longer to recover from anesthesia than heavier snakes, which indicates that body condition has some affect on recovery time. The overall shorter recovery times in heavier snakes may be the result of the affinity for adipose tissue of many injectable agents. In mammals, barbiturate anesthetics distribute sequentially from the blood pool into viscera, lean tissue, and then adipose tissue, before being metabolized hepatically (Gillis et al., 1976; Bickel, 1984). It is largely redistribution from blood into other tissues that terminates the action of the anesthetic (Gillis et al., 1976; Hudson et al., 1983). While the pharmacokinetics of methohexital sodium in reptiles has not been studied, a similar pattern of distribution as that seen in mammals is likely. The shorter duration of anesthesia in heavier snakes may be the result of a greater percentage of the dosage becoming bound in adipose tissue during the early distribution of the drug. Other injectable agents used in reptiles, such as the lipophilic agent propofol, distribute into adipose tissue and may show a similar increased potency in thin reptiles as do the barbiturates.

It is not clear what causes the longer recovery times observed in gravid snakes compared to nongravid snakes. However, this finding may also be the result of differences in body condition. Gravid snakes are often anorexic shortly before giving birth (Gregory et al., 1999), and viviparous snakes can be emaciated after parturition due to the high energetic cost of reproduction (Madsen and Shine, 1993; Luiselli et al., 1996). Both gravid and nongravid snakes increased in mass from June to August (17% and 25%, respectively). Gravid snakes, however, gained mass in the form of developing offspring while nongravid snakes gained largely adipose tissue. When snakes were anesthetized in August, it is likely that gravid females had burned much of their adipose tissue to meet the energetic demands of developing offspring. After parturition, gravid snakes were 24% lighter than their August masses. The reduction in adipose tissue associated with developing offspring may cause the increased recovery time observed in this group. It should be noted that the sample size for this experiment was relatively small (four gravid and four nongravid snakes), and the statistical power of the analysis was lowered than desired. Although the results obtained here should be interpreted with caution, a similar increased effect of methohexital sodium has been anecdotally observed in gravid crotalines (Miller and Gutzke, 1998).

A statistically significant effect of time post-feeding on recovery time was not detected despite the considerable physiological changes snakes undergo during digestion. Metabolic rate, blood flow to digestive organs, and the size of the heart, liver, and intestinal wall are known to increase during digestion in snakes (Secor et al., 2000; Wang et al., 2001; Andersen et al., 2005). The complex physiological changes that snakes undergo during digestion may affect anesthesia a variety of ways, yet we were unable to detect a strong effect with our design. It is still not recommended to anesthetize snakes shortly after feeding due to the risk of regurgitation during recovery and also because the added mass of food in the gut may result in calculating a dosage based on an inaccurate lean body mass.

Snakes with similar body condition scores anesthetized at the same temperature still show considerable variability in recovery time. This suggests that other factors, such as the route of drug administration, may contribute to variability between individuals. Subcutaneous and intramuscular drug administration are considered to be the least precise routes to deliver anesthetic agents since the rate of drug uptake is essentially unknown and may vary between individuals (Mosley, 2005). Subcutaneous and intramuscular injections, however, are the most practical route of drug administration when the accessibility or size of blood vessels in small lizards, snakes and turtles does not allow easy intravenous access and gas anesthetics are not available for use.

The effects of physiological parameters on anesthesia observed in this study may allow the adjustment of dosages of methohexital sodium to create more predictable anesthetic effects. Snakes that are anesthetized at high body temperatures and extremely heavy individuals can safely be given dosages of methohexital sodium higher than 15 mg/kg. We have safely used dosages of 18 to 20 mg/kg in these circumstances. For snakes with mass/SVL ratios below 0.6 and for gravid, sick, or injured snakes it is recommended to use a dose of 12.5 mg/kg, as the reactions of these snakes to methohexital sodium tends to be unpredictable and the risk of anesthetic overdose may be higher. During over 200 anesthesias involving more than 75 different snakes, only one animal was killed by anesthetic overdose using methohexital sodium in the first author's experience.

Of the factors evaluated in this study, body temperature, body condition, and gravidity affect the duration of anesthesia with methohexital sodium in snakes. Other factors undoubtedly play a role in creating variability and future research, such as pharmacokinetic studies, will allow the further improvement of anesthesia protocols and a better understanding of the physiological processes that terminate the duration of action of anesthetics in reptiles.

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