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Efficacy of two methods of intranasal administration of anaesthetic drugs in red-eared terrapins (*Trachemys scripta elegans*)

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ABSTRACT: The aim of the study was to evaluate the efficacy of ketamine, dexmedetomidine, atipamezole and alfaxalone delivered by two methods of intranasal administration in terrapins. The two methods were used in 21 healthy adult female red-eared terrapins: (A) with fully extended neck and restrained head, (B) with head hidden inside the shell. Ketamine (10 mg/kg) and dexmedetomidine (0.2 mg/kg) were delivered using a micropipette in the left and the right naris, respectively. Atipamezole (2 mg/kg) was administered 60 minutes later. Heart rate, head withdrawal reflex, palpebral reflex, toe-pinch reflex on the pelvic limb and glottal control enabling the insertion of the tracheal tube were recorded at 10-minute intervals. After a washout period of six months, alfaxalone (5 mg/kg) was tested. The first measurement in the alfaxalone trial started 5 minutes after the drug was administered and continued at 10-minute intervals. Heart rate decreased significantly in response to both methods of ketamine and dexmedetomidine administration. There were no significant differences between methods in time to loss of reflexes and full recovery of reflexes. Intranasal administration of atipamezole enabled rapid return to full activity. Alfaxalone administration decreased heart rate non-significantly and did not result in loss of evaluated reflexes. Both methods of drug administration of ketamine, dexmedetomidine and atipamezole resulted in a safe form of sedation and recovery. Intranasal administration of 5 mg/kg of alfaxalone was not effective.

Keywords: chelonians; anaesthesia; monitoring; heart rate; reflexes

Chelonians are one of the largest groups of reptiles kept in captivity and they have become very frequent patients in veterinary clinics (McArthur et al. 2004). Due to their defensive (e.g., retraction of the head and limbs into the shell) and offensive strategies (biting), chelonians, especially semi-aquatic chelonians, represent a challenge for veterinarians in handling and sample collection. The dissociative anaesthetic agent ketamine in combination with synergistic agents such as midazolam, opioids and alpha-2 agonist (e.g., medetomidine, dexmedetomidine) at low doses can be used alone or in combination for effective sedation of chelonians (Bennett 1991; Mosley 2005). Atipamezole, a specific alpha-2 antagonist for medetomidine, is used to reverse the action of medetomidine and dexmedetomidine during the recovery period of anaesthesia (Mosley 2005; Sladky and Mans 2012). Atipamezole is typically administered intramuscularly at a dose which is five times greater than

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that of medetomidine (Greer et al. 2001; Olsson and Phalen 2012) and 10 times the dose of dexmedetomidine (Fleming 2014). Ketamine and dexmedetomidine may be administered intramuscularly or subcutaneously in doses of 2–10 mg/kg (Greer et al. 2001; Nevarez 2009; Chu et al. 2014) and 0.05–0.1 mg/kg (Chu et al. 2014; Schumacher and Mans 2014), respectively.

For short term sedation and gentle tracheal tube insertion in terrapins, propofol and alfaxalone may be successfully used (Bennett 1991; Mosley 2005; Shepard et al. 2013; Knotek 2014). The short-acting neurosteroid alfaxalone may be administered to chelonians intravenously in a dose of 5 mg/kg or intramuscularly in doses of 10–20 mg/kg (Kischinovsky et al. 2013; Knotek 2014).

Intranasal drug administration (IN), like intravenous administration, avoids first-pass metabolism by allowing the drug to directly enter into systemic circulation rather than requiring it to be absorbed through the gastrointestinal tract and filtered by the liver. Intranasal administration of fentanyl, ketamine, midazolam and dexmedetomidine is currently used in human medicine, especially for children. This route has proven to be less traumatic for patients, and is easily accepted and safe (Walbergh et al. 1991; Malinovsky et al. 1996; Weber et al. 2003; Yuen et al. 2007; Jia et al. 2013; Rawat et al. 2014).

In veterinary medicine, intranasal administration of anaesthetics has been reported in a small number of avian, mammalian and reptile species (Robertson and Eberhart 1994; Vesal and Eskandari 2006; Vesal and Zare 2006; Moghadam et al. 2009; Al-Shebani 2011; Mans et al. 2012; Schnellbacher et al. 2012; Emery et al. 2014). However, information dealing with intranasal administration of alfaxalone to reptiles is lacking.

We hypothesised that an intranasal combination of ketamine and dexmedetomidine might provide an effective sedation. The aim of the present study was therefore to evaluate the effects and the practical use of ketamine, dexmedetomidine, atipamezole and alfaxalone administered intranasally in red-eared terrapins (*Trachemys scripta elegans*) and to compare two methods of intranasal administration.

MATERIAL AND METHODS

Animals. The study was performed with a group of twenty-one adult captive kept females of red-eared terrapins (*Trachemys scripta elegans*) aged 12–15 years. The animals were housed and handled with the agreement of the Branch Commission for Animal Welfare of the Ministry of Agriculture of the Czech Republic (accreditation No. 45620/2008-17210, 45620/10001). The terrapins were kept in standard husbandry conditions in aquaria (74 cm × 67 cm × 88 cm) with a 12-hour/12-hour day/night cycle provided by 100 W incandescent bulbs, and basking provided by UV/infrared lamps (D3 Basking Lamp 160 W, Arcadia, UK). The temperature in aquaria ranged from 25 to 30 °C, with water temperature ranging from 24 to 27 °C, and air humidity from 70 to 85%. All animals were fasted for 24 h prior to the experiment. Before the procedure, complete physical examination of terrapins was performed and body weight and heart rate (Doppler ultrasonic flow detector UltraTec PD1v, United Kingdom) data were collected for each terrapin. During the experiment, terrapins were housed individually in plastic containers placed on an electric heating pad (Bosch PFP 1031; Bosch, Czech Republic) at a temperature of 37.5 °C.

Study design and procedures. Two variants of intranasal administration were compared: administration of the drug to a terrapin with fully extended neck (EN) and restrained head (Figure 1), and administration of the drug to an animal with the head hidden (HH) inside the shell (Figure 2). The EN variant was performed in 16 terrapins (1.26 ± 0.27 kg). Two terrapins were excluded from the study during the washout period – one due to significant bradycardia and prolonged recovery time and the second due to bite injuries from other animals. The HH variant was performed in 14 ter-

Figure 1. Intranasal administration in a red-eared terrapin (*Trachemys scripta elegans*) with head hidden inside the shell

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Figure 2. Intranasal administration in a red-eared terrapin (*Trachemys scripta elegans*) with fully extended neck and restrained head

rapins (1.23 ± 0.28 kg) after a washout period of four months. The control group consisted of five red-eared terrapins (1.32 ± 0.13 kg).

The animals were held vertically. All drugs used in this study were delivered very slowly, for approximately 30 seconds, to minimise their loss (Schnellbacher et al. 2012). With the use of a micropipette (Nichipet EX, Nichiryo, Japan), ketamine (10 mg/kg; Narkamon, 50 mg/ml; Bioveta, Czech Republic) and dexmedetomidine (0.2 mg/kg; Dexdomitor, 0.5 mg/ml; Orion Pharma, Finland) were delivered in the left and right nares, respectively. In the control group, sterile saline solution (0.9% NaCl, Braun) was intranasally administered in the same volume as anaesthetic drugs using the same technique. After drug administration, each animal was placed back in the plastic container.

Sixty minutes after ketamine and dexmedetomidine administration, terrapins were held vertically and atipamezole (2 mg/kg; Antisedan, 5 mg/ml; Orion Pharma, Finland) divided in two equal parts was administered IN into both nares. All terrapins were housed in containers without water for the next 24 hours and all animals were observed for any adverse reactions.

After a washout period of six months, a group of 14 terrapins (1.27 ± 0.26 kg) underwent the study with alfaxalone. Both the EN and HH methods of holding the animal were used. Alfaxalone (5 mg/kg; Alfaxan, 10 mg/ml; Vetoquinol, France) was delivered into both nares (50% of the dose in the left and 50% of the dose in the right naris) using the same approach as with ketamine or dexmedetomidine. All terrapins were housed in containers without water for the next 24 h and all chelonians were observed for any adverse reactions.

Monitoring. Over the whole course of the study, chelonian activity, heart rate and loss of reflexes were recorded by the same person (E.C.). Heart rate was recorded with the use of a Doppler ultrasonic flow detector (UltraTec PD1v, United Kingdom). To evaluate the effects of IN, the following response variables were measured at 10-minute intervals in the trial with ketamine, dexmedetomidine and atipamezole: heart rate, loss and full recovery of the head withdrawal reflex, loss and full recovery of the palpebral reflex, loss and full recovery of the toe-pinch reflex on the pelvic limb, time when the tracheal tube (Braun 14G, Vasofix Safety, Germany) could be easily inserted and the time of full recovery of the glottal reflex. The various time points were designated as T_{KD}^{0} (before intranasal administration of ketamine and dexmedetomidine), T_{KD}^{10}, T_{KD}^{20}, T_{KD}^{30}, T_{KD}^{40}, T_{KD}^{50} and T_{KD}^{60} (10, 20, 30, 40, 50 and 60 minutes after administration of ketamine and dexmedetomidine), and T_{AT}^{10}, T_{AT}^{20}, T_{AT}^{30}, T_{AT}^{40}, T_{AT}^{50} and T_{AT}^{60} (10, 20, 30, 40, 50 and 60 minutes after administration of atipamezole).

The control group underwent the same monitoring timeline – T_{S}^{10}, T_{S}^{20}, T_{S}^{30}, T_{S}^{40}, T_{S}^{50} and T_{S}^{60} (10, 20, 30, 40, 50 and 60 minutes after administration of saline solution).

In the alfaxalone study, drug effects were determined 5, 10, 20, 30, 40, 50 and 60 minutes after the drug was administered (T_{AL}^{5}, T_{AL}^{10}, T_{AL}^{20}, T_{AL}^{30}, T_{AL}^{40}, T_{AL}^{50} and T_{AL}^{60}).

Statistical analysis. All statistical tests were performed in MedCalc PC-based software for Windows, version 14 (MedCalc Software, Ostend, Belgium). Descriptive statistics (mean ± SD, minimum, maximum) were used for the analysis of data. Based on an assessment of the normality (Shapiro-Wilk test), repeated measures one-way ANOVA was used to assess the differences in heart rate. If violations of sphericity occurred, Greenhouse-Geisser or Huynh-Feldt correction was appropriately used according to Girden (1992). A paired samples *t*-test was used to compare the time to loss of reflexes and full recovery of reflexes in the EN and HH groups. Correlations between drug volume and sedation effects were determined using the Pearson correlation coefficient. A significance (α) level of 0.05 was used in this study.

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RESULTS

There were no significant differences between groups (EN, HH) in body weight and volume of administered drugs (Table 1). Saline administration in the control group did not decrease the heart rate (Table 2), and also did not result in loss of any of the reflexes that were evaluated. There was a significant difference in heart rate between EN and HH in T_{KD}^{10}. In EN, the heart rate decreased significantly ($P < 0.0001$) between T_{KD}^{0} and T_{KD}^{20}–T_{KD}^{60} (Table 3). In HH, the heart rate decreased significantly ($P = 0.0156$) between T_{KD}^{0} and T_{KD}^{10} and significantly ($P < 0.0001$) between T_{KD}^{0} and T_{KD}^{20}–T_{KD}^{60}, respectively (Table 4).

Intranasal administration of atipamezole enabled rapid return to full activity in all terrapins, and counteracted the sedation and decreasing heart rate promoted by ketamine and dexmedetomidine within 10 minutes of application. In EN, the heart rate increased significantly ($P < 0.0001$) from 25 ± 5 [13–32] at T_{KD}^{60} to 54 ± 11 [32–68] bpm at T_{AT}^{10}. Heart rate did not differ between T_{AT}^{10} and T_{KD}^{0}. In HH, the heart rate increased significantly ($P < 0.0001$) from 24 ± 3 [19–32] at T_{KD}^{60} to 48 ± 15 [29–72] bpm at T_{AT}^{10}. Heart rate did not differ between T_{AT}^{10} and T_{KD}^{0}.

Alfaxalone administration decreased the heart rate, but did not result in loss of any of the reflexes that were evaluated. The differences in heart rate between T_{AL}^{0} and T_{AL}^{5}–T_{AL}^{60} were non-significant in both groups (EN, HH). There were no significant differences in heart rate between groups (EN, HH).

Both methods (EN, HH) of intranasal administration of ketamine and dexmedetomidine resulted in safe forms of sedation (Table 5). There were no significant differences between groups (EN, HH) in time to loss of reflexes and full recovery of reflexes. Loss of the head withdrawal reflex was recorded within 10–40 minutes and 10–60 minutes with the

Table 1. Intranasal administration of saline, dexmedetomidine, ketamine, atipamezole and alfaxalone in red-eared terrapins

Descriptive parameters	Terrapins with fully extended neck		Terrapins with hidden head	
	n	mean ± SD [min.–max.]	*n*	mean ± SD [min.–max.]
Saline (ml)	5	0.79 ± 0.08 [0.71–0.90]	5	0.80 ± 0.07 [0.72–0.89]
Dexmedetomidine (ml)	16	0.50 ± 0.11 [0.36–0.80]	14	0.49 ± 0.11 [0.33–0.79]
Ketamine (ml)	16	0.25 ± 0.06 [0.18–0.40]	14	0.25 ± 0.06 [0.16–0.39]
Atipamezole (ml)	16	0.50 ± 0.11 [0.36–0.80]	14	0.49 ± 0.11 [0.33–0.79]
Alfaxalone (ml)	7	0.64 ± 0.16 [0.52–0.99]	7	0.63 ± 0.10 [0.47–0.77]

Table 2. Heart rate frequency (bpm; mean ± SD [min.–max.]) in red-eared terrapins after intranasal administration of saline. Terrapins with fully extended neck (EN) and with hidden head (HH)

Variant	T_S^0	T_S^{10}	T_S^{20}	T_S^{30}	T_S^{40}	T_S^{50}	T_S^{60}
EN (*n* = 5)	46 ± 3 [42–50]	44 ± 2 [42–46]	45 ± 2 [43–47]	46 ± 2 [44–48]	49 ± 2 [47–50]	48 ± 3 [44–52]	46 ± 3 [43–49]
HH (*n* = 5)	46 ± 2 [44–50]	44 ± 3 [40–47]	43 ± 2 [41–46]	45 ± 2 [42–47]	46 ± 3 [41–48]	47 ± 3 [44–50]	46 ± 4 [40–51]

T_S^0 = heart rate before saline administration; $T_S^{10–60}$ = heart rate in minutes after administration

Table 3. Heart rate frequency (bpm; mean ± SD [min.–max.]) in red-eared terrapins after intranasal administration of anaesthetic drugs. Terrapins with fully extended neck

Drug	T_{KD}^0, T_{AL}^0	T_{AL}^5	T_{KD}^{10}, T_{AL}^{10}	T_{KD}^{20}, T_{AL}^{20}	T_{KD}^{30}, T_{AL}^{30}	T_{KD}^{40}, T_{AL}^{40}	T_{KD}^{50}, T_{AL}^{50}	T_{KD}^{60}, T_{AL}^{60}
Alfaxalone (*n* = 7)	50 ± 6 [38–58]	38 ± 7 [27–49]	40 ± 6 [33–50]	38 ± 9 [22–52]	36 ± 8 [24–45]	38 ± 8 [30–52]	42 ± 7 [32–51]	44 ± 7 [32–53]
Dexmedetomidine + ketamine (*n* = 16)	50 ± 8 [30–62]		36 ± 13 [18–62]	32 ± 7[a] [23–50]	30 ± 5[a] [20–38]	27 ± 5[a] [17–36]	26 ± 5[a] [14–32]	25 ± 5[a] [13–32]

T_{KD}^0, T_{AL}^0 = heart rate before drug administration; $T_{KD}^{10–60}$, $T_{AL}^{5–60}$ = heart rate in minutes after administration
[a]Values significantly different from T_{KD}^0 ($P < 0.0001$)

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Table 4. Heart rate frequency (bpm; mean ± SD [min.–max.]) in red-eared terrapins after intranasal administration of anaesthetic drugs. Terrapins with hidden head

Drug	T_{KD}^{0}, T_{AL}^{0}	T_{AL}^{5}	T_{KD}^{10}, T_{AL}^{10}	T_{KD}^{20}, T_{AL}^{20}	T_{KD}^{30}, T_{AL}^{30}	T_{KD}^{40}, T_{AL}^{40}	T_{KD}^{50}, T_{AL}^{50}	T_{KD}^{60}, T_{AL}^{60}
Alfaxalone (n = 7)	42 ± 7 [32–52]	36 ± 4 [30–40]	40 ± 4 [34–44]	38 ± 7 [30–46]	38 ± 6 [30–44]	40 ± 5 [34–48]	36 ± 6 [28–44]	38 ± 8 [24–44]
Dexmedetomidine + ketamine (n = 14)	53 ± 8 [40–64]		34 ± 12[a] [17–68]	32 ± 8[b] [24–56]	27 ± 5[b] [18–35]	26 ± 4[b] [20–32]	26 ± 4[b] [20–31]	24 ± 3[b] [19–32]

T_{KD}^{0}, T_{AL}^{0} = heart rate before drug administration; $T_{KD}^{10–60}$, $T_{AL}^{5–60}$ = heart rate in minutes after administration
[a]Values significantly different from T_{KD}^{0} (P = 0.0156)
[b]Values significantly different from T_{KD}^{0} (P < 0.0001)

Table 5. Monitoring of reflexes in red-eared terrapins after intranasal administration of anaesthetic drugs

Parameter	Terrapins with fully extended neck		Terrapins with hidden head	
	n	mean ± SD [min.–max.]	n	mean ± SD [min.–max.]
Loss of head withdrawal reflex after ketamine and dexmedetomidine delivery (min)	16	24 ± 7 [10–40]	14	27 ± 17 [10–60]
Full recovery of head withdrawal reflex after atipamezole delivery (min)	16	17 ± 6 [10–30]	14	17 ± 9 [10–30]
Loss of toe-pinch reflex loss after ketamine and dexmedetomidine delivery (min)	6	35 ± 5 [30–40]	6	48 ± 12 [30–60]
Full recovery of toe-pinch reflex after atipamezole delivery (min)	6	13 ± 5 [10–20]	6	13 ± 8 [10–30]
Tracheal tube insertion time after ketamine and dexmedetomidine delivery (min)	16	45 ± 15 [20–60]	14	50 ± 13 [20–60]
Full recovery of glottal reflex after atipamezole delivery (min)	16	11 ± 3 [10–20]	14	11 ± 3 [10–20]

use of EN and HH methods, respectively. It was possible to insert the endotracheal tube within 20–60 minutes (EN, HH). The palpebral reflex was weak but still present in 10 and six terrapins using the EN and HH methods, respectively. The toe-pinch reflex was lost in six terrapins within 30–40 minutes and 30–60 minutes with the use of EN and HH methods, respectively. There was no significant negative relationship between drug volume and time to loss of reflexes. After the study, all terrapins recovered well.

DISCUSSION

The sedative and anaesthetic effects of intranasal drug administration in chelonians have so far been tested in only a limited number of studies (Schnellbacher et al. 2012; Emery et al. 2014).

Intranasal administration of midazolam (0.5 mg/kg and 1.5 mg/kg) and dexmedetomidine (0.05 mg/kg and 0.15 mg/kg) failed to produce effective sedation in two species of tortoises (Emery et al. 2014). The practical use of IN in reptile clinical practice could be negatively influenced by the large volume of drug that must be administered (Schnellbacher et al. 2012; Emery et al. 2014). Slow administration of a large volume of drug is uncomfortable for the patient and significantly increases drug loss during administration.

Using both methods of animal fixation (EN, HH), all red-eared terrapins (*T. scripta elegans*) showed statistically significant decreases in heart rate after IN application of ketamine and dexmedetomidine. The use of the HH method resulted in a significantly decreased heart rate at the 10th minute after the administration of anaesthetics, and the decreased heart rate persisted until the 60th minute, when atipamezole was applied. When using the EN method, a significant decrease in the heart rate was observed 20 minutes after the administration of anaesthetics, and the decreased heart rate persisted until the 60th minute, when atipamezole was applied. The gradual decreases in the heart rate between the 10th

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and 60th minutes (HH), and between the 20th and 60th minute (EN) were not statistically significant.

Using both methods of animal fixation (EN, HH), the head withdrawal reflex and glottal reflex disappeared in all monitored red-eared terrapins (*T. scripta elegans*) after IN application of ketamine and dexmedetomidine. The palpebral reflex was maintained in 10 out of 16 turtles using the EN method and in six out of 14 turtles using the HH method. The toe-pinch reflex was maintained in 10 of 16 turtles using the EN method and in eight of 14 turtles using the HH method. Intranasal administration of ketamine and dexmedetomidine promoted a turtle condition that enabled safe insertion of the tracheal tube. Neither of the examined application methods (EN, HH) provided sedation that was sufficiently deep to allow the performing of simple surgery. The above observations are in agreement with Schnellbacher et al. (2012). The method of intranasal ketamine and dexmedetomidine application, mainly in the HH form, can therefore be considered to be appropriate in the case of sedation required for tracheal tube introduction before the start of inhalation anaesthesia for aquatic terrapins. Intranasal application of atipamezole yielded positive results in both methods (EN, HH) and ensured rapid return to full activity in all terrapins within 10 minutes of application. For terrestrial tortoises, however, the above methods did not prove viable (Emery et al. 2014; Knotek and Cermakova 2014); anatomical and physiological differences may play a role. Both methods (EN, HH) of intranasal administration of ketamine, dexmedetomidine and atipamezole resulted in a form of sedation that enabled safe tracheal tube insertion. Alfaxalone did not result in the loss of any of the reflexes that were evaluated. Intranasal administration of alfaxalone at the dose used in this study is not recommended.

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