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The influence of mechanical ventilation on physiological parameters in ball pythons (*Python regius*)

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ABSTRACT

Mechanical ventilation is widely recommended for reptiles during anesthesia, and while it is well-known that their low ectothermic metabolism requires much lower ventilation than in mammals, very little is known about the influence of ventilation protocol on the recovery from anesthesia. Here, 15 ball pythons (*Python regius*) were induced and maintained with isoflurane for 60 min at one of three ventilation protocols (30, 125, or 250 ml min^{-1} kg^{-1} body mass) while an arterial catheter was inserted, and ventilation was then continued on 100% oxygen at the specified rate until voluntary extubation. Mean arterial blood pressure and heart rate (HR) were measured, and arterial blood samples collected at 60, 80, 180 min and 12 and 24 h after intubation. In all three groups, there was evidence of a metabolic acidosis, and snakes maintained at 30 ml min^{-1} kg^{-1} experienced an additional respiratory acidosis, while the two other ventilation protocols resulted in normal or low arterial PCO_2. In general, normal acid-base status was restored within 12 h in all three protocols. HR increased by 143 ± 64% during anesthesia with high mechanical ventilation (250 ml min^{-1} kg^{-1}) in comparison with recovered values. Recovery times after mechanical ventilation at 30, 125, or 250 ml min^{-1} kg^{-1} were 289 ± 70, 126 ± 16, and 68 ± 7 min, respectively. Mild overventilation may result in a faster recovery, and the associated lowering of arterial PCO_2 normalised arterial pH in the face of metabolic acidosis.

1. Introduction

A variety of common procedures in reptile medicine and physiological studies, ranging from simple clinical examinations to surgical interventions, require chemical restraint or general anesthesia. Inhalation anesthesia is commonly used for reptiles (e.g., Bertelsen, 2014; Enok et al., 2013; Jensen et al., 2011; Mosley, 2005), but little is known about the ventilatory requirements during anesthesia, and even less is known about the recovery of acid-base status after surgeries involving respiratory disturbances (Bertelsen et al., 2014). Given their low ectothermic metabolism, conscious reptiles often exhibit prolonged breath holding, and many reptiles are endowed with high hypoxia tolerance (Bickler and Buck, 2007; Milsom, 1991). Nevertheless, it is widely recommended to provide mechanical ventilation during anesthesia when spontaneous ventilation subsides (Bertelsen, 2014; Sladky and Mans, 2012). While ventilation rates of 2–6 breaths min^{-1} have been recommended with a maximum airway pressure of 10–20 cm H_2O (Mosley, 2005; Sladky and Mans, 2012), it remains a challenge to define an appropriate ventilation regime that secures oxygen requirements during anesthesia, while avoiding respiratory acid-base disturbances due to hypo- or hyperventilation. Both respiratory acidosis (i.e., increased arterial PCO_2) and respiratory alkalosis (i.e., decreased $PaCO_2$) exert negative effects on the heart and the central nervous system (Mitchell et al., 1972).

We recently showed that mechanical ventilation protocols during brief anesthesia with propofol and recovery (total of 60 min) in South American rattlesnakes have profound effects on acid-base balance and mean arterial pressure during the following 24 h (Bertelsen et al., 2014). Further investigations are required for inhalation anesthesia of longer duration, likely to form the mainstay of surgical intervention in practice, and with a variance of tidal volume. During and after inhalation anesthesia, ventilation provides the major means of eliminating the anesthetic agent and the effects of ventilation protocol may differ from those observed after intravenous anesthesia. Here, we report the influence of three levels of ventilation (30, 125 and 250 ml min^{-1} kg^{-1}) on cardiovascular parameters and arterial blood gases in ball pythons during inhalation anesthesia and during the subsequent recovery period. Pythons are common pet species and used extensively in comparative physiology (Enok et al., 2012; Jensen et al., 2011, 2010; Overgaard and Wang, 2002), providing physiological data available for comparison. The cardiovascular anatomy of pythons also differs from that of

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rattlesnakes, as pythons are unique amongst snakes by being endowed with ventricular pressure separation and a minimal capacity for cardiac shunts (Jensen et al., 2010).

2. Materials and methods

2.1. Experimental animals

15 juvenile ball pythons (*Python regius*) of undetermined sex (mean ± SD body mass: 980 ± 30 g) were obtained from commercial suppliers and housed in thermostatically controlled rooms (26–27 °C, 75% humidity, 12 h/12 h light/dark cycle) in terrariums with an internal temperature gradient from 26 to 32 °C, constant access to water and hiding area, within the animal care facility at Bioscience, Aarhus University. The animals were assessed as healthy by external clinical examination and gained weight at a normal rate. Snakes were fed whole dead mice every two weeks and were fasted for at least one week prior to the experiment. The investigation was carried out under permit from the Danish Animal Inspectorate (license number 2013-15-2934-00847) in accordance with EU Directive 2010/63/EU for animal experiments.

2.2. Anesthesia and ventilation

Snakes were manually restrained and anesthetized with isoflurane (IsoFlo® vet., Orion Pharma Animal Health, Denmark) by placing a plastic bag containing a swab with 0.5–1 ml of isoflurane over the head of the snake until the righting reflex subsided. Animals were then endotracheally intubated with an appropriately sized uncuffed tube and randomly assigned to one of three ventilation regimes: 30 (7.5 ml kg^{-1}, 4 breaths min^{-1}), 125 (25 ml kg^{-1}, 5 breaths min^{-1}), or 250 (50 ml kg^{-1}, 5 breaths min^{-1}) ml min^{-1} kg^{-1} using a small animal ventilator (Hallowell EMC Model AWSTM Veterinary Anesthesia Workstation, MA, USA), and a maximum airway pressure of 15 cm H_2O. Isoflurane was delivered in 100% oxygen using a Fluotec Mark 3 vaporizer (Simonsen & Weel A/S, Denmark). The snakes remained on 4–5% isoflurane until the catheter was placed, after which the vaporizer setting was reduced to 2% for 15–20 min for suturing until surgery ended and isoflurane administration ceased, approximately 60 min after intubation (time to catheter placement was 41 ± 19 min), and ventilation protocols did not differ significantly in catheter placement time (one way ANOVA, F value 0.3402, $p = 0.5697$). Recovery time was defined as the interval from the end of isoflurane administration to voluntary extubation, which occurred as the snake actively withdrew its head from the endotracheal tube. During recovery, mechanical ventilation as indicated was continued.

2.3. Instrumentation

Using aseptic technique, a 5 cm ventral incision was made either anterior to the heart or anterior to the cloaca to allow access to the vertebral artery or dorsal aorta respectively (Enok et al., 2012; Olesen et al., 2008). An occlusive cannula (PE50 0.58 mm internal diameter polythene; Smiths Medical Danmark ApS, Denmark) containing heparinized saline (50 iu ml^{-1}, Heparin, in 0.9% NaCl; Fresenius Kabi Norge AS, Norway) was placed to obtain blood samples and measure mean arterial pressure (MAP) and heart rate (HR). Given the very low capacity for cardiac shunts in pythons, there is no reason to expect differences in blood gases at these two sites of catheterization and there is no difference in MAP at the two sites (personal communication T Wang). The catheter was externalized and secured dorsally with three sutures (4/0 coated silk Braun, Aesculap, Germany). The incision was sutured (4/0 silk) in an everting mattress pattern. Body temperature of the snake was maintained at 30 °C by a heating mat (Melissa Electric Heating Pad 631-015; Adexi A/S, Denmark) during surgery and by placing the snakes in a climate chamber postoperatively. In this chamber, the snakes were shielded from visual and auditory disturbance induced by the presence of the investigators.

2.4. Measurements of blood pressure and arterial blood gases

MAP was measured by connecting the arterial catheter to a pressure transducer (Baxter Edward, model PX600, Irvine, CA, USA) positioned at heart level and calibrated against a static water column. The signal was amplified and collected on a Biopac MP100 data acquisition system (ACQKnowledge 3.9.1; BIOPAC Systems, Inc., CA, USA). HR was derived from the pulsatile pressure signal. MAP was measured continuously from catheter placement to extubation, and at 60, 80 and 180 min as well as 12 and 24 h after intubation. The first samples were timed so that all animals were sampled under isoflurane anesthesia (60 min sample at end of surgery), and early in recovery while still mechanically ventilated with oxygen (80 min sample).

Heparinized arterial blood samples were drawn from the catheter at 60 min after intubation (corresponding to the end of surgery), and then at 80 and 180 min, as well as 12 and 24 h after intubation. Samples were analyzed using a GEM Premier 3500 blood gas analyzer measuring pH_a, $PaCO_2$, PaO_2, $[Na^+]$, $[K^+]$, $[Ca^{++}]$ and hematocrit (hct) and machine corrected to 30 °C. These values were then further corrected for species by applying recently derived correction equations for python blood (Malte et al., 2014). Plasma $[HCO_3^-]$ was calculated as $[HCO_3^-] = 10^{pH-pK'} \cdot pCO_2 \cdot \alpha_{CO_2}$, using an α_{CO_2} (CO_2 solubility in plasma) of 0.0366 mmol l^{-1} (Heisler, 1984) and a pK′ ($pK' = -0.0763 \cdot pH + 6.72830$) from Overgaard and Wang (2002).

2.5. Data analysis

Recovery times for the different ventilation protocols were compared by means of a one-way ANOVA with Welch's correction and Games-Howell post-hoc analysis, to account for the heterogeneity in variance between groups, and are reported as F ratio with corresponding p value. The values for the remaining variables in the 3 treatments were compared by means of linear mixed modelling (using R package nlme), with snake ID forming a random effect, and initial model specification including an interaction between time and ventilation where significant, and a type AR-1 temporal autocorrelation to account for the unequally spaced measurement times. Histograms of residuals and plots of residuals vs fitted values were visually examined to ensure conditions for testing (normality and homoscedasticity) were met. PaO_2 was analyzed by gamma family generalized linear mixed model, as linear modelling failed to fulfil the necessary conditions. Tukey's HSD was used for pairwise comparison to reveal differences between ventilation regimes at each time point, and within ventilation regime to the recovered 24 h value. The recovered value for all parameters is taken as the mean value of the 24 h measurements for that ventilation regime. The level of significance was set at $p < 0.05$, and all values are presented as means ± SD. Results are reported as with z value test statistics for the fixed effect of ventilation regime or time, testing whether their coefficients are significantly different from zero, with their respective p value. All statistical analyses were performed in R Studio (0.99.842), using R 2015 (RStudio Team (2015), R Core Team (2015)).

3. Results

3.1. Arterial blood gases and acid-base balance

Values for PaO_2, $PaCO_2$, pH_a and plasma $[HCO_3^-]$ during recovery from anesthesia are presented in Fig. 1 for each ventilation regime. Within all ventilation protocols, PaO_2 was significantly elevated during the period of 100% oxygen administration (60–180 min) compared to the recovered value, such that PaO_2 at 12 h was not significantly different from the recovered value at 24 h of 9.15 ± 2.11 kPa as seen in Fig. 1 (z values in pooled protocols for comparison 80 min vs 24 h = 10.735,