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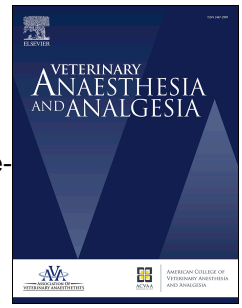

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RESEARCH STUDY

Cardiovascular effects of intravenous vatinoxan (MK-467) in medetomidine-tiletamine-zolazepam anaesthetized red deer (*Cervus elaphus*)

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Authors' contributions

Conceptualization of the study: GS, UA, JP, MR; **Performed experiments and data sampling:** JE, JP, FR, UA, GS; **Statistical analysis:** KG; **Data interpretation**: JE, KG, GS, JP; **Funding acquisition, resources:** GS, UA; **Writing – original draft:** JE, GS; **Review & editing:** all authors

Conflict of interest statement

The authors declare no competing financial interests and no conflict of interests. Vetcare Oy, Finland, provided the vatinoxan used during the study. However, the company played no role in the study design or in the data collection, analysis and interpretation. None of the authors has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Abstract

Objective To determine the effect of intravenous vatinoxan administration on bradycardia, hypertension and level of anaesthesia induced by medetomidine-tiletamine-zolazepam in red deer (*Cervus elaphus*).

Study design and animals A total of 10 healthy red deer were enrolled in a randomized, controlled, experimental, crossover study.

Methods Deer were administered a combination of 0.1 mg kg^{-1} medetomidine hydrochloride and 2.5 mg kg^{-1} tiletamine-zolazepam intramuscularly, followed by 0.1 mg kg^{-1} vatinoxan hydrochloride or equivalent volume of saline intravenously (IV) 35 minutes after anaesthetic induction. Heart rate (HR), mean arterial blood pressure (MAP), respiration rate (f_R), end-tidal CO_2 ($P_{E'}CO_2$), arterial oxygen saturation (SpO_2), rectal temperature (RT) and level of anaesthesia were assessed before saline/vatinoxan administration (baseline) and at intervals for 25 minutes thereafter. Differences within treatments (change from baseline) and between treatments were analysed with linear mixed effect models ($p < 0.05$).

Results Maximal (81 ± 10 beats $minute^{-1}$) HR occurred 90 seconds after vatinoxan injection and remained significantly above baseline (42 ± 4 beats $minute^{-1}$) for 15 minutes. MAP significantly decreased from baseline (122 ± 10 mmHg) to a minimum MAP of 83 ± 6 mmHg 60 seconds after vatinoxan and remained below baseline until end of anaesthesia. HR remained unchanged from baseline (43 ± 5 beats $minute^{-1}$) with the saline treatment, while MAP decreased significantly (112 ± 16 mmHg) from baseline after 20 minutes. $P_{E'}CO_2$, f_R, and SpO_2 showed no significant differences between treatments, while RT decreased significantly 25 minutes after vatinoxan. Level of anaesthesia was not significantly influenced by vatinoxan.

Conclusion and clinical relevance Vatinoxan reversed hypertension and bradycardia induced by medetomidine without causing hypotension or affecting the level of anaesthesia in red deer. However, the effect on HR subsided 15 minutes after vatinoxan IV administration.

Vatinoxan has the potential to reduce anaesthetic side effects in non-domestic ruminants immobilized with medetomidine-tiletamine-zolazepam.

Keywords bradycardia, hypertension, medetomidine, red deer, vatinoxan

Introduction (Word count 3668)

Chemical immobilization and anaesthesia are often essential for handling and medical interventions of captive and free-ranging non-domestic species. Induction of balanced anaesthesia in non-domestic species by injectable anaesthetics often requires high doses of drug combinations such as dissociative anaesthetics combined with α_2-adrenoceptor agonists (Grimm & Lamont 2007). In non-domestic ruminants, α_2-adrenoceptor agonists, such as medetomidine, are the most frequently represented drug class (Masters 2015), and are commonly combined with ketamine (Arnemo et al. 2005) or tiletamine-zolazepam (Barasona et al. 2013).

While the sedative and analgesic effects of α_2-adrenoceptor agonists are mediated by central α_2-adrenoceptors located in the central nervous system, the activation of peripheral α_2-adrenoceptors causes peripheral vasoconstriction and a consequent increase in arterial blood pressure (Langer et al. 1980). In non-domestic ruminants, this can result in severe hypertension (Sainmaa et al. 2019). Furthermore, baroreceptor mediated sinus bradycardia together with a decreased cardiac output regularly occur in animals sedated with medetomidine (Bryant et al. 1996; Murrell & Hellebrekers 2005). The dose-dependent cardiovascular side effects should be considered, since the doses of α_2-adrenoceptor agonists used in non-domestic ruminant anaesthesia (Williams et al. 2018) often exceed those required for their domestic relatives, *e.g.* sheep (Bryant et al. 1996) by 3 - 4 times.

The peripheral α_2-adrenoceptor antagonist vatinoxan (MK-467) alleviates the peripheral cardiovascular and pulmonary effects of α_2-adrenoceptor agonists while maintaining sedation, as shown in many domestic species, such as dogs (Honkavaara et al. 2008; Honkavaara et al. 2011), cats (Honkavaara et al. 2017b; Pypendop et al. 2017), horses (Bryant et al. 1998; de Vries et al. 2016) and sheep (Bryant et al. 1998; Raekallio et al. 2010; Adam et al. 2018a). In contrast to the widely used antagonist for α_2-adrenoceptor agonists atipamezole, it mainly affects peripheral receptors due to its minimal ability to cross the

blood-brain barrier (Clineschmidt et al. 1988). Therefore, vatinoxan does not substantially affect the sedation mediated by α_2-adrenoceptors located in the central nervous system (Honkavaara et al. 2008). Moreover, vatinoxan can be safely used with atipamezole as demonstrated in medetomidine sedated sheep (Adam et al. 2018a).

The aim of this study was to evaluate the effect of vatinoxan on heart rate (HR) and blood pressure after intravenous (IV) bolus administration in red deer (*Cervus elaphus*) anaesthetized with medetomidine-tiletamine-zolazepam. We hypothesized that vatinoxan would alleviate the bradycardia and hypertension induced by medetomidine in red deer without markedly affecting the level of anaesthesia.

Material and methods

A total of 10 healthy 7-month-old red deer (male = 4; female = 6) with a mean body weight of 69.6 kg (standard deviation: ± 10.3 kg; range: 53 - 89 kg) were included in the study. The animals originated from the red deer population kept in a 45 hectare enclosure adjacent to the Research Institute of Wildlife Ecology (48.21°N, 16.37°E), under conditions that approximate those in the wild. All procedures and experiments were approved by the institutional ethics and animal welfare committee and the Animal experimental committee of the Federal Ministry of Science, Research and Economy in accordance to the Austrian Animal Experimentation Act (Tierversuchsgesetz 2012) (BMWFW-68.205/0191-WF/V/3B/2017), the guidelines for good scientific practice and national legislation.

The study was carried out as a randomized, controlled, experimental, crossover study, with a wash-out period between treatments of 14 - 16 days. Each animal was studied on two occasions and randomly allocated to two treatments by flipping a coin in the first trial round. Animals were given the opposite treatment in the second trial round. Allocation into the specific treatment was carried out by the same anaesthetist (JP), who was responsible for drug calculations and preparations and was not involved in data acquisition. The blinded data acquisition was done by another investigator (JE) throughout the project. The exclusion

criterion was incomplete administration of the initial medetomidine-tiletamine-zolazepam dose, which occurred in one animal.

Anaesthesia was induced with 0.1 mg kg^{-1} medetomidine (20 mg mL^{-1}, Medetomidine-hydrochloride 2%, magistral formula by Richter Pharma AG, Austria) combined with 2.5 mg kg^{-1} tiletamine-zolazepam (Zoletil, Virbac Österreich GmbH, Austria) administered intramuscularly (IM) followed by **1)** 0.1 mg kg^{-1} vatinoxan (VAT) (Vetcare Finland Oy, Finland) (referred to as VAT) or **2)** equivalent volume of saline 0.9 % in mL (Isotonic sodium chloride solution 0.9 % ad us. vet., B. Braun GmbH, Austria) (referred to as Control). Vatinoxan HCl, in powder form, was dissolved in sterile 0.9 % saline solution to a final concentration of 2.5 mg mL^{-1}. Both treatments were administered IV 35 minutes after medetomidine-tiletamine-zolazepam.

Animals were led into a walled corral located in their enclosure by a professional animal trainer. This procedure was practiced before by positive reinforcement training in order to familiarise the animals with the procedure and provide stress-free handling and anaesthetic induction. The animals were then remotely injected using a filled dart (3 mL dart syringe, Dan-Inject, Denmark) projected into the caudo-lateral aspect of the pelvic limb (*Musculus biceps femoris)* via blowpipe (BLOW 1.25 Model Zoo, Dan-Inject, Denmark) with medetomidine-tiletamine-zolazepam mixed in the same syringe. When the dart had fully discharged this was recorded as the start of anaesthesia and measured using a stopwatch. Subsequent sampling timepoints were taken from time the stopwatch was started. To avoid any visual or auditory stimulation by the research team, the animal was observed by one person who monitored it through a small opening in the corral wall. As soon as the recumbent deer became unresponsive to auditory stimuli, the corral was entered by one veterinarian to confirm an adequate level of anaesthesia (i.e. no response to physical stimulation, assessment of pupil dilation and loss of palpebral reflex). Deer were then blindfolded, placed in right lateral recumbency and transported to the clinic within 10 minutes. The animals were

intubated (HS Endotracheal Tube, Inner Diameter 8.0 mm, Length 32 cm Murphy, Henry Schein Animal Health, Czech Republic) and were given 100 % oxygen (2 L $minute^{-1}$) until the end of anaesthesia. An arterial catheter (Insyste-A Arterienkatheter 22 Gauge, Length 38 mm, Becton Dickinson, Germany) was placed in the *Arteria auricularis caudalis* and a venous catheter (Vasofix Safety 18 Gauge, Length 45 mm, B. Braun Austria GesmbH, Austria) in the *Vena jugularis*. The transducer of the arterial catheter was calibrated before each experiment against a mercury column and zeroed to atmospheric pressure at the level of the sternum with the red deer in lateral recumbency.

Baseline values were recorded 30 minutes after the injection of medetomidine-tiletamine-zolazepam and included HR, respiratory rate (f_R), end-tidal CO_2 ($P_{E'}CO_2$), electrocardiogram (ECG), haemoglobin oxygen saturation using pulse oximetry (SpO_2), rectal temperature (RT), and direct mean (MAP), systolic (SAP) and diastolic (DAP) arterial blood pressures. The variables $P_{E'}CO_2$, f_R, SpO_2 (Root with Noninvasive Blood Pressure and Temperature Monitoring; Radical-7; Phasein ISA; Masimo Corporation, CA, USA) and continuous lead II ECG, HR, MAP, SAP, DAP (PM-8000 Express, Mindray Medical Germany GmbH, Germany) were measured using multiparameter monitors. Rectal temperature was determined using a digital thermometer (HS Digital Veterinary Thermometer, Henry Schein Animal Health, Czech Republic).

Anaesthetic level was assessed by using the following scoring system: Degree of hypnosis was assessed by the palpebral and perineal reflexes tested by tactile stimulation (up to 3 times within 10 seconds) of the naso-ventral canthus of the eye and the perianal skin. Reflex response was scored with 0 = absent; 1 = slight (reflex/response could be induced only by repeated stimulus); 2 = slight but definitely present response; 3 = brisk, normal response. Evaluation of antinociception was assessed by the pedal reflex in response to pinching (up to 3 times within 10 seconds) the interdigital space using the same scoring system as described earlier.

Treatment with VAT or an equal volume of saline followed 35 minutes after induction, and was injected over a 30 second period (T = -0.5 to T = 0), at 5 minutes after baseline.

Immediately after the end of treatment application (T = 0) (VAT or Control), SAP, DAP, MAP, ECG and HR were documented at 10 second-intervals for 2 minutes, 60 second-intervals for the following 8 minutes until T = 10 and 5 minute-intervals for the remaining 15 minutes (T = 15-T = 25).

The variables $P_{E'}CO_2$, f_R, RT, SpO_2 and depth of anaesthesia were documented 5 minutes before (baseline T = -5) and 5 (T = 5), 10 (T = 10), 15 (T = 15), 20 (T = 20) and 25 minutes (T = 25) after VAT or saline treatment, resulting in a total anaesthesia duration of 60 minutes. The animals were returned to their enclosure, extubated and injected with atipamezole (Antisedan, Vetoquinol GmbH, Germany) dosed at 5 µg for each 1 µg of medetomidine IM into the lateral muscles of the shoulder girdle (*Musculus deltoideus*). All animals were observed until complete recovery.

Statistical analysis

Statistical analyses were performed by use of RStudio (R version 3.4.1; R Core Team 2017). Power analysis based on data previously obtained in our laboratory suggested that 10 deer would permit the detection of a 24% difference in MAP with a standard deviation (SD) of 19 mmHg between VAT and control treatment, with an alpha level of 0.05 and a power of 0.95.

The Shapiro-Wilk normality test was used to assess data distributions. The normal distribution of model residuals was visually determined with qq-plots and histograms. To adjust for repeated measurements and to avoid pseudo-replication, differences between treatments were evaluated with a repeated measures analysis of linear mixed effects models (nlme package, Pinheiro et al. 2017) with multiple comparisons (VAT *versus* Control). Changes in physiological variables were analysed with a *post-hoc* test (Tukey Honestly Significant Difference) over-time within treatments (change from baseline) and between

treatments, as well as at selected time points for time x treatment interaction effects (lsmeans package, Lenth 2016). Regarding level of anaesthesia, data were not normally distributed, therefore a nonparametric test (Kruskal-Wallis test) was used to assess differences between treatments. Data are reported as mean ± SD. Statistical significance was set at $p < 0.05$.

Results

The VAT administration resulted in a significant decrease in arterial blood pressure and a significant increase in HR. The MAP (Fig. 1), DAP (Fig. 2) and SAP (Fig. 3) significantly decreased by a mean of 34 % from baseline after VAT and remained below baseline values until end of anaesthesia. Minimum values were obtained 110 seconds after VAT injection.

Blood pressures differed significantly between Control and VAT at all time points (Figs. 1-3, Table 1). In the Control treatment, MAP, SAP and DAP decreased significantly below baseline 20 minutes after saline injection (Figs. 1-3).

For HR, a significant difference between Control and VAT treatment was detected 10 seconds after injection lasting for 15 minutes (Fig. 4, Table 1). The HR significantly increased 10 seconds after injection of VAT by an average of 102% 3 minutes after injection and remained significantly above baseline for 15 minutes (Fig. 4). In the Control treatment no significant difference for HR from baseline was detected (Fig. 4).

The SpO_2 was significantly increased in both treatments at all time points after treatment compared with baseline (Table 1). No significant difference between treatments was observed for SpO_2.

The RT (Table 1) was significantly lower with VAT treatment than with Control treatment. RT started to decrease significantly below baseline 5 minutes after VAT treatment and 25 minutes after Control treatment.

No significant difference within and between VAT and Control treatment occurred for $P_{E'}CO_2$ and f_R during the observation period (Table 1).

Except for respiratory sinus arrhythmia with both treatments (VAT: 8 animals; Control: 7 animals), no ECG abnormality was detected.

Level of anaesthesia showed neither a difference between treatments nor a time-treatment interaction or influence of time. No perineal- or pedal reflexes were detected, but all animals showed moderate palpebral eye reflexes (range: 0-2; mean: 1.4 ± 0.5) throughout the observation period.

Discussion

The combination of medetomidine-tiletamine-zolazepam induced both bradycardia and hypertension in all deer in the present study. IV administration of VAT alleviated these changes without causing hypotension or affecting the degree of anaesthesia. However, while the reduction of blood pressure lasted until the end of the anaesthesia, the effect on HR subsided 15 minutes after VAT. Respiratory variables f_R, $P_{E'}CO_2$, and SpO_2 were within physiological ranges and showed no significant difference between the treatments in anaesthetized deer supplemented with oxygen.

Physiological reference values for blood pressure and HR in conscious deer are unavailable, as the majority of data collection in wildlife species takes place in anaesthetized animals. In the present study, resting HR for deer (mean: 84 ± 3 beats $minute^{-1}$) and cut-off points for bradycardia and tachycardia were calculated according to the formula 241* $bodyweight^{-0.25}$ (Heard 2007), as in a study by Sainmaa et al. (2019) in markhors. A HR more than 20% below or above this value was interpreted as brady- or tachycardia (Heard 2007). Thus, baseline HR of all animals in the present study may be considered bradycardic. Baseline MAP values after medetomidine-tiletamine-zolazepam administration were increased compared to normotensive values in other mammalian species (*e.g.* goats and sheep: MAP 75–100 mmHg; Riebold 2015) and therefore considered hypertensive. Both hypertension and bradycardia are well-known side effects of medetomidine (Bryant et al. 1996; Murrell & Hellebrekers 2005). Nevertheless, tiletamine-zolazepam used in the present

study could have additionally impacted blood pressure and HR (Lin et al. 1989; Lin et al. 1993). However, tiletamine causes an increase in HR or even tachycardia due to an increased sympathetic tone in species such as dogs (Cullen & Reynoldson 1997) and cats (Yanmaz et al. 2017). Therefore, the bradycardic effect in this study can probably be mostly attributed to medetomidine. Furthermore, a study in horses showed an increase in HR after tiletamine-zolazepam application reversing detomidine induced bradycardia (Wan et al. 1992).

However, tiletamine-zolazepam could impact blood pressure, due to increased sympathetic systemic vascular resistance (Lin et al. 1993). In calves (Lin et al. 1989) administered tiletamine-zolazepam, arterial blood pressure was characterized by biphasic decreases followed by an increase. In other ruminants such as sheep no significant changes in blood pressure and HR were detected even with up to five times higher doses (15 mg kg^{-1} tiletamine-zolazepam) than that used in the present study (Taylor et al. 1992). Nevertheless, an enhancing effect by tiletamine-zolazepam on hypertension in the present study due to the sympathomimetic effects of the dissociative drug tiletamine cannot be discounted. While VAT reversed hypertension and bradycardia in this study, no hypotension occurred.

No effect of VAT administration was detected on the level of anaesthesia. However, a shortened duration of the sedative effect of medetomidine may have been undetectable in our study as the anaesthetic effect of tiletamine-zolazepam was present during the observation period. The shorter duration of sedation has previously described in dogs administered dexmedetomidine (Honkavaara et al. 2012). Furthermore, assessment of anaesthetic level by reflex testing as performed in the present study might be limited and could be further refined in future studies.

While blood pressure remained significantly below baseline until the end of anaesthesia, HR returned to baseline values 15 minutes after VAT. This is in contrast to other studies in dogs, where the influence of VAT on HR was sustained for longer time periods of 60 - 90 minutes (Honkavaara et al. 2011; Restitutti et al. 2017). The comparatively short

effect of VAT on HR in this study might be attributed to differences in the pharmacokinetics between IM medetomidine and IV VAT, resulting in diverging changes in their plasma concentrations over time. For example in dogs, the concentration of VAT in plasma decreased rapidly during the first 10 minutes after an IV injection (Honkavaara et al. 2012) whereas the concentration of medetomidine changed relatively little between 30 and 90 minutes after IM administration (Restitutti et al. 2017). This corresponds to the time frame when VAT was administered to the red deer. Therefore, in our study, the concentration of VAT might have been only high enough to compete with medetomidine and to replace it at the receptor sites briefly. However, differences in duration of cardiovascular effects might also be attributed to species differences in the half-lives of VAT (IV). Plasma half-life of VAT in dogs is approximately 40 - 60 minutes (Honkavaara et al. 2012), whereas in horses plasma half-lives of 140 - 170 minutes have been reported (de Vries et al. 2016). Therefore, studies of plasma drug concentration profiles would be desirable in order to understand bioavailability and pharmacokinetics according to administration route and species. Furthermore, species-specific sensitivity towards VAT and α_2-adrenoceptor agonists might also explain differences in effects on HR. Studies in dogs (Honkavaara et al. 2008), and in sheep (Raekallio et al. 2010) with the same dose ratios of dexmedetomidine (0.005 mg kg^{-1}) and VAT (0.25 mg kg^{-1}) indicate a species-specific effect of VAT on HR. Duration of changes in HR after dexmedetomidine/VAT administration lasted longer in dogs (40 minutes) than in sheep (20 minutes). Moreover, Bryant et al. (1998) showed a greater attenuation of medetomidine-induced hypertension in sheep given VAT than that observed in horses. A greater sensitivity to the vasodilatory effects of VAT in the presence of dexmedetomidine has furthermore been proposed for cats when compared with dogs (Honkavaara et al. 2011). Additional studies in wildlife species such as red deer are required to investigate the different mechanisms underlying this variation in species-specific sensitivity. However, the return of HR to baseline values independently from the sustained normotension in this study may be attributed to a

combination of low sympathetic tone due to medetomidine-induced central sympatholysis in combination with a diminishing baroreceptor reflex. Hypertension induced by α_2-adrenoceptor agonists is known to subside over time (Savola 1989). Therefore, blood pressure probably remained low due to central effects of medetomidine (*e.g.* sedation, central sympatholysis and the resulting bradycardia), despite the potentially vanishing effect of VAT in this study. This proposed mechanism is supported by the significant decrease of MAP below baseline after 20 minutes in the control treatment. Even though challenging in wildlife species it would be desirable to assess cardiovascular variables shortly after induction of anaesthesia in order to better understand the cardiovascular dynamics as well as the effect of an earlier or concomitant VAT application. In contrast to the majority of studies that administered VAT concomitantly with an α_2-adrenoceptor agonist in the induction phase (Honkavaara et al. 2017b; Pypendop et al. 2017), animals in this study were given VAT 35 minutes after medetomidine-tiletamine-zolazepam application.

Cardiovascular effects of VAT are furthermore dose dependent in several species, such as dogs (Honkavaara et al. 2011; Restitutti et al. 2017), and cats (Honkavaara et al. 2017a; Honkavaara et al. 2017b). The comparable low dose of VAT (0.1 mg kg^{-1} IV) used in our study in contrast to previous studies administering VAT IV in cats for example (Honkavaara et al. 2017a; Pypendop et al. 2017), dogs (Honkavaara et al. 2008) and sheep (Raekallio et al. 2010) might have also influenced the duration of the effect on HR. However, Tapio et al. (2018) showed in horses, using a comparable dose of VAT (0.15 mg kg^{-1} IV) 10 minutes after detomidine (0.02 mg kg^{-1} IV), a significant increase in HR that remained elevated for 90 minutes. Sainmaa et al. (2019), who administered various doses of VAT (0.117-0.297 mg kg^{-1}) to markhors, did not detect a dose-dependent effect on HR, although the decrease in MAP correlated significantly with the dose of VAT. Thus, it needs to be verified, whether, and if so, higher doses influence the efficiency of VAT in red deer.

A decrease in RT over time was observed in the present study although no hypothermia (RT < 37°C) was detected. Hypothermia can occur in sedated and anaesthetized animals due to impaired thermoregulation and decreased metabolic activity (MacDonald et al. 1988; Grimm & Lamont 2007). In the present study, RT decreased significantly from baseline with both treatments. However, with VAT treatment a decrease occurred sooner. In dogs treated with medetomidine/butorphanol, thermographic imaging of superficial temperature suggested that VAT may increase peripheral heat loss (Vainionpää et al. 2013), possibly counteracting increased peripheral vasoconstriction by α_2-adrenoceptor agonists (Honkavaara et al. 2011). Therefore, regular monitoring of core temperature is emphasized when VAT is combined with α_2-adrenoceptor agonists.

$P_{E'}CO_2$, f_R and SpO_2 were within physiological limits and showed no significant differences between treatments in this study. However, all animals were given supplemental oxygen during the experiment. Therefore, a potential effect of VAT on ventilatory variables could not be assessed. Low prebaseline SpO_2 levels may be attributed to the lack of direct oxygen supply as the animals were not given supplemental oxygen during transport. Once the animals were intubated and connected to oxygen, SpO_2 values increased to clinically desirable levels. $P_{E'}CO_2$ was slightly elevated in all animals, which is commonly reported in deer anaesthetized with α_2-adrenoceptor agonists (Boesch et al. 2011). Elevated $P_{E'}CO_2$ in the present study may have occurred due to central respiratory depression (*e.g.* from the anaesthetic drugs) or to increased production of CO_2 (*e.g.* from exertion) or both causes. Impairment of gas exchange could have been further compromised by α_2-adrenoceptor agonist induced pulmonary oedema, which is commonly described in ruminants (Kästner et al. 2007). Blood gas analysis and further investigation of oxygen delivery and utilization, that were not performed in the present study, are desirable in order to understand underlying pathophysiological effects that might impact pulmonary variables and oxygenation.

The recovery from anaesthesia after atipamezole administration was smooth and rapid without differences between treatments, as reported in previous studies in dogs and sheep (Honkavaara et al. 2008; Adam et al. 2018b). No prolonged recoveries due to tiletamine-zolazepam or renarcotization was observed.

Limitations of this study include the delayed assessment of cardiovascular variables and application of VAT due to working with a wildlife species. Restrictions and potential subjective bias limit the assessment of anaesthetic level. In addition, the lack of further assessment of effects on the cardiopulmonary system, *e.g.* arterial blood gases analysis restrains the information on the influence of VAT on oxygenation.

Conclusion

The IV administration of vatinoxan alleviated cardiovascular side effects, such as hypertension and bradycardia, in immobilized non-domestic ruminants with medetomidine-tiletamine-zolazepam. There was no significant effect of vatinoxan on the degree of anaesthesia and reversal of sedation by atipamezole. Vatinoxan has the potential to reduce anaesthetic side effects in immobilized non-domestic mammals.

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Figure 1 Mean ± standard deviation of direct mean arterial blood pressure (mmHg) in 10 red deer administered intravenous either: 0.1 mg kg^{-1} vatinoxan or an equivalent volume of saline. The animals were anaesthetized with medetomidine (0.1 mg kg^{-1}) and tiletamine/zolazepam (2.5 mg kg^{-1} IM) 35 minutes before vatinoxan or saline injection. Spaces between the graphs and timelines (x-axis) represent a change in time intervals. *VAT group (dotted line) significantly different (all $p < 0.05$) from baseline (-5 minutes) at the respective time points. †Significantly different (all $p < 0.05$) from Control at the respective time points. Arrows represent: B = baseline (5 minutes before injection of vatinoxan or saline), SI = start injection of vatinoxan/saline, EI = end injection of vatinoxan or saline.

Figure 2 Mean ± standard deviation direct systolic arterial blood pressure (mmHg) in 10 red deer receiving IV either: 0.1 mg kg^{-1} vatinoxan or an equivalent amount of saline. (See Fig. 1 legend for medetomidine-tiletamine-zolazepam doses). Spaces between the graphs and timelines (x-axis) represent a change in time intervals. *VAT group (dotted line) significantly different (all $p < 0.05$) from baseline (-5 minutes) at the respective time points. †Significantly different (all $p < 0.05$) from Control at the respective time points. Arrows represent: B = baseline (5 minutes before injection of vatinoxan or saline), SI = start injection of vatinoxan or saline, EI = end injection of vatinoxan or saline.

Figure 3 Mean ± standard deviation direct diastolic arterial blood pressure (mmHg) in 10 red deer receiving IV either: 0.1 mg kg^{-1} vatinoxan or an equivalent amount of saline. (see Fig. 1 legend for medetomidine-tiletamine-zolazepam doses). Spaces between the graphs and timelines (x-axis) represent a change in time intervals. *VAT group (dotted line) significantly

different (all $p < 0.05$) from baseline (-5 minutes) at the respective time points. †Significantly different (all $p < 0.05$) from Control at the respective time points. Arrows represent: B = baseline (5 minutes before injection of vatinoxan or saline), SI = start injection of vatinoxan or saline, EI = end injection of vatinoxan or saline.

Figure 4 Mean ± standard deviation heart rate (beats minute^{-1}) in 10 red deer given intravenously either: 0.1 mg kg^{-1} vatinoxan or an equivalent amount of saline. (see Fig. 1 legend for medetomidine-tiletamine-zolazepam doses). Spaces between the graphs and timelines (x-axis) represent a change in time intervals. *VAT group (dotted line) significantly different (all $p < 0.05$) from baseline (-5 minutes) at the respective time points. †Significantly different (all $p < 0.05$) from Control at the respective time points. Arrows represent: B = baseline (5 minutes before injection of vatinoxan or saline), SI = start injection of vatinoxan or saline, EI = end injection of vatinoxan or saline.

Table 1 Changes in physiological variables in red deer before and after intravenous administration of 0.1 mg kg^{-1} vatinoxan (VAT; n = 10) or saline (equal amount of mL of vatinoxan; Control; n = 10). End of injected treatment was at time 0. Data are presented as means ± standard deviation. All animals were administered supplemental oxygen.

Variable	Treatment	Time (minutes)										
		-5 (baseline)	0	1	2	3	4	5	10	15	20	25
HR (beats minute^{-1})	Control	43 ± 5	42 ± 6	43 ± 5	45 ± 5	47 ± 9*	43 ± 5	44 ± 6	46 ± 8*	45 ± 7	45 ± 7	46 ± 7
	VAT	42 ± 4	44 ± 6	80 ± 9*†	81 ± 12*†	79 ± 14*†	73 ± 15*†	67 ± 17*†	57 ± 21*†	51 ± 17*	42 ± 3	44 ± 5
SAP (mmHg)	Control	145 ± 15	145 ± 15	144 ± 17	145 ± 16	145 ± 17	146 ± 17	145 ± 18	145 ± 18	144 ± 18	142 ± 17*	142 ± 17*
	VAT	151 ± 8	145 ± 8*	116 ± 8*†	119 ± 9*†	122 ± 10*†	126 ± 11*†	128 ± 10*†	131 ± 9*†	133 ± 8*†	131 ± 9*†	131 ± 9*†
MAP (mmHg)	Control	116 ± 15	117 ± 13	116 ± 15	117 ± 15	117 ± 12	116 ± 15	115 ± 16	114 ± 19	115 ± 15	112 ± 16*	110 ± 18*
	VAT	122 ± 10	116 ± 10*	83 ± 6*†	85 ± 6*†	88 ± 7*†	91 ± 6*†	94 ± 6*†	98 ± 9*†	102 ± 11*†	99 ± 10*†	99 ± 10*†
DAP (mmHg)	Control	103 ± 15	103 ± 12	101 ± 14	103 ± 15	104 ± 10	102 ± 13	100 ± 16	99 ± 19	100 ± 15	96 ± 15*	94 ± 19*
	VAT	109 ± 15	103 ± 16	68 ± 11*†	69 ± 11*†	72 ± 12*†	75 ± 12*†	79 ± 11*†	83 ± 15*†	87 ± 16*†	85 ± 14*†	84 ± 15*
f_R (breaths minute^{-1})	Control	24 ± 8	24 ± 6	NA	NA	NA	NA	24 ± 7	28 ± 11	29 ± 11	30 ± 11*	29 ± 10
	VAT	21 ± 9	24 ± 14	NA	NA	NA	NA	23 ± 7	23 ± 9	21 ± 6†	24 ± 11	28 ± 9
$P_{E'}CO_2$ (mmHg)	Control	55 ± 9	57 ± 8	NA	NA	NA	NA	59 ± 5	55 ± 6	57 ± 5	57 ± 6	58 ± 8
	VAT	56 ± 7	59 ± 8	NA	NA	NA	NA	60 ± 4	58 ± 6	59 ± 3	56 ± 8	54 ± 7
$P_{E'}CO_2$ (kPa)	Control	7.3 ± 1.2	7.6 ± 1.0	NA	NA	NA	NA	7.9 ± 0.7	7.3 ± 0.9	7.7 ± 0.7	7.6 ± 0.8	7.7 ± 1.0
	VAT	7.5 ± 1.0	7.9 ± 1.1	NA	NA	NA	NA	8.0 ± 0.5	7.3 ± 0.9	7.9 ± 0.4	7.3 ± 1.2	7.2 ± 0.9
SpO_2 (%)	Control	90 ± 13	96 ± 4*	NA	NA	NA	NA	97 ± 2*	97 ± 2*	97 ± 2*	97 ± 2*	97 ± 1*
	VAT	93 ± 8	95 ± 3	NA	NA	NA	NA	96 ± 2*	97 ± 2*	97 ± 2*	97 ± 2*	97 ± 2*
RT (°C)	Control	39.3 ± 0.7	39.3 ± 0.8	NA	NA	NA	NA	39.2 ± 0.8	39.0 ± 0.7	38.9 ± 0.9*	39.0 ± 1.0*	39.0 ± 0.9
	VAT	38.9 ± 0.5	38.8 ± 0.5	NA	NA	NA	NA	38.7 ± 0.6*	38.6 ± 0.5*	38.6 ± 0.6*	38.5 ± 0.7*	38.4 ± 0.7*†

IV: intravenous; NA: not available; HR: heart rate; SAP: systolic arterial pressure (invasive); MAP: mean arterial pressure (invasive); DAP: diastolic arterial pressure (invasive); f_R: respiratory rate; $PE'CO_2$: end-tidal CO_2; SpO_2: oxygen saturation of arterial blood measured by pulse oximetry; RT: rectal temperature. *Significantly different from baseline (-5 minutes) ($p < 0.05$). †Significantly different from Control at this time point ($p < 0.05$).