Sedative and antinociceptive effects of different detomidine constant rate infusions, with or without methadone in standing horses

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Summary

Background: Standing surgery avoids the risks of general anaesthesia in horses.

Objectives: To assess sedation, antinociception and gastrointestinal motility in standing horses after a detomidine loading dose and 2-hours constant rate intravenous (i.v.) infusion, with or without methadone.

Study design: Blinded, randomised, crossover with seven healthy adult cross-bred horses, three geldings and four females (404 ± 22 kg).

Methods: Five i.v. treatments were administered to all horses with 1-week washout period: saline (SAL), detomidine low (2.5 µg/kg bwt + 6.25 µg/kg bwt/h) (DL) and high doses (5 µg/kg bwt + 12.5 µg/kg bwt/h) (DH) alone or combined with methadone (0.2 mg/kg bwt + 0.05 mg/kg bwt/h), (DLM) and (DHM), respectively. Height of head above the ground (HHAG), electrical (ET), thermal (TT) and mechanical (MT) nociceptive thresholds and gastrointestinal motility were evaluated at predetermined times between 5 and 240 minutes. A mixed effect model and Kruskal-Wallis test were used to analyse normally and non-normally distributed data, respectively.

Results: Sedation (<50% basal HHAG) was achieved for the duration of the infusion, and for an additional 15 minutes in DH and DHM groups. Nociceptive thresholds were higher than baseline, to the greatest degree and the longest duration, with DHM (ET and TT for 135 minutes and MT for 150 minutes). After DH, TT was significantly higher than baseline from 30 to 120 minutes and MT from 15 to 135 minutes. After DLM, ET was increased at 90

minutes, TT at 30 minutes and MT for 120 minutes. Gastrointestinal motility was reduced for up to 135 minutes after DL, 150 minutes after DLM and 210 minutes after DH and DHM.

Main limitations: Nociceptive thresholds are not equivalent to surgical stimuli.

Conclusion: Methadone with the highest detomidine dose (DHM) may provide sufficient sedation and analgesia for standing surgical procedures and warrants further investigation.

Introduction

In equine practice, procedures such as laparoscopy, dentistry, sinus trephination and eye enucleations can be performed with the aid of intravenous (i.v.) sedative and analgesic constant rate infusions (CRIs) combined with local anaesthesia [1,2]. The aim of using an i.v. CRI is to maintain steady plasma drug concentrations, leading to consistent sedation and analgesia. Infusions of α_2-agonists alone [3-6] or in combination with opioids [4-7] are commonly used for this purpose.

Detomidine CRI has been used under clinical conditions after loading doses of 7.5 – 15 µg/kg bwt in prospective studies [8-11], and of 7.5 ± 1.87 µg/kg bwt (mean ± s.d.) in a retrospective study with 51 horses [12]. These reports describe a variety of protocols with mean infusion rates varying from 6.6 to 36 µg/kg bwt/h, with surgery duration of up to 170 minutes. An in-depth literature review revealed no publications evaluating the effects of simultaneous CRIs of detomidine and an opioid such as methadone in standing horses.

Based on data from previous experimental studies in standing horses [13], pharmacokinetic/pharmacodynamic (PK/PD) modelling [14] was used to simulate the effects of different doses and rates of detomidine and methadone on sedation, antinociception and

gastrointestinal motility [15]. Leading on from this, we aimed to identify a protocol under experimental conditions that provides antinociception without excessive sedation. Loading doses of detomidine at 2.5 and 5 µg/kg bwt combined with 0.2 mg/kg bwt methadone followed by infusion of detomidine (6.25 or 12.5 µg/kg bwt/h) and methadone (0.05 mg/kg bwt/h) were chosen according to the results of simulation [15]. Our hypothesis was that the higher doses and infusion rates of detomidine, when combined with methadone would produce antinociception with adequate sedation that should be suitable for evaluation under clinical conditions.

Materials and methods

The study was designed as a randomised, placebo-controlled, observer-blinded, crossover experiment. Each horse received all treatments with a 1-week washout period between treatments. The study was performed during March and April of 2017, at the farm facilities of the School of Veterinary Medicine and Animal Science, São Paulo State University (UNESP), Botucatu (Brazil) where all the horses were born and lived. Each horse was always treated at the same time of day.

Animals

Seven docile, healthy adult cross-bred horses (three geldings, four mares), 9 – 11 years and weighing 372 – 450 kg were enrolled in the study. All had been trained using positive reinforcement and used in similar antinociceptive studies. The horses were kept at pasture and brought in to covered pens with outdoor access for at least 12 hours, with water *ad libitum*, commercial feed and hay provided until each experiment began. The animals were classified as healthy ASA I (American Society of Anesthesiologists) according to physical, musculoskeletal and lameness examination, haematology and biochemistry (complete

haemogram, blood urea nitrogen, aspartate and alanine transaminases, gamma glutamyl transferase and alkaline phosphatase), performed one to 2 weeks before beginning the study. No drugs such as sedatives, analgesics, corticosteroids or nonsteroidal anti-inflammatory drugs were administered by any route for at least one month prior to the study.

The sample size was estimated using the sedative [height of head above the ground (HHAG)] and antinociceptive (thermal, mechanical and electrical) results of a pilot study and data obtained from the same group of horses in previous studies [13,16,17]. Five treatments and the differences between means and expected general s.d. (https://www.statstodo.com/SSizUnpairedDiff_Pgm.php) ($\alpha = 0.05$, $\beta = 0.80$) were used.

Study design

On the day of the experiment, each animal was weighed and fly repellent was sprayed onto the skin. The hair over the left jugular vein was clipped and the skin disinfected. Thereafter, 1 ml of 2% lidocaine (Xylestesin 2%)[a] was injected subcutaneously and one 14-gauge catheter (G14 x 70 mm)[b] was placed for drug administration.

The hair over the middle third of the dorsal aspect of both metacarpals was clipped for placement of the thermal probe (right) and mechanical actuator (left). The hair proximal to the coronary band of the left thoracic limb was clipped for placement of the electrodes. A strict clipping and cleaning protocol was followed in order to ensure good contact and to minimise between-electrode resistance to less than 3 kiloohms (kΩ) [13,18].

Each horse was then led to the 6-square metres experimental room, without windows, to which fly repellent had been previously applied. A period of thirty minutes was allowed for acclimatisation of the horses to the working environment. During this period, two adhesive electrodes (2223BRQ)[c] were placed on the clipped and cleaned area of the left coronary band; the electrodes were placed 8 cm from each other and fixed with adhesive

strips around the hoof. A commercial horse-blanket[d] was placed on the back of the horse and the thermal (WTT2)[d] and mechanical (WMT1)[d] control units, remotely controlled with infrared signals, were attached with Velcro. The thermal probe and mechanical actuator were attached to the limbs and connected to the control units after the horse had been placed in the restraining stocks. A blood pressure cuff (DURA-CUF CRITIKON 17–25 cm)[e], positioned at the base of the tail, was used to measure heart rate (HR) and systolic arterial blood pressure (SAP) with a non-invasive Doppler system (Model 812)[f].

The five treatments were all comprised of a bolus injection followed by a 2-hour CRI, assigned using a randomisation website[g]. The treatments were: saline (SAL) (Cloreto de sódio 0.9%)[h], detomidine (Eqdomin, 10 mg/ml)[i] low dose (DL, 2.5 µg/kg bwt followed by a CRI of 6.25 µg/kg bwt/h), detomidine high dose (DH, 5 µg/kg bwt followed by a CRI of 12.5 µg/kg bwt/h), detomidine low dose with methadone (Mytedom 10 mg/ml)[j] (DLM, 2.5 µg/kg bwt detomidine + 0.2 mg/kg bwt methadone followed by 2 concurrent CRIs of detomidine 6.25 µg/kg bwt/h + methadone 0.05 mg/kg bwt/h), and detomidine high dose with methadone (DHM, 5 µg/kg bwt detomidine + 0.2 mg/kg bwt methadone followed by 2 concurrent CRIs of detomidine 12.5 µg/kg bwt/h + methadone 0.05 mg/kg bwt/h). All bolus doses and infusions were diluted with saline by an assistant investigator (M.W.F.) to 10 and 18 ml for boluses and infusions, respectively, to keep the main investigator blinded. The CRIs were delivered by two calibrated syringe drivers (DigiPump SR8x[k] and Pilot Anestesia[l]).

Evaluation of the sedation variables and the responses to noxious stimuli were evaluated 3 times before drug administration (T0, baseline values), and once only at T5 (5 minutes after drug administration), T15, T30, T60, T90, T120, T135, T150, T180, T210 and T240. Starting at T15, gastrointestinal motility was scored at the same time points. Evaluation of each variable is described in detail elsewhere [13] and summarised briefly below:

Sedation

One investigator (A.R.O.), unaware of treatment group assignment, measured the HHAG to determine the degree of sedation [4,5]. Quality of sedation was scored using numerical rating scales [13,19] for ataxia and responses to tactile, acoustic and visual stimuli, always in this order (Supplementary Item 1).

Nociceptive threshold testing

Nociceptive stimuli were applied at each time point, always in the same order: electrical, thermal and mechanical, immediately after the sedation scoring. All the responses were evaluated by the main investigator (M.G.M.), who was unaware of the treatment group assignment. Aversive reactions were considered positive responses when the horse lifted its foot, pawed the ground, stamped, flexed the limb or walked to avoid the stimulus [20]. Further details for electrical, thermal and mechanical threshold testing and devices are described elsewhere [13,18,21,22].

Cardiopulmonary variables

Measured SAP values were corrected by adding the height difference between the right atrium of the heart (scapulohumeral joint in the standing horse) and the cuff. A height difference of 10.2 cm was considered equivalent to 7.5 mmHg. The respiratory rate (f_R) was measured by observation of the thoracic movements over 15 seconds.

Abdominal auscultation

The main investigator (M.G.M.) auscultated the four abdominal quadrants immediately for one minute each (right dorsal, right ventral, left dorsal and left ventral), and awarded a

motility score, from 0 to 5 according to Boscan *et al.* [23]. The sum of the scores was recorded, ranging from 0 to 20 (0 no motility, 20 maximal motility possible).

Data Analysis

Normality was evaluated graphically and with normality tests (Shapiro-Wilk) for each variable. Descriptive measurements were generated. For parametric variables, a mixed model ANOVA with Tukey's post-hoc test was used to evaluate the differences between treatments and time points; data are shown as mean ± s.d. For non-parametric variables, Friedman's test with Dunn's post-hoc test was used; data are shown as median (range). A significance level less than 0.05 was used for all analysis and the calculations were made with the aid of Statistical Analysis Software[m].

Results

All the horses received all treatments and completed the study. Appetite and faecal output were within normal limits at the end of each session. None of the horses showed signs of colic. One horse reacted with mild head-shaking to acoustic and visual stimuli up to T30 when receiving treatment DLM. No other complications were observed.

Sedation

Sufficient sedation (<50% of basal HHAG) [5] was achieved for the duration of the infusion and for an additional 15 minutes in DH and DHM groups only. Significant differences within and between treatments are shown in Figure 1.

Significant differences within and between treatments for ataxia scores are shown in Table 1. With regard to the scores for responses to the different stimuli, few significant differences were observed: 1) responses to tactile stimuli had lower scores (diminished

response) after DHM compared to SAL at T5 [DHM 1 (0 – 3)] *versus* [SAL 3 (2 – 3)], T30 [DHM 0.5 (0 – 2)] *versus* [SAL 3 (2 – 3)], T60 [DHM 1 (0 – 3)] *versus* [SAL 3 (2 – 3)] and T90 [DHM 1.5 (0 – 3)] *versus* [SAL 3 (1 – 3)]; and 2) responses to visual stimuli had lower scores (diminished response) than baseline [2 (1 – 3)] for treatment DL at T30 and T60 [0.5 (0 – 1) at both time points]. Between treatments, scores for visual stimuli were lower after DL [0.5 (0 – 1)] than SAL [2 (1 – 2)] at T30. No differences within or between treatments were observed for acoustic stimuli.

Nociceptive threshold testing

Differences within and between treatments are shown in Figures 2, 3 and 4 for electrical, thermal and mechanical thresholds, respectively. Treatment DHM resulted in the highest thresholds for all the 3 stimuli. Thermal and mechanical cut-out was reached at most time points during the DHM infusion. All nociceptive thresholds increased to the greatest degree and for the longest duration with DHM compared to the other treatments (electrical and thermal for 135 minutes and mechanical for 150 minutes). Treatments DH and DLM provided similar antinociception.

Abdominal auscultation, cardiovascular, and respiratory function

Differences within and between treatments for abdominal auscultation are shown in Figure 5. Intestinal motility was reduced for up to 135 minutes after DL, 150 minutes after DLM and 210 minutes after DH and DHM.

Throughout the study period, cardiovascular function was maintained well within acceptable limits in all horses undergoing all treatments (Table 2), with no differences from baseline and between treatments for HR, and only one single difference at one time point from baseline for SAP. All treatments decreased the f_R. This reduction persisted after the end

of the infusion significantly for 60 minutes in DL and DH groups and 90 minutes in DLM and DHM groups.

Discussion

The results of this study indicate that the higher detomidine dose combined with methadone (DHM) produced the most intense and persistent antinociceptive effects of all the protocols for the three stimuli. Sedation and effects on gastrointestinal motility were similar to the high dose of detomidine without methadone (DH). Low doses of detomidine with or without methadone (DLM and DL) produced less antinociception and sedation but still reduced gastrointestinal motility for the duration of the infusion, although scores returned to baseline sooner than for DH and DHM. Minimal cardiovascular and respiratory effects were observed for all the treatments.

Treatment DHM produced the most intense antinociceptive effects and these were maintained during the 120-minute infusion period. The low dose of detomidine (DL) did not produce any antinociception as described previously [13,16,17]. However, when combined with methadone (DLM), antinociception was mild and similar to that observed with the high dose of detomidine alone (DH). This may be explained by the different effects of the interaction of methadone on the effect of detomidine, which were of positive potentiation for the electrical and thermal stimuli and from synergistic to additive for the thermal stimulus [15].

Sedation, indicated by more than or equal to a 50% reduction in HHAG, was similar to that observed after a 5 µg/kg bwt bolus of detomidine [13], and described as 'sufficient' sedation [5]. According to this criterion, overall, only horses treated with the high dose of detomidine (DH and DHM) were 'sufficiently' sedated. In contrast, low doses of detomidine, regardless of the inclusion of methadone (DL and DLM), produced insufficient sedation. This

is important if these protocols are to be used in horses undergoing standing surgery when an adequate degree of sedation is essential. When using treatment DHM, a certain degree of ataxia must be anticipated, with a reduction in the responses to stimuli. Treatments using 2.5 µg/kg bwt of detomidine appear unlikely to provide adequate sedation for the invasive procedures.

All treatments reduced gastrointestinal motility. The higher doses of detomidine (DH and DHM) produced the greatest effects and for the longest period after the end of the infusion. Similar results were also observed when these drugs were given as *boli* [13], and by simulation based on PK/PD models [15]. Although no signs of colic were observed, the reduction in motility scores suggests that horses would benefit from close monitoring during the first 12 – 24 hours after treatment.

Detomidine at 5 µg/kg bwt with 0.2 mg/kg bwt of methadone also provided antinociception with adequate sedation in a previous report [13]. For prolonged maintenance of sedation and antinociception, the 12.5 µg/kg bwt/h rate for detomidine co-administered with methadone (DHM) appeared to be the best of all the treatments. When given alone, detomidine infusion rates of around 9 µg/kg bwt/h were successfully used for laparoscopy [8,9], whereas rates of approximately 20 µg/kg bwt/h were required for dental or sinus procedures [10,11]. Clinical studies are needed to determine if the DHM protocol might be useful, or if the rate should be adapted to different surgical procedures.

Methadone was used to enhance the sedative properties of detomidine, provide analgesia [24], and reduce the dose and infusion rate of the α_2-agonist. To date, the use of methadone as a co-infusion with an α_2-agonist has not been reported in horses. Excitatory effects, such as head-shaking, linked to the use of 0.2 mg/kg bwt of methadone [16] may occur, especially when using low doses of detomidine. In the current study, only one horse showed mild head-shaking in response to external stimuli after the bolus in treatment DLM.

A low dose of 2.5 μg/kg bwt may not always overcome the potential excitatory effects of opioids. The κ- agonist, butorphanol, is the opioid most widely studied for use as a CRI [4-7], but methadone, a full μ-agonist, might be more appropriate for more painful surgical procedures. Two reports suggest that buprenorphine, a partial μ-agonist opioid, when combined with detomidine infusion, although contributing to antinociception, may lead to abdominal pain and increase locomotor activity [10,11]. However, minimal complications were observed in a large clinical study of horses sedated with romifidine and buprenorphine before elective general anaesthesia [25].

Most equine standing sedation protocols are based on clinical experience, experimental [3-9], clinical [10,11] or retrospective [12] studies. Our study is innovative because we used the data from our previous *boli* study [13] to perform PK/PD modelling [14,15]. Recent refinements in sequential PK/PD modelling allow concentration-effect data to be determined and the effects on different PD variables to be predicted according to plasma concentrations. In our PK/PD study [15], we were able to determine the different interactions between both drugs for each PD variable, and even to simulate the effects of different dose and rate ranges regarding sedation (HHAG), antinociception and gastrointestinal motility [15]. This demonstrates that PK/PD modelling can be used to predict more precisely the expected effects of future equine clinical trials.

The main limitation of our study related to the extrapolation of the results to a clinical scenario as: (i) nociceptive stimuli are not real surgical stimuli, even when validated for experimental studies in standing horses [20]; (ii) surgical pain in different locations may differ in intensity due to specific innervation; and (iii) understanding of the magnitude of inter-animal variability is limited from a group of 7 horses. Study of a larger clinical population undergoing a range of standing surgical procedures is needed to confirm the predicted clinical effects.

Conclusions

In conclusion, the high dose of detomidine combined with methadone produced the most potent and persistent antinociception, with minimal adverse effects. This protocol appears likely to be useful for clinical surgical procedures and future studies to evaluate its clinical applicability are justified.

Authors' declaration of interests

Except for Dr P.M. Taylor, who is a director of Topcat Metrology Ltd., none of the authors declare any competing interests.

Ethical animal research

The study complied with requirements described by the Ethical Committee for The Use of Animals in Research of São Paulo State University (UNESP), School of Veterinary Medicine and Animal Science, Botucatu, SP, Brazil (0210/2016).

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Authorship

M. Gozalo-Marcilla contributed to study design, acquisition, management and interpretation of data, writing of the manuscript and critical review of the manuscript. A. Rodrigues de Oliveira contributed to study design, data acquisition and critical review of the manuscript. M. Werneck Fonseca contributed to study design and data acquisition. F. Sossai Possebon contributed to statistical analysis, management and interpretation of data. L. Pelligand, P. Taylor and S.P. Loureiro Luna contributed to study design, interpretation of data and critical review of the manuscript.

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Figure legends

Fig 1: Height of head above the ground (HHAG) in 7 standing horses given i.v. saline (SAL), detomidine low (DL) (2.5 µg/kg + 6.25 µg/kg bwt/h) and high doses alone (DH) (5 µg/kg + 12.5 µg/kg bwt/h) or combined with methadone (0.2 mg/kg + 0.05 mg/kg bwt/h) (DLM and DHM) for 120 minutes. Dotted line at T120 indicates the end of infusion. Data are mean ± s.d. Significant differences were the following: * Values lower than time 0 within the same treatment. † Values after DL, DH, DLM and DHM lower than SAL. ‡ Values after DL, DH and DHM lower than SAL. § Values after DH and DHM lower than SAL. α Values after DHM lower than DLM. β Values after DH lower than DLM. δ Values after DH lower than DL. # Values after DHM lower than DL. ($p<0.05$).

Fig 2: Electrical [upper safety limit 20 Volts (V)] thresholds in 7 standing horses given i.v. saline (SAL), detomidine low (DL) (2.5 µg/kg bwt + 6.25 µg/kg bwt/h) and high doses alone (DH) (5 µg/kg bwt + 12.5 µg/kg bwt/h) or combined with methadone (0.2 mg/kg bwt + 0.05 mg/kg bwt/h) (DLM and DHM) for 120 minutes. Dotted line at T120 indicates the end of infusion. Data are mean ± s.d. Significant differences were the following: * Values higher than time 0 within the same treatment. † Values after DHM higher than DH, DLM, DL and SAL. ‡ Values after DHM higher than DL and SAL. § Values after DH higher than SAL. α Values after DLM higher than SAL. ($p<0.05$).

Fig 3: Thermal [upper safety limit 60 degrees Celsius (°C)] thresholds in 7 standing horses given i.v. saline (SAL), detomidine low (DL) (2.5 µg/kg bwt + 6.25 µg/kg bwt/h) and high doses alone (DH) (5 µg/kg bwt + 12.5 µg/kg bwt/h) or combined with methadone (0.2 mg/kg bwt + 0.05 mg/kg bwt/h) (DLM and DHM) for 120 minutes. Dotted line at T120 indicates the

end of infusion. Data are mean ± s.d. Significant differences were the following: * Values higher than time 0 within the same treatment. † Values after DHM, DH and DLM higher than SAL. ‡ Values after DHM higher than SAL. § Values after DHM higher than DL. α Values after DH higher than DL. β Values after DLM higher than DL. ($p<0.05$).

Fig 4: Mechanical [upper safety limit 20 Newtons (N)] thresholds in 7 standing horses given i.v. saline (SAL), detomidine low (DL) (2.5 µg/kg bwt + 6.25 µg/kg bwt/h) and high doses alone (DH) (5 µg/kg bwt + 12.5 µg/kg bwt/h) or combined with methadone (0.2 mg/kg bwt + 0.05 mg/kg bwt/h) (DLM and DHM) for 120 minutes. Dotted line at T120 indicates the end of infusion. Data are mean ± s.d. Significant differences were the following: * Values higher than time 0 within the same treatment. † Values after DHM higher than DL and SAL. ‡ Values after DHM higher than DH. § Values after DHM higher than DLM. α Values after DH and DLM higher than SAL. β Values after DH higher than DL. δ Values after DLM higher than SAL. # Values after DLM higher than DL. ($p<0.05$).

Fig 5: Intestinal motility in 7 standing horses given i.v. saline (SAL), detomidine low (DL) (2.5 µg/kg + 6.25 µg/kg bwt/h) and high doses alone (DH) (5 µg/kg + 12.5 µg/kg bwt/h) or combined with methadone (0.2 mg/kg + 0.05 mg/kg bwt/h) (DLM and DHM) for 120 minutes. Dotted line at T120 indicates the end of infusion. Data are mean ± s.d. Significant differences were the following: * Values lower than time 0 within the same treatment. † Scores after SAL higher than DL, DLM, DH and DHM. ‡ Scores after SAL higher than DH and DHM. α Scores after DL higher than DH. β Scores after DL higher than DHM. § Scores after DL and DLM higher than DHM. # Scores after DLM higher than DH. ($p<0.05$).

Supporting Information

Supplementary Item 1: Numerical rating scales.

Table 1: Ataxia scores in 7 standing horses administered intravenous saline intravenously (SAL), detomidine low (DL) (2.5 µg/kg bwt + 6.25 µg/kg bwt/h) and high doses alone (DH) (5 µg/kg bwt + 12.5 µg/kg bwt/h) or combined with methadone (0.2 mg/kg bwt + 0.05 mg/kg bwt/h) (DLM and DHM) for 120 minutes. Data are presented in [median (range)], where 0 indicates, no ataxia and 3 indicates severe ataxia sedation and ataxia (Supplementary Item 1).

Variable	Time (minutes)	Treatments				
		SAL	DL	DH	DLM	DHM
Ataxia	0	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)
	5	0 (0 - 0)	0 (0 - 1)	0.5 (0 - 2)	0 (0 - 1)	0.5 (0 - 3)
	15	0 (0 - 1)^{a}	0 (0 - 1)^{a}	1 (0 - 3)^{ab}	0 (0 - 1)^{ab}	2 (1 - 3)^{b}
	30	0 (0 - 0)^{a}	1 (0 - 2)^{ab}	1 (0 - 2)^{ab}	1 (0 - 1)^{ab}	2 (1 - 3)^{*b}
	60	0 (0 - 0)^{a}	1 (0 - 2)^{ab}	1 (0 - 2) ^{ab}	1 (0 - 2)^{ab}	2.5 (1 - 3)^{*b}
	90	0 (0 - 0)^{a}	1 (0 - 2)^{ab}	1 (0 - 2)^{ab}	0.5 (0 - 2)^{ab}	1.5 (1 - 3)^{b}
	120	0 (0 - 0)^{a}	0.5 (0 - 1)^{ab}	1 (0 - 3) ^{ab}	1 (0 - 2)^{ab}	2 (1 - 3)^{b}
	135	0 (0 - 0)	0 (0 - 2)	0.5 (0 - 3)	0.5 (0 - 1)	1.5 (0 - 3)
	150	0 (0 - 0)	0 (0 - 1)	0 (0 - 3)	0 (0 - 0)	1 (0 - 2)
	180	0 (0 - 0)	0 (0 - 0)	0 (0 - 2)	0 (0 - 0)	0 (0 - 1)
	210	0 (0 - 0)	0 (0 - 0)	0 (0 - 1)	0 (0 - 0)	0 (0 - 1)
	240	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)

*Significantly different from baseline (time 0) within a treatment ($p<0.05$).

^{abc} Different superscript letters indicate significant differences between treatments at that time point ($p<0.05$). Dotted line at T120 indicates the end of infusion.

Table 2: Heart rate (HR), systolic arterial blood pressure (SAP) and respiratory rate (f_R) (mean ± s.d.) in 7 standing horses administered i.v. saline (SAL), detomidine low (DL) (2.5 µg/kg bwt + 6.25 µg/kg bwt/h) and high doses alone (DH) (5 µg/kg bwt + 12.5 µg/kg bwt/h) or combined with methadone (0.2 mg/kg bwt + 0.05 mg/kg bwt/h) (DLM and DHM) for 120 minutes. Data are presented as mean ± s.d.

Variable	Time (minutes)	Treatment				
		SAL	DL	DH	DLM	DHM
HR (bpm)	0	46 ± 7	43 ± 4	46 ± 6	47 ± 7	44 ± 7
	5	48 ± 11	41 ± 7	42 ± 9	45 ± 12	39 ± 8
	15	47 ± 9	42 ± 5	37 ± 5	44 ± 12	41 ± 13
	30	46 ± 10	41 ± 6	41 ± 11	43 ± 13	37 ± 11
	60	45 ±10	41 ± 6	41 ± 13	44 ± 15	40 ± 12
	90	44 ± 9	41 ± 6	40 ± 8	44 ± 17	41 ± 12
	120	45 ± 10	41 ± 8	41 ± 9	45 ± 17	41 ± 14
	135	46 ± 10	43 ± 8	42 ± 7	44 ± 8	42 ± 12
	150	45 ± 12	43 ± 7	43 ± 6	43 ± 12	43 ± 12
	180	46 ± 12	47 ± 9	44 ± 10	47 ± 13	47 ± 13
	210	47 ± 13	43 ± 6	45 ± 9	46 ± 14	46 ± 13
	240	45 ± 11	41 ± 6	45 ± 9	44 ± 12	46 ± 14
SAP (mmHg)	0	141 ± 12	137 ± 10	140 ± 15	143 ± 12	136 ± 9
	5	130 ± 9	130 ± 10	148 ± 16	149 ± 13	136 ± 14
	15	135 ± 14	123 ± 9	144 ± 12	139 ± 20	138 ± 21
	30	130 ± 14	125 ± 9	144 ± 21	139 ± 22	142 ± 19
	60	135 ± 16	124 ± 11	142 ± 18	132 ± 14	141 ± 19
	90	131 ± 11	137 ± 12	136 ± 17	129 ± 15	142 ± 16
	120	134 ± 17	130 ± 14	130 ± 15	125 ± 10	131 ± 14
	135	131 ± 17	124 ± 11	123 ± 10	111 ± 9*	120 ± 11
	150	139 ± 19	116 ± 8	116 ± 7	121 ± 11	119 ± 10
	180	138 ± 14	121 ± 10	114 ± 8	130 ± 16	116 ± 6
	210	133 ± 14	130 ± 12	118 ± 12	135 ± 11	119 ± 5
	240	135 ± 12	122 ± 10	122 ± 12	138 ± 11	121 ± 11

f_R (rpm)	0	19 ± 3	20 ± 3	18 ± 2	20 ± 3	18 ± 3
	5	20 ± 4^{a}	16 ± 3^{ab}	13 ± 4^{b}	15 ± 5^{ab}	11 ± 2^{*b}
	15	19 ± 4^{a}	16 ± 4^{ab}	11 ± 1^{*bc}	16 ± 5^{ab}	9 ± 1^{*c}
	30	19 ± 4^{a}	14 ± 3^{*b}	10 ± 2^{*bc}	14 ± 3^{*b}	8 ± 1^{*c}
	60	19 ± 2^{a}	12 ± 3^{*b}	10 ± 2^{*b}	11 ± 2^{*b}	9 ± 2^{*b}
	90	20 ± 1^{a}	11 ± 3^{*b}	10 ± 2^{*b}	12 ± 2^{*b}	8 ± 1^{*b}
	120	21 ± 2^{a}	10 ± 3^{*b}	9 ± 2^{*b}	10 ± 2^{*b}	9 ± 1^{*b}
	135	19 ± 1^{a}	12 ± 2^{*b}	9 ± 2^{*b}	10 ± 1^{*b}	9 ± 1^{*b}
	150	18 ± 2^{a}	12 ± 2^{*b}	10 ± 2^{*b}	12 ± 2^{*b}	10 ± 2^{*b}
	180	20 ± 2^{a}	15 ± 2^{*ab}	12 ± 3^{*b}	14 ± 3^{*b}	10 ± 2^{*b}
	210	19 ±2^{a}	16 ± 1^{ab}	13 ± 3^{b}	15 ± 5^{*ab}	11 ± 2^{*b}
	240	20 ± 2^{a}	16 ± 2^{ab}	14 ± 3^{b}	16 ± 2^{ab}	14 ± 3^{b}

*Significantly different from baseline (time 0) within a treatment ($p<0.05$).

^{abc} Different superscript letters indicate significant differences between treatments at that time point ($p<0.05$). Dotted line at T120 indicates the end of infusion.