TITLE: Sedative and antinociceptive effects of different combinations of detomidine and methadone in standing horses

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JOURNAL: Veterinary Anaesthesia and Analgesia

PUBLISHER: Elsevier

PUBLICATION DATE: 17 April 2017 (online)

DOI: 10.1016/j.vaa.2017.03.009

RESEARCH PAPER

Running head (author) *M Gozalo-Marcilla et al.*

Running head (Short title) Detomidine-methadone combinations in horses

Sedative and antinociceptive effects of different combinations of detomidine and methadone in standing horses

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Abstract

Objective To evaluate intravenous (IV) detomidine with methadone in horses to identify a combination which provides sedation and antinociception without adverse effects.

Study design Randomized, placebo-controlled, blinded, crossover.

Animals Eight adult healthy horses aged (mean ± standard deviation) 7 ± 2 years and 372 ± 27 kg.

Methods Six treatments were administered IV: saline (SAL); detomidine (5 µg kg^{-1}; DET); methadone (0.2 mg kg^{-1}; MET) alone or combined with detomidine [2.5 (MLD), 5 (MMD) or 10 (MHD) µg kg^{-1}]. Thermal, mechanical and electrical nociceptive thresholds (NT) were measured, and sedation, head height above ground (HHAG), cardiopulmonary variables and intestinal motility were evaluated at 5, 15, 30, 45, 60, 75, 90, 120, 180 minutes. Normal data were analyzed by mixed-model ANOVA and non-normal by Kruskal-Wallis ($p < 0.05$).

Results Nociceptive thresholds in horses administered methadone with the higher doses of detomidine (MMD, MHD) were increased above baseline to a greater degree and for longer duration (MMD: 15-30 minutes, MHD: 30-60 minutes) than in horses administered low dose with methadone or detomidine alone (MLD, DET: 5-15 minutes). No increases in NT were recorded in SAL or MET. Compared with baseline, HHAG was lower for 30 minutes in MMD and DET, and for 45 minutes in MHD. No significant sedation was observed in SAL, MET or MLD. Intestinal motility was reduced for 75 minutes in MHD and for 30 minutes in all other treatments.

Conclusions Methadone (0.2 mg kg^{-1}) potentiated the antinociception produced by detomidine (5 µg kg^{-1}), with minimal sedative effects.

Clinical relevance Detomidine (5 µg kg^{-1}) with methadone (0.2 mg kg^{-1}) produced antinociception without the adverse effects of higher doses of detomidine.

Keywords detomidine, electrical stimulus, equine, mechanical stimulus, methadone, thermal stimulus

Introduction

It is common practice to administer drug combinations for chemical restraint of standing horses, avoiding the potential complications and costs of general anaesthesia. The α_2-agonist detomidine has been extensively used to produce sedation and analgesia. However, adverse effects associated with detomidine administration, especially occurring after intravenous (IV) bolus administration, include ataxia, bradycardia, arrhythmias, increased systemic vascular resistance and reduction in cardiac output, respiratory rate (f_R), arterial oxygen tension and intestinal motility (Yamashita et al. 2000; Daunt & Steffey 2002; Valverde 2010).

Combinations of an α_2-agonist with an opioid provide synergistic analgesic effects with predictable sedation (Marly et al. 2014; Oliveira et al. 2014; Taylor et al. 2014; Lopes et al. 2016), while potentially reducing adverse effects. Opioids play an important role in analgesia in other species, however, their use in horses is controversial owing to the potential for excitement and intestinal hypomotility, especially when administered IV (Bennett & Steffey 2002; Clutton 2010; Schauvliege 2014). Methadone, a synthetic μ-agonist opioid with similar properties to morphine, also provides analgesia by antagonizing *N*-methyl-D-aspartate (NMDA) receptors and has delta opioid receptor activity (Pollock et al. 2011).

Methadone (0.5 mg kg^{-1}) administered IV alone increased spontaneous locomotor activity and impaired coordination in horses (Combie et al. 1979; Oliveira et al. 2014), and the duration of thermal antinociception was short at 30 minutes (Oliveira et al. 2014). Lower doses (0.2 mg kg^{-1}) administered IV have resulted in less adverse behavioral effect but provided insufficient antinociception (Pippi & Lumb 1979; Oliveira et al. 2014). More recent studies reported that methadone (0.2 mg kg^{-1}) did not produce antinociception when administered alone, but potentiated antinociception induced by detomidine (10 μg kg^{-1}) (Oliveira et al. 2014; Lopes et al. 2016). No adverse effects were reported after administration of methadone (0.15 mg kg^{-1}) IV, but antinociception was not reported (Linardi

et al. 2012). These studies suggest that further evaluation of lower doses of methadone in combination with detomidine in horses may be worthwhile.

Our hypothesis was that the combination of low (2.5 µg kg^{-1}) and medium (5 µg kg^{-1}) doses of detomidine with methadone (0.2 mg kg^{-1}) would result in sedation and analgesia with less adverse effects when compared with the administration of each drug alone. The aim of the study was to identify a combination resulting in antinociception with minimal adverse effects by evaluating the sedative, antinociceptive and potential side effects of some combinations of detomidine and methadone.

Materials and methods

The study was designed as a prospective, randomized, placebo-controlled, observer blinded crossover, with a washout period of ≥1 week between treatments. It was approved by the Institutional Ethical Committee for the Use of Animals in Research of the Faculty of Veterinary Medicine and Animal Science of the State of São Paulo University (UNESP), Botucatu (EC no. 08/2015).

Animals

Eight healthy adult cross-bred horses (four castrated males, four females), aged [mean ± standard deviation (SD) (range)] 7 ± 2 years (3-9 years), weighing 372 ± 27 kg (325-420 kg) were studied. The animals were kept at grass and brought to covered pens with outdoor access at least 24 hours before each experiment. Commercial horse feed, hay and *ad libitum* water were provided. The horses were considered healthy based upon physical examination and laboratory investigations performed 1-2 weeks before the study started. No sedatives or analgesics were administered for at least 1 month before the beginning of the study.

The sample size was estimated using OpenEpi Software (www.openepi.com) based on the results of a pilot study with three horses and from previous data (Oliveira et al. 2014; Lopes et al. 2016). The expected mean differences between the antinociceptive variables in the different treatments were assessed. A test power of 80% and a significance level of 5% were employed.

Study design

The horses were weighed on the day of each experiment and fly repellent was applied to the skin. The hair over both jugular veins was clipped for catheter placement. After skin disinfection, 1 mL of 2% lidocaine (Xylestesin 2% ; Cristália Produtos Químicos Farmacêuticos Ltda, SP, Brazil) was injected subcutaneously and two 14 gauge catheters were placed at each site, in the direction of the blood flow. The one in the right jugular vein was used for drug administration (14 gauge, 48 mm BD Angiocath; Becton Dickinson Ind., MG, Brazil) and in the left (14 gauge, 70 mm, Delta Med Srl, Italy) for blood sampling for pharmacokinetic analysis (results reported elsewhere). Hair was clipped over the dorsal aspects of both metacarpi for the thermal and mechanical devices, and immediately proximal to the coronary band of the left thoracic limb for the electrical electrodes. To obtain a good contact for the electrodes and to reduce resistance, the clipped coronary skin was washed with water and degreased with chlorhexidine containing surfactants (RIOHEX 2% ; Industria Farmaceutica Rioquimica Ltda, SP, Brazil). This process was repeated twice.

After that, the horse was brought to the experimental room (6 m^2, without windows), previously sprayed with fly repellent, and allowed 30 minutes for familiarization. A blood pressure cuff [DURA-CUF CRITIKON (12–19 and 17–25 cm); GE Healthcare, Finland] was placed at the base of the tail, to measure heart rate (HR) and systolic arterial blood pressure (SAP) with a noninvasive Doppler (Model 812; Parks Medical Electronics, Inc., OR, USA).

The prepared area at the left coronary band was twice cleaned with alcohol (Álcool 96; Farmácia Santa Cruz, SP, Brazil) and dried before attaching two adhesive electrodes (2223BRQ; 3M do Brasil Ltda, SP, Brazil) 8 cm apart, secured with four adhesive wrap strips around the hoof. The thermal (WTT2; Topcat Metrology Ltd, UK) and mechanical (WMT1; Topcat Metrology Ltd) control units (Topcat Metrology Ltd) were attached with Velcro onto a commercial blanket (Topcat Metrology Ltd) on the horse's back. The units were remotely controlled by infrared signals. Temperature and force were recorded in degrees Celsius (°C) and newtons (N), respectively. Finally, the thermal sensor on the right clipped metacarpus and mechanical pin on the left were placed and connected to the control units.

Six treatments were randomly assigned using Microsoft Excel: saline (Cloreto de sódio 0.9%; Fresenius Kabi, SP, Brazil; treatment SAL), detomidine (5 µg kg^{-1}; Eqdomin, 10 mg mL^{-1}; Ourofino Saúde Animal, SP, Brazil; treatment DET), methadone (0.2 mg kg^{-1}; Mytedom 10 mg mL^{-1}, Cristália Produtos Químicos Farmacêuticos Ltda, SP, Brazil; treatment MET), and methadone at the same dose combined with detomidine (2.5 µg kg^{-1}; treatment MLD), detomidine (5 µg kg^{-1}; treatment MMD) and detomidine (10 µg kg^{-1}; treatment MHD). The final volume was adjusted to 10 mL by adding saline and administered IV by hand at T0 over 10 seconds.

Sedation and responses to antinociceptive stimuli were evaluated in triplicate at baseline (T0) before and at 5, 15, 30, 45, 60, 75, 90, 120 and 180 minutes after drug administration. Intestinal motility was scored at the same time points starting at 15 minutes.

Degree and quality of sedation

Sedation was evaluated using the height of the head above the ground (HHAG) by measuring the level of the lower lip against a scale on the wall (Ringer et al. 2012; Marly et al. 2014). Quality of sedation was scored first by evaluating the degree of ataxia and second by

responses to tactile and audiovisual stimulation, always in the same order. The scoring system was a numerical rating scale (NRS) ranging from 0 to 3 for ataxia (0 no ataxia, 3 maximal ataxia) and responses to audiovisual stimuli (0 no response, 3 maximal response) (Appendix A). A subjective visual analogue scale (VAS) was assigned consisting of a 10 cm line where 0 cm represented no sedation or ataxia and 10 cm represented maximal sedation and/or maximal ataxia. All the sedation variables were always evaluated by the main investigator (MGM) unaware of the treatment.

Cardiopulmonary variables

SAP was corrected according to the height difference between the cuff and shoulder joint, the latter location representing the level of the right atrium of the heart. The f_R was measured by observation of chest movements over 30 seconds.

Nociceptive threshold testing

Nociceptive stimuli were applied at each time point immediately after sedation was scored and cardiopulmonary variables had been recorded. Stimuli were always applied in the same order; first electrical, followed by thermal and mechanical stimulation. Aversive responses were recorded when the horse lifted its foot, pawed the ground, stamped, flexed the limb or walked to avoid the stimulus (Luna et al. 2015). The stimulus was immediately withdrawn at this end point and the value in volts (V), °C and N at threshold recorded. If cut-out was reached, this value was recorded as the threshold.

Electrical threshold testing

The resistance between electrodes was measured using a digital multimeter (ET 1100 ; Minipa do Brasil Ltda, SP, Brazil) to confirm it was below 3 kΩ. The electrodes were

connected to an electrical stimulator (Grass S-48; Astro-Med, Inc., RI, USA) adjusted to deliver pulsatile current square waves of 10 ms at 10 Hz. The voltage started at 1 V, then was increased by 1 V every 5 seconds. Stimulation was stopped immediately when an avoidance response was observed or the voltage reached 20 V.

Thermal threshold testing

A thermal probe with a heating element encased in an 8 mm long brass tube (internal diameter 2.4 mm and external diameter 3.2 mm) (probe 3; Dixon et al. 2016) was placed on the clipped area of the right metacarpus and attached with an elasticated band secured by Velcro. The probe includes a heater and a temperature sensor and is connected to the thermal control unit. At least 5 minutes were allowed for equilibration with body temperature. After recording skin temperature, the ramped stimulus was applied, heating at 0.8 °C second^{-1} until a positive response was observed or the cut-out at 60 °C was reached (Luna et al. 2015; Lopes et al. 2016). After each stimulus, the probe was moved 1-2 cm proximally on the limb to avoid focal tissue damage.

Mechanical threshold testing

A pneumatic actuator containing a 1 mm round-ended pin was secured with a brushing boot on the left clipped metacarpal area, held against the leg with an elasticated band secured with Velcro and connected to the control unit with non-distensible tubing (Taylor et al. 2016). The automatic control system increased the force of the pin pressing on the skin surface at 0.8 N second^{-1}. The stimulus was stopped when an aversive response was observed or the cut-out value of 20 N reached.

Abdominal auscultation

Intestinal sounds were assessed by auscultation over one minute in each of the four abdominal quadrants: right dorsal, right ventral, left dorsal and left ventral. The total motility score was the sum of the scores from each quadrant (Boscan et al. 2006) (Appendix B).

Statistical analysis

For each variable, normality was assessed by the Shapiro-Wilk test and graphical analysis. Effects of time and treatment were analyzed with a mixed-model analysis of variance (ANOVA) followed by Tukey´s test for HHAG, cardiopulmonary variables, thermal stimulus and intestinal motility (mean ± SD). Data from electrical and mechanical stimuli were not normally distributed. Thus, logarithmic transformation was performed, and these data are presented as geometric mean (back-transformed bounds of the 95% confidence interval). Different covariance structures were tested. Data for ataxia, responses to tactile and audiovisual stimuli and VAS, non-normally distributed, were analyzed by Kruskal-Wallis with Dunn´s tests and are presented as median (range). Significance level was set at 0.05.

Results

The HHAG was lower than baseline (sedation was greater) after DET (66 ± 26 *versus* 98 ± 10 cm) and MMD (77 ± 19 *versus* 99 ± 3 cm) for 30 minutes and after MHD (68 ± 26 *versus* 99 ± 9 cm) for 45 minutes (Fig. 1). Comparing treatments, HHAG with DET was lower than SAL, MET and MLD for 30 minutes. The HHAG with MMD was lower than SAL and MLD for 15 minutes and MET for 30 minutes, respectively. The HHAG with MHD was lower than SAL, MET, MLD for 45 minutes and between 15 and 45 compared with MMD. There were no differences between DET and MHD.

Visual analogue scores were significantly higher than baseline for 30 minutes only in treatments DET, MMD and MHD (Table 1). Comparing treatments, MHD scores were not

different from MMD or DET at any time point, but were higher than MLD for 45 minutes, and higher than SAL and MET for 60 minutes (Table 1). Scores in MMD were not different from DET or MLD at any time point, but were higher than SAL and MET for 30 minutes.

The ataxia scores were higher than baseline [0 (0 – 0)] with DET [1 (0 – 1)], MMD [1 (0 – 2)] and MHD [1 (0 – 3)] up to 15 minutes ($p < 0.05$). Comparing treatments, ataxia was more pronounced with MHD [1 (0 – 3)] than with SAL [0 (0 – 0)] and MET [0 (0 – 0)] for 30 minutes ($p < 0.05$). Ataxia scores with MHD [2 (1 – 3)] were also higher than MLD [1 (0 – 3)] at 5 minutes ($p < 0.05$).

Scores for responses to tactile stimuli were lower than baseline [3 (2 – 3)] with MMD [1.5 (0 – 2)] for 5 minutes and up to 30 minutes in MHD [1 (0 – 2)] ($p < 0.05$). Comparing treatments, scores were lower with MHD [2 (0 – 3)] than SAL [3 (3 – 3)] and MET [3 (3 – 3)] up to 45 minutes, lower than MLD at 15 [[0 (0 – 1)] *versus* [3 (1 – 3)]] and at 30 minutes [[1 (0 – 2)] *versus* [3 (1 – 3)] and lower than DET at 15 [[0 (0 – 1)] *versus* [2.5 (1 – 3)]] and 45 minutes [[2 (0 – 3)] *versus* [3 (3 – 3)]] ($p < 0.05$). Scores were also lower with MMD [1.5 (0 – 2)] than SAL [3 (3 – 3)] and MET [3 (2 – 3)] at 5 minutes ($p < 0.05$).

Scores of responses to audiovisual stimuli were lower than baseline [1 (1 – 2)] for 15 minutes in MHD [0 (0 – 1)] ($p < 0.05$). Comparing treatments, scores were lower with MHD [0 (0 – 1)] than SAL [1 (1 – 2)] and MET [1 (1 – 2)] for up to 15 minutes and lower than MLD at 5 minutes [0 (0 – 0)] *versus* [1 (1 – 2)] ($p < 0.05$).

HR were not significantly changed from baseline within all treatments (Table 2). Statistically significant changes in SAP and f_R were recorded in some treatments (Table 2).

Electrical thresholds in treatments MHD, MMD, MLD and DET were significantly higher than baseline for 30, 15, 5 and 15 minutes, respectively (Table 3). Comparing treatments, MHD thresholds were higher than MMD at 15 and 30 minutes only, but were higher than DET, MLD and SAL for 30 minutes and MET for 45 minutes. After MMD,

thresholds were higher than MET and SAL only for 15 minutes. Thresholds at, and after 60 minutes were not different from baseline and between treatments.

Thermal thresholds in treatments MHD, MMD, MLD and DET were significantly higher than baseline for 45, 30, 15 and 15 minutes, respectively (Table 3). Comparing treatments, MHD thresholds were higher than MMD only at 30 minutes, higher than MLD and DET from 15-45 minutes and higher than SAL and MET at all time points up to 45 minutes. Thermal thresholds in MMD were higher than DET only at 30 minutes, and higher than SAL and MET for 30 minutes. Thresholds in MLD and DET were higher than MET for 5 minutes and SAL for 15 minutes. Thresholds at, and after 60 minutes were not different from baseline and between treatments.

Mechanical thresholds in MHD, MMD, MLD and DET were significantly higher than baseline for 60, 30, 15 and 5 minutes, respectively (Table 3). Comparing treatments, MHD thresholds were higher than MLD only at 30 minutes, higher than DET at 15-60 minutes, higher than MET for 45 minutes, and higher than SAL for up to 60 minutes. Mechanical thresholds in MMD were higher than DET only at 15 minutes, and higher than MET and SAL for 15 minutes. Thresholds at, and after 75 minutes were not different from baseline and between treatments.

Intestinal motility was below baseline in MET, DET, MLD and MMD for 30 minutes and up to 75 minutes in MHD (Fig. 2). Comparing treatments, MET, DET, MMD and MLD were lower than SAL for 30 minutes, and MHD was lower for 60 minutes. MHD was lower than MET and MMD for 30 minutes and lower than MLD and DET for 45 minutes.

Methadone alone (MET) resulted in mild behavioral effects in the first 5-15 minutes: two horses were restless, two flicked their lower lips, one made minor attempts to move and one was sedated. No adverse effects were observed in the remaining two horses. No abnormal

behavioral effects were observed after any other treatments except in MLD where one horse flicked the lower lips and one briefly became very alert to the environment.

One horse became deeply sedated in response to both drugs, either alone or in combination. In this horse only, some superficial skin lesions became evident a few hours after application of thermal stimuli in the third treatment, when thresholds close to the cut-out temperature were reached. The lesions started as edematous wheals mimicking the footprint of the probe. The outer surface of the skin was lost after two to three days, followed subsequently by corneal flaking which resolved in 5 days. The lesions were initially slightly painful to touch and were treated with wet cold towels, dried and followed by an ointment containing methyl salicylate, Peru balsam (exudation of *Myroxilon peruiferum*) and zinc oxide (Balsamex; Chemitec Agro-Veterinária Ltda, SP, Brazil). These injured local sites were not used again for further thermal stimulation for the remaining treatments.

Discussion

Detomidine (10 µg kg^{-1}) with methadone produced the most intense and prolonged antinociception. When the detomidine dose was reduced to 5 µg kg^{-1}, antinociception was of similar intensity but of shorter duration, the horses were less sedated and intestinal motility better preserved. This observation confirms that methadone potentiates detomidine-induced antinociception, not only at the high detomidine dose of 10 µg kg^{-1} (Oliveira et al. 2014; Lopes et al. 2016), but also at 5 µg kg^{-1}, while sedative effects are minimized. Moreover, the lowest dose of detomidine (2.5 µg kg^{-1}) combined with methadone produced antinociception similar to that after detomidine alone (5 µg kg^{-1}), yet without producing measurable sedation. No conclusion as to potentiation can be drawn regarding this combination as treatment with detomidine alone at 2.5 µg kg^{-1} was not studied.

Nociceptive electrical, thermal and mechanical stimulation on the thoracic limbs is easy to apply and interpret, is reliable, sensitive and specific, and has been validated in horses (Luna et al. 2015). Electrical stimuli activate not only the nociceptive Aδ and C fibres, but also large diameter Aβ fibres not directly involved in antinociception (Le Bars et al. 2001). Thermal stimulation with a slow heating rate of 0.8 °C second^{-1} predominantly activates C fibres (Yeomans & Proudfit 1996; Lopes et al. 2016) and mechanical stimuli activate both nociceptive Aδ and C fibres (Le Bars et al. 2001). Since opioids and α_2-agonists have different mechanisms of action, the use of a variety of nociceptive stimuli improves sensitivity and specificity and therefore produces more accurate data (Luna et al. 2015; Lopes et al. 2016). All three nociceptive testing modalities were sufficiently sensitive to detect different degrees of antinociception between treatments, and, in general, followed a similar trend.

The equipment employed for application of nociceptive stimuli was the same as previously reported (Luna et al. 2015; Lopes et al. 2016). Additional features were included for the electrical stimulus: before application, a strict protocol of clipping, washing, degreasing and cleaning was followed. Since current intensity is equal to voltage divided by resistance (Ohm´s law), increases in skin resistance reduce intensity. Studies where resistance was not measured showed heterogeneous, less accurate data (Lopes et al. 2016). Thus, proper control of resistance is mandatory, not only by preparing the area and maintaining the same distance between electrodes (7-8 cm on the coronary band), but also by keeping resistance values below 3 kΩ (Hopster et al. 2014; Risberg et al. 2014).

The drug doses used in this study were chosen to identify a combination that would produce adequate antinociception with minimal adverse effects. Detomidine (10-20 µg kg^{-1}) and methadone (0.1-0.2 mg kg^{-1}) are commonly used clinical doses. In the present study, methadone alone (0.2 mg kg^{-1}) administered IV resulted in only transitory, minor behavioral

effects and no antinociception. Similar results were reported by Oliveira et al. (2014), where mild ataxia and head shaking were observed for 30 minutes, without consistent antinociception. However, the addition of methadone (0.2 mg kg^{-1}) to detomidine (5 µg kg^{-1}) as described here, and to 10 µg kg^{-1} as reported by Oliveira et al. (2014) and Lopes et al. (2016), prolonged the detomidine-induced antinociception and abated the methadone-induced behavioral effects. Combination with detomidine (2.5 µg kg^{-1}) also appeared to reduce the adverse effects of methadone, resulting in minimal adverse reactions in only two of the horses.

Antinociception was observed in all treatments that included detomidine. In general, thresholds followed a similar pattern in the two treatments DET and MLD and in MMD and MHD, with longer durations of antinociception when methadone was combined with 5 or 10 µg kg^{-1} detomidine. The results indicate that the combination of methadone (0.2 mg kg^{-1}) with detomidine (5 µg kg^{-1}) could provide useful antinociceptive effects for some clinical procedures lasting around 30 minutes.

Excessively deep sedation and severe ataxia may be considered adverse effects under clinical conditions. In the study presented here, a reduction in the detomidine dose from 10 in MHD to 5 µg kg^{-1} in MMD resulted in a similar degree of antinociception, but less sedation (HHAG). Sedation in DET (5 µg kg^{-1}) was comparable to MHD, but of shorter duration. The lowest detomidine dose combined with methadone (MLD) produced similar antinociception to detomidine alone (DET), without apparent sedation. The HHAG variable has been used to measure the degree of sedation induced by administration of α_2-agonists. Previous authors have defined ‘sufficient’ sedation as when the horse’s head position is equal to or lower than 50% of the awake position (Ringer et al. 2012; Marly et al. 2014). Using this definition, adequate sedation was achieved only at 15 minutes after detomidine alone and for 30 minutes in MHD, whereas sufficient sedation was not achieved with the other treatments. However,

the term 'sufficient' should be used carefully when an opioid is added to low doses of α_2-agonists, as opioid-linked behavioral effects may overcome the sedative effects of the α_2-agonist, depending on the relative dose ratio of the drugs.

Contrary to HHAG, VAS was similar in DET, MHD and MMD for the first 30 minutes. Moreover, the VAS detected more differences in sedation quality than the NRS used to score ataxia and responses to tactile and audiovisual stimuli. As two of these three tactile stimuli were performed on the limbs, it might be expected that methadone would increase locomotor activity (Oliveira et al. 2014) and, potentially increase the bias of these responses. However, the significant ataxia seen in treatments with detomidine at doses equal to or higher than 5 µg kg^{-1} may have reduced the responses to the tactile stimuli..

Throughout the experiments, the values for HR, SAP and f_R remained within clinically acceptable ranges. As reported by Wagner et al. (1991) with a detomidine dose of 10 µg kg^{-1}, initial increases in arterial pressure, followed by a decrease, was observed only with the combinations including the highest dose of detomidine. Reducing this dose limited the biphasic effect on arterial pressure. f_R decreased below baseline for 60 minutes only in MHD, but this was not statistically different from the other treatments. It is possible that detomidine and its combination with methadone may cause respiratory depression, especially at the highest dose, but arterial blood gases were not measured in this study.

Invasive arterial pressure measurements would have produced more accurate measurements. However, a noninvasive method was used because evaluation of the cardiopulmonary effects of the treatments was not the main aim of the study, and arterial catheterization may have stimulated the horses and affected subsequent sedation and antinociception.

Duration and intensity of intestinal hypomotility was detomidine dose-dependent. The reduction in intestinal motility and its possible consequences are well reported after both α_2-

agonists (Daunt & Steffey 2002; Valverde 2010) and opioids (Bennett & Steffey 2002; Boscan et al. 2006; Clutton 2010; Schauvliege 2014).

One horse developed small lesions at the site of thermal stimulation. Development of skin lesions was unexpected as the cut-out was set at 60 °C, based on previous similar studies, where only occasional superficial, local lesions were seen, usually at a thoracic site and not on the limbs (Luna et al. 2015). A 60 °C cut-out may not be suitable for all conditions as some individuals may be more sensitive to thermal stimulation. A lower cut-out such as 55 °C might be appropriate for different environments, sites and breeds to avoid tissue damage. This horse became deeply sedated after all treatments, which may have influenced the results. However, its exclusion would not be justified as this horse could represent a real effect seen in a certain proportion of the population. It is also possible that allodynia developed after the thermal injury in this horse, changing responses in subsequent experiments. Nonetheless, separate statistical analysis performed without this horse´s data did not significantly modify the nociceptive results.

Conclusions

Methadone (0.2 mg kg^{-1}) potentiated the antinociception produced by detomidine at 5 µg kg^{-1}. However, side effects such as deep sedation, ataxia and prolonged intestinal hypomotiliy were diminished when methadone was combined with 5 µg kg^{-1} detomidine when compared with the combination with 10 µg kg^{-1} detomidine. Further studies are justified to evaluate the value of this combination under clinical conditions.

Acknowledgements

This work was supported by Fundação de Amparo à Pesquisa de São Paulo (FAPESP), Scholarships grant numbers 2010/08967-0 and 2014/00474-5.

The authors thank the pharmaceutical company Ourofino for donating the injectable form of detomidine. The authors also thank Altamiro Rosam for the special care and dedication with the horses used in this study and to Evaldo Mamedes Cândido de Queiroz for his unvaluable assistance, both at the São Paulo State University (UNESP), Botucatu, SP, Brazil.

Authors' contributions

MGM: study design, acquisition, management and interpretation of data, writing and critical review of the manuscript. SPLL: study design, interpretation of data, review of the manuscript. NC: study design, data acquisition, review of the manuscript. JNPF: data acquisition. FSP: statistical analysis and interpretation of data. LP: study design, management and interpretation of data, review of the manuscript. PMT: study design, interpretation of data, review of the manuscript.

Conflict of interest

PM Taylor is director of Topcat Metrology Ltd.

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Appendix A Numerical rating scale (NRS) to assess ataxia and responses to tactile and audiovisual stimulation. Adapted from Bryant et al. (1991) and Ringer et al. (2013).

	Degree of ataxia	**Tactile stimuli**	**Audiovisual stimuli**
	Evaluated visually and by pushing the horse to detect any swaying	Touching an ear with a pen, then pressing the coronary band of thoracic and pelvic limbs with the pen	Acoustic stimulus was hand clapping behind the horse and visual stimulus was shaking a towel in front of the horse
NRS			
0	No ataxia	No response, even with strong pressing	No response, no signs of noise recognition or visual arousal
1	Stable but swaying slightly	Mild response, slightly diminished response to normal or strong pressing	Mild response, minimal movement of ears and elevates head slightly
2	Unstable, swaying markedly	Intermediate response, animal elevates the limb after normal pressing	Intermediate response, subdued reactions and movements, turning slowly

3	Severe ataxia, risk of falling down	Fast response, movement of the limb after touching, with mild pressing	Fast response, animal abruptly turns or moves away

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Appendix B Scoring of intestinal sounds (Boscan et al. 2006).

Score	Criteria
0	No intestinal sounds auscultated
1	Low-pitched crepitant sounds, frequency ≤ 1 minute^{-1}
2	Low-pitched crepitant sounds, frequency > 1 minute^{-1}
3	Long, loud, gurgling sounds, frequency 1 minute^{-1}
4	Long, loud, gurgling sounds, frequency 2 to 4 minute^{-1}
5	Long, loud, gurgling sounds, frequency > 4 minute^{-1}

491 **Table 1** Sedation quality evaluated by visual analogue scale (VAS) [median (range)]. 0 indicates no sedation and no ataxia and 10 indicates maximum
492 sedation and ataxia, in eight standing horses after six treatments administered intravenously: 10 mL saline (SAL), 5 µg kg^{-1} detomidine (DET), 0.2 mg
493 kg^{-1} methadone alone (MET), or combined with 10 (MHD), 5 (MMD) or 2.5 (MLD) µg kg^{-1} detomidine before (time 0) and after (times 5-180
494 minutes) drug administration.

Time	**Treatment**					
(minutes)	**SAL**	**MET**	**DET**	**MLD**	**MMD**	**MHD**
0	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)
5	0 (0 - 0)^{a}	0 (0 - 1.3)^{a}	5.3 (2.1 - 6.4)^{*abc}	0 (0 - 8.7)^{ab}	6.3 (4.9 - 8.8)^{*bc}	7.8 (6.4 -9.9)^{*c}
15	0 (0 - 0)^{a}	0 (0 - 0.9)^{ab}	6.2 (2.9 - 7.5)^{*bcd}	0 (0 - 8.9)^{abc}	6.2 (4.0 - 8.1)^{*cd}	9.1 (6.1 - 9.6)^{*d}
30	0 (0 - 0)^{a}	0 (0 - 0)^{a}	4.6 (0 - 6.4)^{*abc}	0 (0 - 7.1)^{ab}	3.3 (1.4 - 7.9)^{*bc}	6.7 (3.9 - 8.6)^{*c}
45	0 (0 - 0)^{a}	0 (0 - 0)^{a}	1.0 (0 - 4.5)^{ab}	0 (0 - 4.5)^{a}	0.5 (0 - 6.2)^{ab}	3.4 (1.3 - 7.8)^{b}
60	0 (0 - 0)^{a}	0 (0 - 0)^{a}	0 (0 - 1.4)^{ab}	0 (0 - 1.7)^{ab}	0 (0 - 2.2)^{ab}	1.4 (0 - 5.2)^{b}
75	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 3)
90	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 2.1)
120	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)

180	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)

*Significantly different from baseline (time 0) ($p < 0.05$). [abc]Different superscript letters indicate significant differences among treatments at that time point ($p < 0.05$).

500 **Table 2** Heart rate (HR), systolic arterial blood pressure (SAP) and respiratory rate (f_R) (mean ± standard deviation) in eight standing horses
501 administered intravenous saline (SAL), 5 µg kg^{-1} detomidine (DET), 0.2 mg kg^{-1} methadone alone (MET), or combined with 10 (MHD), 5 (MMD) or
502 2.5 (MLD) µg kg^{-1} detomidine.

		Treatments					
Variable	**Time (minutes)**	**SAL**	**MET**	**DET**	**MLD**	**MMD**	**MHD**
HR (beats minute^{-1})	**0**	37 ± 11^{a}	32 ± 4^{b}	34 ± 6^{bc}	36 ± 6^{a}	33 ± 5^{ac}	35 ± 5^{b}
	5	37 ± 14^{a}	33 ± 5^{b}	30 ± 5^{bc}	36 ± 10^{a}	33 ± 8^{ac}	29 ± 5^{b}
	15	37 ± 12^{a}	35 ± 3^{b}	34 ± 10^{bc}	36 ± 9^{a}	35 ± 8^{ac}	31 ± 5^{b}
	30	38 ± 13^{a}	31 ± 4^{b}	34 ± 8^{bc}	38 ± 11^{a}	35 ± 7^{ac}	30 ± 5^{b}
	45	35 ± 12^{a}	31 ± 3^{b}	35 ± 7^{bc}	38 ± 12^{a}	35 ± 7^{ac}	31 ± 4^{b}
	60	37 ± 12^{a}	33 ± 4^{b}	33 ± 6^{bc}	34 ± 6^{a}	35 ± 9^{ac}	32 ± 4^{b}
	75	37 ± 13^{a}	33 ± 3^{b}	34 ± 7^{bc}	37 ± 11^{a}	37 ± 9^{ac}	32 ± 5^{b}
	90	38 ± 13^{a}	32 ± 4^{b}	32 ± 6^{bc}	36 ± 6^{a}	40 ± 8^{ac}	33 ± 4^{b}
	120	37 ± 12^{a}	31 ± 3^{b}	36 ± 8^{bc}	37 ± 10^{a}	35 ± 7^{ac}	33 ± 5^{b}

	180	37 ± 12[a]	32 ± 3[b]	33 ± 6[bc]	38 ± 12[a]	37 ± 8[ac]	33 ± 4[b]
SAP (mmHg)	**0**	139 ± 15	161 ± 11	143 ± 12	122 ± 9	125 ± 6	127 ± 6
	5	133 ± 9[a]	143 ± 13[a]	149 ± 16[a]	148 ± 11[a]	148 ± 8[a]	193 ± 13[*b]
	15	153 ± 22[ab]	156 ± 22[a]	110 ± 6[*c]	137 ± 12[abc]	127 ± 11[bc]	138 ± 7[ab]
	30	154 ± 11[ab]	165 ± 5[a]	149 ± 7[ab]	131 ± 10[b]	141 ± 15[ab]	134 ± 13[b]
	45	132 ± 12	130 ± 4[*]	141 ± 6	143 ± 8	144 ± 7	138 ± 4
	60	168 ± 10[*a]	136 ± 29[bc]	151 ± 41[ab]	137 ± 5[bc]	114 ± 5[c]	143 ± 17[ab]
	75	134 ± 6[a]	131 ± 4[*b]	143 ± 25[ab]	126 ± 21[a]	143 ± 11[ab]	168 ± 9[*b]
	90	141 ± 23	145 ± 13	126 ± 6	137 ± 12	140 ± 12	132 ± 7
	120	141 ± 13	127 ± 11[*]	133 ± 25	127 ± 8	124 ± 9	138 ± 21
	180	127 ± 6[ab]	126 ± 4[ab]	116 ± 9[a]	149 ± 7[b]	139 ± 7[ab]	123 ± 8[ab]
f_R							
(breaths minute^{-1})	**0**	16 ± 4	16 ± 4	16 ± 4	15 ± 3	15 ± 2	17 ± 5
	5	16 ± 4[a]	16 ± 6[ab]	10 ± 2[ab]	12 ± 6[ab]	10 ± 3[ab]	8 ± 2[*b]
	15	16 ± 3[a]	17 ± 7[b]	10 ± 2[ab]	13 ± 6[ab]	9 ± 2[ab]	8 ± 1[*b]
	30	16 ± 4[ab]	19 ± 6[a]	10 ± 2[bc]	13 ± 5[abc]	9 ± 2[bc]	8 ±1[*c]

45	$17 \pm 4^{\mathbf{ab}}$	$18 \pm 6^{\mathbf{a}}$	$11 \pm 2^{\mathbf{bc}}$	$14 \pm 6^{\mathbf{abc}}$	$11 \pm 3^{\mathbf{bc}}$	$10 \pm 2^{\mathbf{*c}}$
60	$17 \pm 4^{\mathbf{ab}}$	$19 \pm 7^{\mathbf{a}}$	$11 \pm 2^{\mathbf{b}}$	$14 \pm 6^{\mathbf{ab}}$	$12 \pm 2^{\mathbf{b}}$	$10 \pm 2^{\mathbf{*b}}$
75	17 ± 5	18 ± 7	12 ± 2	15 ± 4	12 ± 3	11 ± 1
90	17 ± 4	18 ± 6	13 ± 2	15 ± 5	14 ± 2	12 ± 2
120	18 ± 4	18 ± 5	14 ± 3	15 ± 3	14 ± 2	12 ± 2
180	18 ± 4	18 ± 5	14 ± 2	15 ± 3	15 ± 2	13 ± 2

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

504 [abc]Different superscript letters indicate significant differences among treatments at that time point ($p < 0.05$).

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Table 3 Electrical (upper safety limit 20 V), thermal (upper safety limit 60 °C) and mechanical (upper safety limit 20 N) thresholds in eight standing horses treated intravenously with 10 mL saline (SAL), 5 µg kg^{-1} detomidine (DET), 0.2 mg kg^{-1} methadone (MET), or methadone combined with 10 (MHD), 5 (MMD) or 2.5 (MLD) µg kg^{-1} detomidine. Thermal threshold data are mean ± standard deviation; electrical and mechanical threshold data are geometric means (back-transformed bounds of the 95% confidence interval).

Stimulus	Time (minutes)	Treatment					
		SAL	MET	DET	MLD	MMD	MHD
Electrical (V)	**0**	1.7 (1.5, 2.1)	1.6 (1.3, 1.8)	1.4 (1.2, 1.7)	1.7 (1.4, 2.0)	1.7 (1.4, 2.0)	1.8 (1.5, 2.2)
	5	1.7 (1.4, 2.1)^{a}	1.7 (1.3, 2.2)^{a}	2.9 (2.0, 4.1)^{*ab}	3.0 (1.5, 6.1)^{*ab}	4.7 (3.4, 6.6)^{*bc}	6.1 (3.4, 10.9)^{*c}
	15	1.9 (1.6, 2.3)^{a}	1.9 (1.5, 2.4)^{a}	3.1 (2.2, 4.4)^{*ab}	2.8 (1.8, 4.5)^{ab}	4.0 (2.7, 5.9)^{*b}	8.4 (6.0, 11.7)^{*c}
	30	1.9 (1.6, 2.3)^{a}	1.7 (1.4, 2.0)^{a}	1.8 (1.5, 2.2)^{a}	2.4 (1.6, 3.6)^{a}	2.9 (1.9, 4.5)^{a}	6.2 (3.6, 10.8)^{*b}
	45	1.7 (1.3, 2.2)^{ab}	1.7 (1.4, 2.0)^{a}	1.8 (1.4, 2.3)^{ab}	2.0 (1.4, 2.9)^{ab}	2.3 (1.6, 3.2)^{ab}	3.0 (2.7, 3.4)^{b}
Thermal (°C)	**0**	44 ± 4	44 ± 3	44 ± 3	44 ± 3	44 ± 4	44 ± 2
	5	44 ± 5^{a}	44 ± 3^{a}	54 ± 5^{*b}	53 ± 6^{*b}	59 ± 2^{*b}	60 ± 0^{*b}
	15	43 ± 3^{a}	47 ± 6^{ab}	51 ± 5^{*bc}	52 ± 7^{*bc}	56 ± 5^{*cd}	60 ± 0^{*d}

	30	44 ± 4[a]	44 ± 5[a]	45 ± 2[a]	49 ± 5[ab]	52 ± 6[*b]	60 ± 1[*c]
	45	42 ± 4[a]	44 ± 4[a]	45 ± 3[a]	45 ± 3[a]	49 ± 5[ab]	55 ± 5[*b]
Mechanical							
(N)	**0**	1.1 (0.6, 2.2)	0.9 (0.6, 1.3)	0.9 (0.6, 1.2)	0.8 (0.6, 1.2)	1.0 (0.6, 1.7)	0.9 (0.5, 1.5)
	5	1.8 (0.9, 3.5)[a]	1.5 (0.5, 4.0)[a]	4.4 (2.1, 9.5)[*ab]	4.7 (1.8, 12.0)[*ab]	17.4 (12.9, 20.0†)[*b]	20.0 (20.0, 20.0)[*b]
	15	1.3 (0.7, 2.6)[a]	1.6 (0.6, 4.0)[a]	2.5 (0.9, 6.7)[a]	4.8 (2.0, 11.4)[*ab]	15.1 (9.9, 20.0†)[*b]	20.0 (20.0, 20.0)[*b]
	30	1.5 (0.8, 2.9)[a]	1.7 (0.7, 4.0)[a]	2.4 (1.4, 4.0)[a]	3.1 (1.2, 7.9)[a]	6.5 (2.0, 20.0†)[*ab]	19.2 (17.4, 20.0†)[*b]
	45	1.5 (0.8, 2.7)[a]	1.5 (0.6, 3.4)[a]	1.4 (0.9, 2.2)[a]	3.2 (1.2, 8.1)[ab]	3.8 (1.6, 8.7)[ab]	13.1 (8.7, 19.7)[*b]
	60	1.3 (0.8, 2.2)[a]	1.7 (0.9, 3.2)[ab]	1.2 (0.7, 2.1)[a]	1.5 (0.8, 2.8)[ab]	1.9 (0.8, 4.5)[ab]	6.4 (3.2, 12.6)[*b]

*Significantly increased above baseline (time 0) ($p < 0.05$). Different lower case letters at each time point for each variable indicate significant differences among treatments ($p < 0.05$).

†Indicates that the upper limit of the confidence interval was higher than 20, although mechanical thresholds were censored at 20N.

Figure legends

Figure 1 Height of head above the ground (HHAG) in eight standing horses administered intravenously saline (SAL), 5 µg kg^{-1} detomidine (DET), 0.2 mg kg^{-1} methadone alone (MET), or combined with 10 (MHD), 5 (MMD) or 2.5 (MLD) µg kg^{-1} detomidine. Data are mean ± standard deviation.

*Significantly lower from time 0 and lower than MET ($p < 0.05$).

†Significantly lower from time 0 and lower than SAL, MET and MLD ($p < 0.05$).

‡Significantly lower from time 0 and lower than SAL, MET, MLD and MMD ($p < 0.05$).

Figure 2 Intestinal motility scores (mean ± standard deviation) in eight standing horses administered intravenously saline (SAL), 5 µg kg^{-1} detomidine (DET), 0.2 mg kg^{-1} methadone alone (MET), or combined with 10 (MHD), 5 (MMD) or 2.5 (MLD) µg kg^{-1} detomidine.

*Significantly lower from time 0 ($p < 0.05$). †Significantly higher than all other treatments ($p < 0.05$). ‡Significantly higher than MHD ($p < 0.05$). §Significantly lower than MET, DET, MLD, MMD ($p < 0.05$). ¶Significantly lower than DET and MLD ($p < 0.05$).