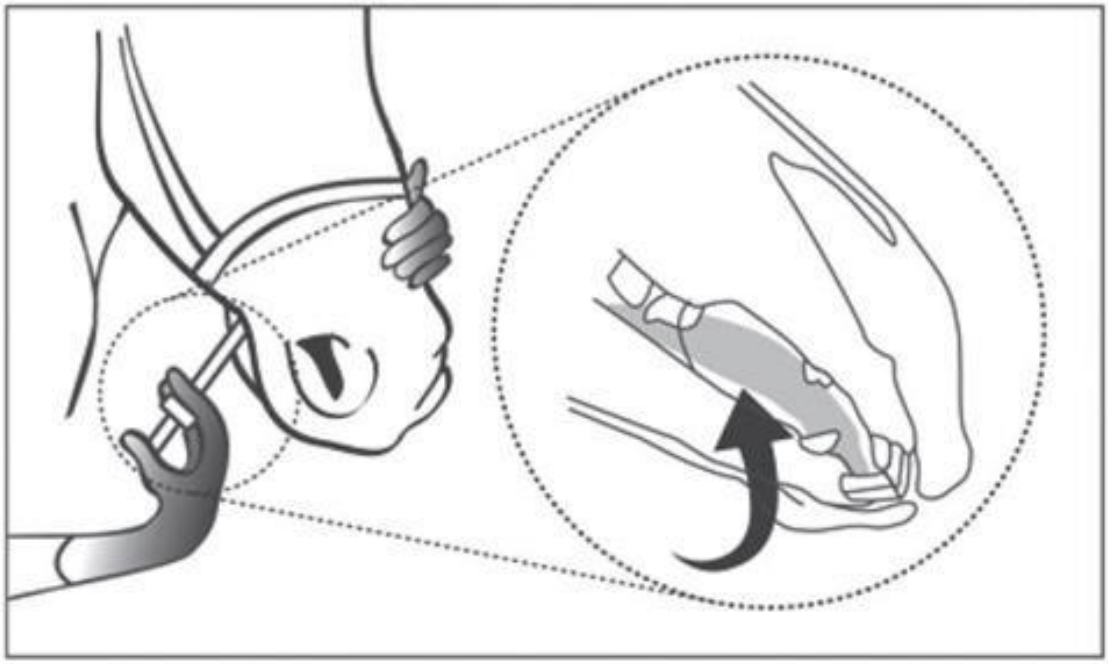
Sublingual administration of detomidine in horses: Sedative effect, analgesia and detection time



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Abstract

A single dose of 40 μg/kg bodyweight (BW) of oromucosal detomidine gel was administered sublingually to 10 healthy Dutch Warmblood mares aged 7 ± 4 years (mean ± SD) and BW 580 ± 69 kg. Blood and urine samples were collected before and for 8 days following administration and evaluated qualitatively in an FEI Reference Laboratory and quantitatively in a research laboratory. Clinical effects were evaluated at baseline and for 24 h after administration. Sedation was determined using head height and scores of reaction to auditory and mixed auditory/sensory stimuli. Mechanical nociceptive thresholds (MNTs) were assessed using pressure algometry to evaluate analgesia. Heart rate (HR) was measured and ataxia scored.
All horses were considered negative for detomidine in blood samples by 48 h post-administration and in urine by 60 h. These results indicated that a safe withdrawal time for detomidine oromucosal gel maybe 72 h following a single sublingual administration of 40 μg/kg BW. Decreases in HR and head height were maximal at 40 and 60 min post-administration, respectively. The maximal decrease in response to stimuli was observed at 100 min. Ataxia was maximal at 60 min. At 40 and 80 min MNTs were significantly increased compared to baseline. All parameters, except the MNTs of two locations, which were decreased, returned to baseline values within 24 h post-administration.

Keywords

Detomidine, sublingual administration, detection time, sedation, analgesia.

Introduction

The use of a-2 agonists is prohibited during competitive events in equestrian sports, as these substances may interfere with fair competition and affect animal welfare. The Fédération Equestre Internationale (FEI) has designated detomidine as a controlled medication substance on the Equine Prohibited Substances List(2012). The FEI provides a list of detection times (DTs) for several of these substances, stating the time the substance may be detected in blood or urine by a laboratory after administration of a specified dose via a specified route of administration.
The DT is influenced by numerous factors, including the route of administration, drug formulation, number of doses administered, individual horse factors (e.g. size of the horse, metabolism and disease), and the detection limit of the screening method used to detect the drug. The concentration above which the sample is officially reported to contain the substance, the limit of reporting (LOR), is harmonised between all FEI reference laboratories. The withdrawal time (WT) is defined as the recommended minimal interval between administration and competition and can be viewed as the published DT plus a safety margin advised by the consulting veterinarian (Toutain, 2010). The DT of detomidine following IV administration is given by the FEI3 as 48 h based on the detection of 3-carboxydetomidine (Machnik et al., 2006). Recently, the DT and WT of detomidine following sublingual administration have been estimated based on data from a non-FEI-accredited laboratory (DiMaio Knych and Stanley, 2011).
The clinical effects of a-2 agonists have been assessed using several variables, such as head height and responses to auditory and sensory input. In previous studies on detomidine, the responses to these stimuli were either scored by an observer (Freeman and England, 2000;Mamaet al., 2009; Rohrbach et al., 2009) or measured by determination of nociceptive thresholds using a model based on a standardised thermal (Elfenbein et al., 2009), electrical (Moens et al., 2003; Rohrbach et al., 2009) or mechanical stimulus (Moens et al., 2003; Elfenbein et al., 2009; Mama et al., 2009).
Clinical effects were present, although delayed in onset, following sublingual administration of the injectable detomidine solution (Malone and Clarke, 1993). An oromucosal gel formulation of detomidine (Domosedan gel, Orion) was granted marketing authorisation for sublingual administration to horses in 2010. In undisturbed horses the mean duration of sedative effect following sublingual administration of this gel was 3 h, and the quality of sedation was profound (Kaukinen et al., 2011; DiMaio Knych and Stanley, 2011). There is no information available about the presence or extent of any analgesic effects of the oromucosal gel.
The objectives of the present study were to determine the detection time of detomidine after sublingual administration, to evaluate the quality and duration of sedation by measuring head height and recording and scoring the reaction to different stimuli, to examine the side effects ataxia and bradycardia, and to evaluate analgesia by assessing mechanical nociceptive thresholds (MNTs), using pressure algometry.

Material and methods

Animals

Ten Dutch Warmblood mares, with a mean age 7 ± 4 years and a mean bodyweight (BW) 580 ± 69 kg, were included in the study. Only mares were selected, as this facilitated frequent urine collection by the use of a urinary catheter at set time points without the need for sedation. All horses were clinically healthy and sound at baseline and did not receive any medication for a minimum of 2 weeks before the experiment. General health status was monitored once daily by brief clinical examination. During the experimental period the horses were housed in individual stalls (4 _ 4 m) in a separate division of the stables at the Utrecht University Equine Clinic. Horses were fed silage and concentrate and exercised once daily. In the week prior to the experiment, all horses were familiarised with their stalls and the stocks for urine collection.
The study was approved by the Animal Ethics Committee (DEC) of Utrecht University (approval number DEC 2010.III.12.142).

Experimental procedures

Detomidine (7.6 mg/mL; Domosedan gel, Orion) doses were administered sublingually according to the table provided in the manufacturer's package leaflet, aiming to give the recommended dosage of 40 μg/kg. Food and water were withheld for 5 h after detomidine administration. Sample collection started 1 day before detomidine administration and was continued for 8 days.

Blood and urine samples

Serum and heparinised plasma samples were collected by jugular venepuncture using a 21 G needle and Vacutainer system on the day before administration and at 6, 12, 24, 36, 48, 60, 72, 84, 96, 120, 144, 168 and 192 h post-administration. Immediately after collection, blood samples were centrifuged at 4000 g for 10 min, and serum and plasma samples were stored at -20 °C.
Urine samples were obtained either by spontaneous voiding evoked by placing the horses in a stable with straw bedding or by catheterisation using a sterile mare urinary catheter on the day before administration and 24, 36, 48, 60, 72, 84, 96, 120, 144, 168 and 192 h (± 10 min) post-administration. Urine samples were stored at -20 °C.

Detection of detomidine and metabolites

Three different limits are used when describing the results of blood and urine analysis. The lower limit of detection (LOD) is the lowest limit at which the substance can be detected using this specific analytical method. The lower limit of quantification (LOQ) is the lowest limit at which the quantitative determination of concentration can be performed reliably, taking into account the measurement error inherent to the method. The limit of reporting (LOR) is the limit above which the sample is officially reported to contain the substance. The LOR is equal to or higher than the LOQ, and determined by FEI regulations.
Serum samples were shipped on dry ice to CRST Bioanalytics, Turku, Finland, for quantitative determination of detomidine, 3-hydroxydetomidine and 3-carboxydetomidine concentrations using high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS). Validation of this quantitative method in equine serum samples was conducted prior to study sample analyses. Assay reproducibility was acceptable, with inter-assay Coefficient of Variation values from 3% to 5% for detomidine, 4% to 13% for 3-hydroxydetomidine and 3% to 14% for 3-carboxydetomidine for quality control samples at concentrations of 0.15, 6.00 and 16.0 ng/mL. Intra-assay accuracy was evaluated by analysing two replicates of the quality control samples at each concentration level. Results were accepted only if the accuracy of the QC samples in the same assay-run was 85–115%/bias ±15%. The LOQ of the method was 0.05 ng/mL for detomidine and 3-hydroxydetomidine, and 0.10 ng/mL for 3-carboxydetomidine.
Plasma and urine samples were shipped on dry ice to Laboratoire des Courses Hippiques (LCH), Verrières le Buisson, France, for qualitative detection of detomidine and 3-carboxydetomidine, as the FEI and European Horserace Scientific Liaison Committee (EHSLC) medication control regulations are based on above-threshold (LOR) detection of the latter in plasma and urine. LCH is an official FEI Reference Laboratory and is the official laboratory of the French Racing Federation (Fédération Nationale des Courses Françaises), which is a member of the EHSLC. Both plasma and urine samples were treated as regular 'medication control samples' and subjected to solid phase extraction (SPE) prior to HPLC–MS/MS analysis on a TSQ ultra Thermo- Fisher tandem mass-spectrometer, with the instrument operated in positive ion multiple reaction monitoring (MRM) mode. LODs of the method were stated as 5 pg/ml and 20 pg/mL for detomidine and 3-carboxydetomidine, respectively, in plasma; and 1 ng/mL for 3-carboxydetomidine detection in urine.

Evaluation of sedative effect

In order to assess the depth of sedation after sublingual detomidine administration, all horses were examined the day prior to sedation and at 20, 40, 60, 80, 100, 120, 140, 180, 240 and 300 min following detomidine administration. A final examination was done the morning after the day of administration (>20 h after administration). At all time-points short video clips were recorded of each horse as follows: (1) at rest in its stable for 30 s; (2) in reaction to an auditory stimulus consisting of a clipping machine (Aesculap Econom II) being activated 1 m from the horse's head; and (3) in reaction to a mixed auditory/sensory stimulus, namely, approaching and touching the horse with the activated clipper over the lateral aspect of the neck.
The tests were executed in a fixed order at each time-point, starting with recording the horse undisturbed in its stable, subsequently measuring head height and heart rate, followed by recording its reaction to auditory and combined auditory and sensory stimuli. For head height the distance from the ground to the lowest aspect of the horse's chin was measured by means of a flexible steel rule. HR was obtained by auscultation of the heart.

Evaluation of ataxia

The day before administration of detomidine, as well as 60, 120, 180, 240 and 300 min after administration and the following morning, the horse was removed from its stable to assess the level of ataxia by a brief neurologic examination. A video clip was made of the examination consisting of walking the horse in a straight line, circling the horse to both sides, and pulling the tail to both sides while walking in a straight line.

Scoring system

All video clips of all horses were randomized per test, and investigators were blinded for time after administration. The video clips of the undisturbed horses and all reactions to auditory and mixed auditory/sensory stimuli were scored by a veterinary anaesthetist, while scoring of ataxia was performed by an orthopaedic surgeon using the scales mentioned in Table 1.

Table 1
Scales used for scoring clinical effects of sublingual administration of detomidine.

Sedation of the undisturbed horse in its stable
0 = no sedation
1 = mild sedation
2 = moderate sedation
3 = deep sedation
Scoring the reaction to the various stimuli
0 = no reaction
1 = change in position of head
2 = body movement <1 m
3 = body movement >1 m
4 = violent flight response
Scoring of ataxia
0 = no ataxia
1 = slightly swaying gait, only visible at circle and when tail pulled
2 = obviously swaying gait, mainly visible at circle and when tail pulled
3 = obviously swaying gait, visible at straight line
4 = serious instability, with stumbling and nearly falling at straight line
5 = animal becomes recumbent

Mechanical nociceptive thresholds

Before administration of detomidine and 40, 80, 140 and 240 min postadministration, as well as the following morning, the MNTs were assessed, subsequent to evaluation of sedative effects, using a non-electrical pressure algometer (FPK 60, Wagner Instruments) with a 1 cm^2 tip and a range of 3–30 kgf. Measuring procedures were based on those described by Haussler and Erb (2006). The force was increased perpendicularly to the body surface at a constant rate of 5 $kg/cm^2/s$.
The following predetermined landmarks were adopted from Haussler and Erb (2006) and marked with paint: the caudolateral aspect of the scapular spine in the mid-portion bilaterally (ScapL and ScapR), the dorsal apex of the spinous process of L6 (L6) and bilaterally on the medial gluteal muscles 7 cm abaxial to L6 (GlutMedL and GlutMedR). An avoidance reaction was regarded as a positive response. When a positive response was observed the application of pressure was ceased immediately and the corresponding value recorded. The measurements were repeated three times with a 3–4 s interval, with the median value assumed as the site-specific MNT (Haussler and Erb, 2006). All algometer measurements were performed in a fixed order, starting on the left side of the horse, in a craniocaudal direction, with L6 being tested from the left side, before the GlutMedL.

Statistics

Data are presented as means ± SD, with t values or z values provided as appropriate. Detomidine or metabolite detection is presented as concentration (ng/mL) or as a positive or negative test result. Statistical analysis was performed using R (Team-R, Foundation for Statistical Computing). Continuous variables were evaluated for normality of distribution and logtransformed where needed. Head height and log (HR) were analysed using a linear mixed model with horse as a random effect and time as a fixed effect. The sedation score and the reactions to the auditory and mixed auditory/sensory stimuli were likewise analysed with a generalised linear mixed model with horse as a random effect and time as a fixed effect, using a Poisson distribution. Ataxia was analysed with a linear mixed model with horse as a random effect and time as a fixed effect. The MNTs were analysed with a linear mixed model with horse as a random effect and time, place and interaction as fixed effects.

Results

Blood and urine

The quantitative results of 3-carboxydetomidine analysis in serum samples are given in Table 2. Detomidine was only above LOQ at 6 h post-administration. 3-Hydroxy detomidine was only above LOQ in samples from 6 horses at 6 h and 1 horse at 12 h postadministration. All other samples were below LOQ for both detomidine and 3-hydroxydetomidine. The qualitative results of 3-carboxydetomidine analysis as determined in the official FEI reference laboratory are presented in Table 3 for plasma and in Table 4 for urine. All blood and urine samples obtained before the experiment were clear of detomidine and its metabolites. From 6 to 24 h after detomidine administration all horses tested above LOR for 3-carboxydetomidine in both plasma and urine. At 48 h, plasma samples from three horses were below LOR but above LOD, while from seven horses the plasma samples were below LOD. At 60 h all plasma samples tested below LOD.
Urine samples from all horses were above LOR at 24 h after detomidine administration for 3-carboxydetomidine. At 48 h, urine samples of three horses remained above LOR, samples from four horses tested below LOR but above LOD and samples from three horses were below LOD. All urine samples at 60, 72 and 84 h and at 4, 5, 6, 7 and 8 days were below LOD. Given these results, plasma samples taken at 84 h or later were not examined.

Table 2
Concentration of 3-carboxydetomidine (ng/mL) in serum samples of 10 horses following sublingual administration of detomidine (40 µg/kg BW).[a,b]

Time after administration (h)	Horse 1	Horse 2	Horse 3	Horse 4	Horse 5	Horse 6	Horse 7	Horse 8	Horse 9	Horse 10
0	<LOQ [a]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
6	0.856	2.15	1.34	1.45	2.29	4.35	0.439	1.51	2.53	3.94
12	1.11	1.4	1.49	1.28	0.916	1.42	0.481	0.518	1.41	0.983
24	0.177	0.691	0.203	0.851	0.668	0.167	0.947	<LOQ	0.201	0.121
36	<LOQ	0.103	<LOQ	0.134	<LOQ	<LOQ	0.269	<LOQ	<LOQ	<LOQ
48	<LOQ [b]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

[a] <LOQ (lower limit of quantification), 3-carboxydetomidine concentration <0.100 ng/mL.
[b] All samples <LOQ at 48, 60, 72, 84, 96, 120, 144, 168 and 192 h post-administration.

Table 3
Detection of 3-carboxydetomidine in plasma samples of 10 horses following sublingual administration of detomidine (40 µg/kg BW).[a,b,c,d]

Time after administration (h)	Horse 1	Horse 2	Horse 3	Horse 4	Horse 5	Horse 6	Horse 7	Horse 8	Horse 9	Horse 10
0	<LOD [a]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
6	+ [b]	+	+	+	+	+	+	+	+	+
12	+	+	+	+	+	+	+	+	+	+
24	+	+	+	+	+	+	+	+	+	+
36	- [c]	+	-	+	+	<LOD	+	<LOD	+	-
48	<LOD	<LOD	<LOD	-	-	<LOD	-	<LOD	<LOD	<LOD
60	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
72	<LOD [d]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

[a] <LOD (lower limit of detection), 3-carboxydetomidine concentration <20 pg/mL.
[b] +, above LOR (limit of reporting) according to FEI regulations.
[c] –, below LOR, according to FEI regulations, but above LOD.
[d] All samples <LOD at 72 h post-administration, samples at 84, 96, 120, 144, 168 and 192 h post-administration not analysed.

Table 4
Detection of 3-carboxydetomidine in urine samples of 10 horses following sublingual administration of detomidine (40 µg/kg BW).[a,b,c,d]

Time after administration (h)	Horse 1	Horse 2	Horse 3	Horse 4	Horse 5	Horse 6	Horse 7	Horse 8	Horse 9	Horse 10
0	<LOD [a]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
24	+ [b]	+	+	+	+	+	+	+	+	+
36	+	+	+	+	+	+	+	-	+	+
48	<LOD	- [c]	<LOD	+	-	-	+	<LOD	-	+
60	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
72	<LOD [d]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

[a] <LOD (lower limit of detection), 3-carboxydetomidine concentration <1 ng/mL.
[b] +, above LOR (limit of reporting) according to FEI regulations.
[c] –, below LOR, according to FEI regulations, but above LOD.
[d] All samples <LOD at 72, 84, 96, 120, 144, 168 and 192 h post-administration.

Evaluation of sedative effects

Sedation scores, head height and HR (Fig. 1) showed onset of effect at 20 min post-administration and maximal effect at 60, 80 and 60 min respectively.
Reaction to an auditory stimulus (Fig. 2a) tended to diminish after 20 min, but this effect only reached statistical significance at 80, 100 and 120 min post-administration. Reaction to a combined auditory and sensory stimulus, (Fig. 2b) was significantly diminished at 60, 80, 100 and 120 min. The response to both stimuli was maximally decreased at 100 min post-administration.

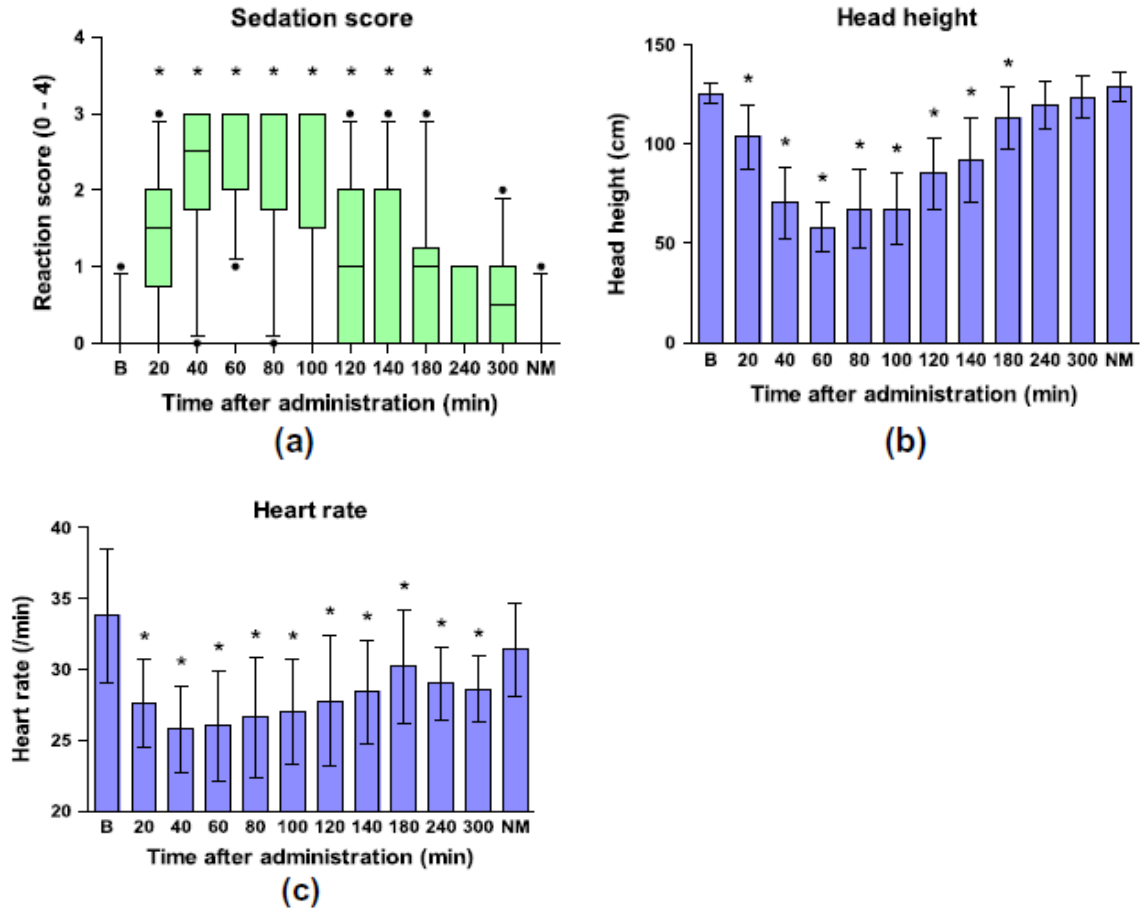

Fig. 1. Sedation score, head height and heart rate of 10 horses undisturbed in the stable at different time points after sublingual administration of 40 µg/kg BW detomidine. (a) Median sedation scores are depicted with boxes with interquartile ranges (25–75%); whiskers are 10–90% of data; dots represent outliers. (b) Head height and (c) heart rate are given as mean ± SD. B represents baseline and NM represents next morning. *Significantly different from baseline ($z > 2$, or $t > 2$ or < -2).

Ataxia

The effect of sublingual administration of detomidine on the degree of ataxia (Fig. 2c) was significant at 60, 120 and 180 min postadministration.

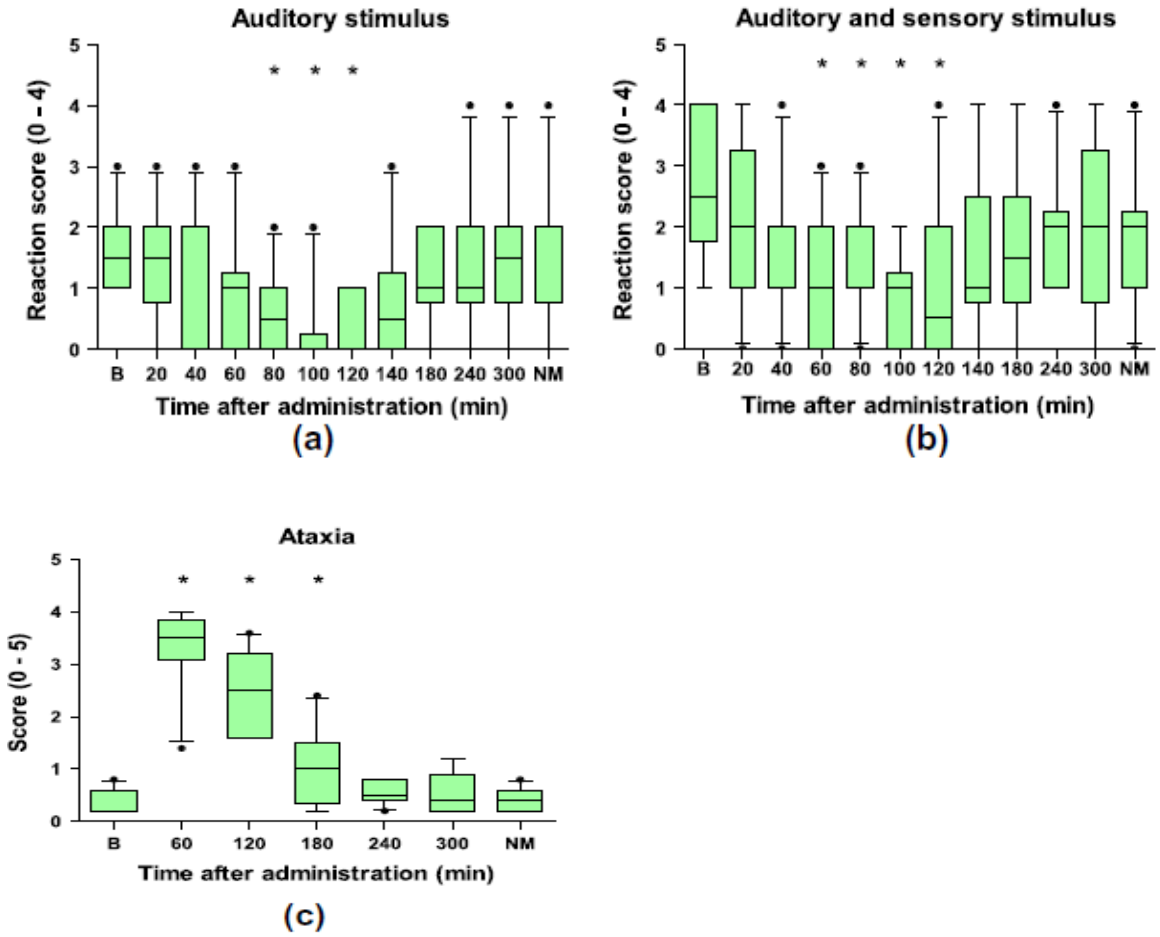

Fig. 2. Scores for 10 horses at different time points after sublingual administration of 40 μg/kg BW detomidine: (a) reaction to an auditory stimulus, (b) reaction to a combined auditory and sensory stimulus, and (c) ataxia. Boxes represent the median with interquartile ranges (25–75%), and whiskers represent 10–90% of data. Dots represent outliers. B represents baseline and NM represents next morning. *Significantly different from baseline values ($z > 2$).

Mechanical nociceptive thresholds

The reproducibility of MNT measurement was adequate, with a range of 2.57 ± 1.72 kgf across all measurements. At 40 min postadministration MNTs were increased at all sites (Fig. 3) except for ScapL, where no significant increase in MNT was found at any time point. The MNT at ScapR was not increased at 80 and 140 min, in contrast to the MNTs at L6 and GlutMed on the left and right side. Significantly lower MNTs compared to baseline were found after 240 min at GlutMedR, and the next morning at both ScapL and GlutMedR.

Discussion

The present study determines the DT of detomidine administered sublingually, while also presenting extensive data about the sedative and analgesic effects as well as two major side effects, all important for its use in equine practice.

Since no obvious differences in metabolism of detomidine were mentioned in previous studies on pharmacokinetics of detomidine using horses from both genders (DiMaio Knych and Stanley, 2011; Kaukinen et al., 2011; Grimsrud et al., 2009; Mama et al., 2009), the authors presume no problems in using only mares in the current study. The choice for mares was due to study design, since repeated urine collection through catheterisation in unsedated horses was needed.

Each horse acted as its own control and measurements before drug administration were considered normal so no negative control horses (e.g. placebo-treated) were used. Positive controls, i.e., dosed with detomidine IV, were not included because of practical and financial constraints. All scoring (sedative effects and ataxia) was done by independent experts in anaesthesia or orthopaedics who were completely blinded for each time point: all video clips were offered for scoring in random order.

To address the issue of different screening methods used in different laboratories the analyses for the present study were performed in duplicate, both qualitatively and quantitatively, in separate laboratories. DTs differed between laboratories. For further discussion, the longest DT (that of the FEI laboratory) will be adopted. Moreover, the advice of the treating veterinarian will be more appropriate for a competition horse when based on the DT of an official FEI laboratory.

Kaukinen et al. (2011) showed that the terminal half-life of detomidine after IV, IM and sublingual administration (40 μg/kg BW) was 0.86 ± 0.15 h, 1.08 ± 0.28 h, and 1.27 ± 0.24 h, respectively. However, these authors did not estimate maximal DTs in plasma or urine, nor did they determine any metabolites, which are often essential in medication control.

A recent study by DiMaio Knych and Stanley (2011) suggested a 48-h and 3-day WT for sublingual administration of detomidine gel (40 μg/kg BW) based on detection in plasma and urine samples, respectively. These data, seem to be in accordance with the DTs found in the present study. However, it should be noted that a separate WT for plasma only is not useful for equine practitioners, as no detomidine (metabolite) is allowed to exceed the prescribed LOR in blood or urine samples, and the WT for urine is the longest.

Onset and duration of sedation after sublingual administration of detomidine in the present study were in line with data obtained by DiMaio Knych and Stanley (2011), Kaukinen et al. (2011) and Malone and Clarke (1993). At 1 day post-administration, no significant alterations in head height, HR, sedation (measured both undisturbed and in reaction to several stimuli), ataxia or elevations of MNTs could be detected.

The determination of MNTs by pressure algometry proved to be quick, leaving the horses undisturbed between measurements long enough to produce a substantial level of sedation. The drawbacks of the method are operator bias and variations in the rate of application (Love et al., 2011). Sufficient training and the use of a single examiner and independent observer to read the outcomes minimised these risks. Another limitation of pressure algometry is the possible influence of a depressed mental state on the reaction of the horse. Although acepromazine does not produce the same sedative effect as detomidine, it is the only sedative without analgesic effects detomidine has been compared to using MNTs. Chambers et al. (1990) reported that MNTs were elevated after IV administration of detomidine, whereas acepromazine had no effect on MNTs.

These findings were recently confirmed by data of Love et al. (2012). A significant decrease in MNTs found at 4 h after administration of detomidine and/or the following morning on the ScapL and the GlutMedR indicated sensitization. This may have been the result of bruising as a consequence of earlier measurements.

This phenomenon has not been described in horses and might be attributed to the repeated application of high pressures because of the analgesia caused by detomidine.

Ataxia was found to be present at time points with concurrent significant decreases in head height and reaction scores, while the decrease in HR remained significant after loss of sedative effects. The prolonged decrease in HR compared to sedation parameters has also been reported by DiMaio Knych and Stanley (2011) and Kaukinen et al. (2011) following the sublingual administration of detomidine (40 μg/kg BW).

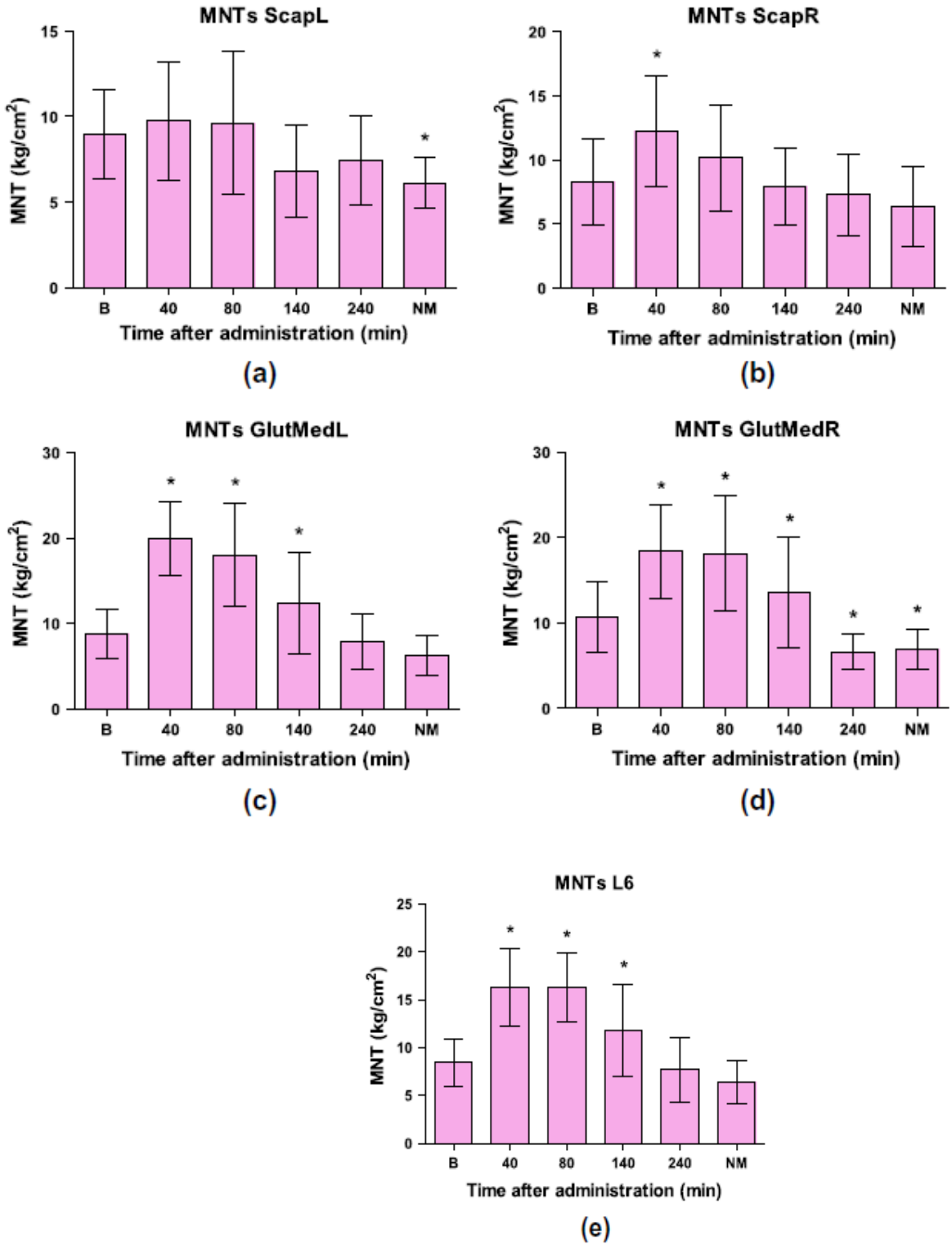

Fig. 3. Mean ± SD of the mechanical nociceptive thresholds (MNTs) of 10 horses at the location of (a) the left scapula (ScapL), (b) the right scapula (ScapR), (c) the left medial gluteal muscle (GlutMedL), (d) the right medial gluteal muscle (GlutMedR), and (e) the spinous process of lumbar vertebra 6 (L6) at different time points after sublingual administration of 40 μg/kg BW detomidine. B represents baseline and NM represents next morning. *Significant difference with baseline ($t > 2$ or < -2).

Conclusions

The present study showed that DT after sublingual administration to horses of detomidine oromucosal gel was less than 60 h in plasma and urine. A safe WT after a single sublingual administration of 40 µg/kg BW may therefore be 72 h. The maximal sedative effect of a single sublingual dose of detomidine was observed between 60 and 100 min post-administration. Four hours after a single sublingual dose, no significant effects on head height, sedation scores or ataxia remained, nor were significant signs of sedation or analgesia detected the next morning.

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